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Low volume PCR (LV-PCR) for STR typing of forensic casework samples

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Abstract. In this study 1 μL low volume PCRs (LV-PCR) using a commercially available chip in microscopic slide format have been performed to test a wide range of forensic casework samples besides sensitivity and mixtures with different STR kits. Moreover critical low copy number and mixed DNA samples were dispensed into 20 individual LV-PCR assays to obtain a combined DNA profile.

Keywords: Low volume PCR; Low copy number; Degraded DNA; STR; Chip

1. Introduction

Commercial multiplex STR typing kits are often used with reduced PCR volumes. A volume reduction of 30–50% normally does not result in a significant loss of quality regarding signal intensity, allele balance, etc. This is especially true for reference samples extracted from blood or buccal swabs where sufficient DNA of good quality is available. But even in low copy number (LCN) amplifications reproducible results can be obtained. This may be due to the assumption that DNA in a lower PCR volume could get into better contact with primer or polymerase molecules because the overall amount of DNA is less diluted than in a higher volume. Otherwise the volume of extracted DNA that can be used for the PCR assay is limited.

In the days of nanotechnology everything is getting smaller. In this case commercial PCR chips have been developed where multiplex PCR can be performed in a 1 μL PCR volume on a 60 well glass chip in microscopic slide format. Circular hydrophilic wells are separated by hydrophobic regions to ensure that the liquid PCR components do not get

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into contact with each other and stay in a drop form comparable to the 'lotus effect'. The 1 μl drops are then covered by mineral oil to prevent evaporation before the slide is put on a suitable in-situ PCR adapter that fits into a common 96-well thermocycler.

2. Material and methods

The "PCR-Chip" AmpliGrid A60 (Alopex, Germany) was used to perform PCR on a Mastercycler (Eppendorf) using an appropriate in situ adapter. Ampf*l*str® Blue™, SEfiler™, Identifiler™-Kits (Applied Biosystems) were used for testing. PCR reaction mix consisted of 0.5 μL master mix and 0.5 μL DNA sample. Master mix and cycling protocol were set up according to manufacturer with 28 cycles. The PCR reaction mix was covered with 5 μL covering solution supplied with the PCR slide. For comparison standard 25 μL PCRs were carried out as well using the same samples. 1 μL PCR product was analyzed with a 3100Avant Genetic Analyzer. The following tests were carried out in duplicates: sensitivity tests—2.5 ng down to 10 pg, mixture tests—blood and DNA mixtures 10:1 to 1:10, amplified in duplicates, forensic samples—blood, saliva, semen, muscle tissue, Achilles tendon, hair, bone, cigarette butts, swabbed skin debris, Isocode™ cards. LCN and degraded DNA samples from routine casework were analyzed by dispensing a 10 μL DNA sample into 20 separate PCR reactions. DNA samples were extracted with standard Qiagen "DNA mini"-Kit and M48 DNA extraction robot (MagAttract DNA Blood Kit), Phenol/Chloroform or the Isocode procedure and quantified with Quantifiler™ on 7000 Sequence Detection System (Applied Biosystems).

3. Results and discussion

As the chip is usable only once on every anchor spot a high sample throughput is necessary to be cost efficient. The chip itself can be used multiple times, but one must be aware of a possible conflict of pre-/post-PCR separation. A maximum of 5 μL of the covering solution should be used to prevent merging of two samples. For correct pipetting 0.5 μL pipette tips (0.1–10 μL) are obligate as larger tips are very imprecise for this application. Due to the small working volume a high risk of evaporation is given so that only 2–5 samples should be prepared at once and covered immediately. A higher contamination risk compared to tubes can be expected while working on the open surface of the slide but was not observed. Besides this, the ability to set up to 30 LV-PCR reactions with the master mix of one standard 25 μL-PCR is comparatively cheap.

