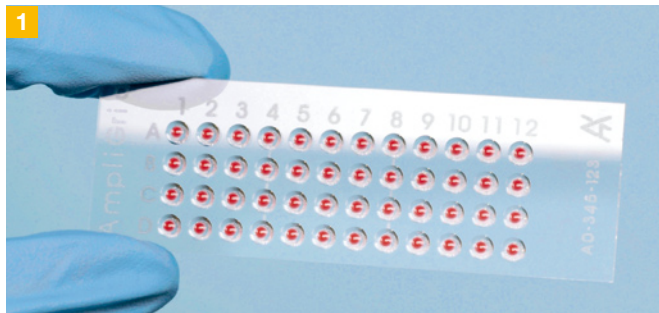


# Getting full profiles from highly redundant low-volume amplifications (LV-PCR)<sup>1</sup>

In forensic DNA analysis, processes such as identification testing, stain analysis and kinship testing, all involve the investigation of short tandem repeat (STR) markers of nuclear DNA, which have proven to be a valuable and sensitive technique (Lutz-Bonengel *et al.*, 2006). Genomic DNA typing in forensic casework is often limited by samples with low template DNA content, leading to incomplete genetic profiles (drop-outs).

Here, we describe a method that enables full PCR profiles to be obtained from samples that show drop outs as a result of a low amount of template. Instead of a single one tube reaction, several redundant amplifications are carried out on the Advalytix AmpliGrid slide. The AmpliGrid slide contains 48 lithographically defined reaction sites (fig. 1) where PCR can be carried out without cross-contamination and evaporation.

Although the limited material is even more subdivided by this procedure, the intensity of positive alleles after pooling the series of reactions for one capillary electrophoresis run are dramatically increased. Moreover the number of drop-outs is reduced.



AmpliGrid AG480F

## Partitioning DNA samples

A set of serial dilutions of the human genomic DNA 9947A (Promega) was prepared in PCR master mix (tab. A): template amounts of 62 pg, 31 pg, 16 pg and 8 pg were each made up to a total volume of 20  $\mu$ L using master mix (tab. A). From each dilution, one aliquot of 10  $\mu$ L was transferred into a conventional PCR reaction tube (control). In parallel 1  $\mu$ L of each dilution was pipetted on 10 AmpliGrid reaction sites respectively.

## Amplification reaction

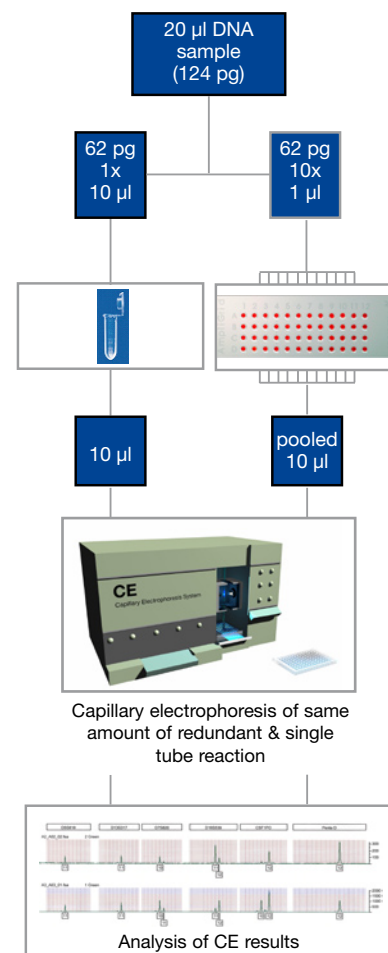
The PowerPlex®16 master mix (Promega) was prepared as shown in table A and applied with specified template amounts as described above. A total of 15 replicate tube reactions were compared with 15 pooled LV-PCR ranks. The tube reactions were carried out using PowerPlex®16 standard conditions. Samples on the AmpliGrid slides were

covered immediately with 5  $\mu$ L of sealing solution before starting the amplification reaction.

## Analysis of amplification products

After the thermal cycling process (tab. B), the 10 samples of each dilution on the AmpliGrid were pooled together. The one tube reactions were used directly for downstream processing. The amplified products were mixed with 1  $\mu$ L Internal Lane Standard 600 (Promega) and 19  $\mu$ L formamide, denatured and detected using a Genetic Analyzer<sup>2</sup>. The whole workflow is shown in figure 2.

### 2 Workflow low volume amplification



**A** Table A: Composition of master mix

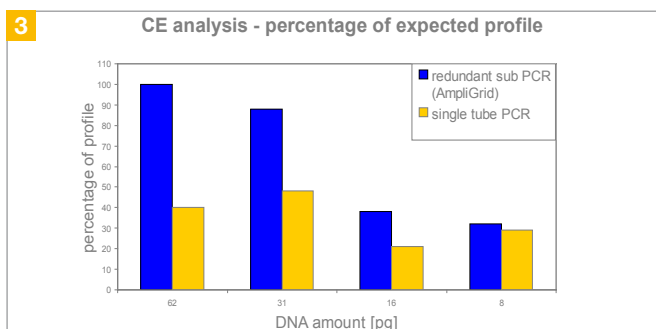
Component	Master mix
Nuclease-Free Water	19.2 µL
Gold St r 10x Buffer	2.5 µL
PowerPlex® 16 10x Primer Pair Mix	2.5 µL
AmpliQa Gold® DNA polymerase	0.8 µL
Total volume	25 µL

**B** Table B: Amplification programme

Temperature	Duration	
95°C	15 min	
96°C	1 min	
94°C	30 sec	
60°C	30 sec	10 cycles
70°C	45 sec	
90°C	30 sec	
60°C	30 sec	20 cycles
70°C	45 sec	
60°C	30 min	
20°C	5	
8°C	∞	

## Results

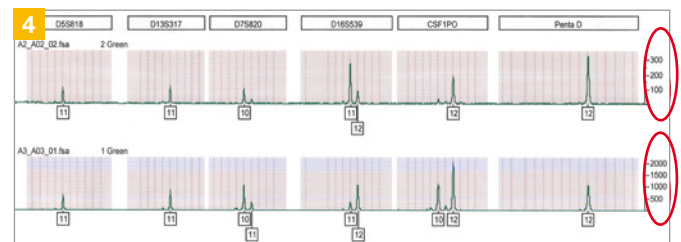
According to Promega's certificate of analysis, all 25 different alleles must be detectable at 500 pg for the DNA