All kits showed an increase of sensitivity (see Table 1), as full profiles were obtained using 80–90 pg, e.g. for the SEfiler®. However, in this range especially peak imbalances could be observed. This may be due to pipetting artifacts, as a volume of 0.5 μL always is a random selection of molecules out of the DNA sample so that stochastic effects can be expected. To obtain reliable results we

Table 1
Sensitivity (in pg) for different STR kits

Kit	Partial profiles	Full profile with peak imbalances	Full balanced profile
Blue	10–20	40–50	100–150
SEfiler	10–20	80–90	150–200
Identifiler	10–20	150–200	300–350

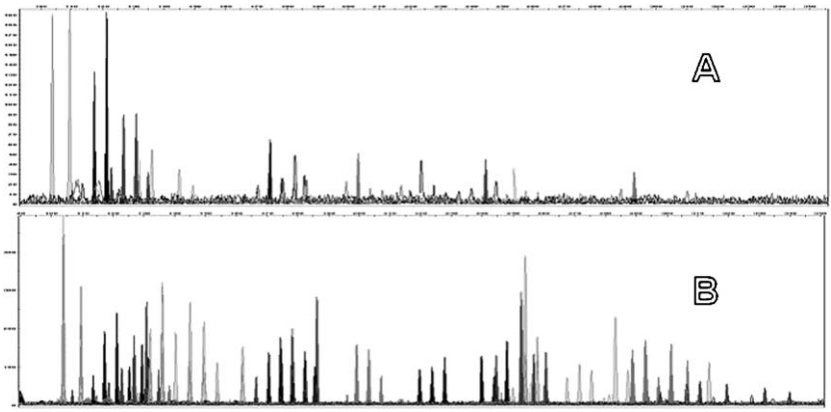

Fig. 1. Typing results of a DNA mixture sample (124 pg/μL, skin debris from a headphone). (A) Obtained profile from a common 25 μL PCR, (B) combined DNA profile including the results of 20 autonomous LV-PCRs.

recommend to set up PCR in duplicates or triplicates for confirmation. Compared to single 25 μL PCR reactions we could observe better peak heights analyzing the same amount of PCR product. The reason for this may be a higher amplification efficiency in the smaller PCR volume. When more than 500–600 pg DNA are amplified, such high amounts of DNA will lead to overloading and possible pull-up artifacts. Good quantification results prior to PCR are necessary.

Mixtures were detected successfully in the whole range up to a ratio of 10:1, for blood and DNA mixtures, respectively.

The typing of forensic casework samples showed no significant deviation from the results obtained with larger PCR volumes (25 μL). Again we observed peak imbalances when low DNA amounts (<100 pg) were used. Low copy number and degraded DNA samples additionally showed allelic or STR system dropouts but as already described above a higher PCR efficiency.

In a pre-testing study selected forensic casework samples (mixture, LCN and degraded DNA) were typed by dispensing 10 μL of the DNA sample into 20 individual LV-PCRs. To compare the results 10 μl of the same DNA sample were amplified in a single 25 μL PCR. The results of the 20 LV-PCRs were combined into one DNA profile by overlaying the electropherograms. Fig. 1 shows the example of a DNA mixture sample (124 pg/μL) typed by a single 25 μL PCR (A) and by combining the results of 20 individual LV-PCRs. Excluding Amelogenin, with the single 25 μL PCR only seven alleles above 50 rfu were obtained.

In contrast, the combined analysis of the 20 LV-PCRs resulted in 55 different alleles. Of these, 30 alleles were confirmed by peak signals above 50 rfu in at least two of the 20 LV-PCRs leading to a much more representative STR profile of the sample. Other DNA samples showed comparable results that were confirmed by reference samples. Only when the DNA was too degraded did the analysis not lead to better results.

The technique of combining a DNA profile from numerous LV-PCRs seems to be a promising method to obtain more information. Further investigations will have to address the contamination risk and the analysis of a higher number of LCN, mixture samples and artificially degraded DNA to verify the present results based on a small number of selected samples.

In conclusion, this study shows that LV-PCR for typing routine and paternity samples is reliable and cost efficient. Forensic casework samples are typed reliably down to 100–150 pg DNA, for lower DNA amounts duplicate or triplicate amplifications are required. Combined DNA profiling as described above may be a possible solution for difficult LCN or degraded DNA samples.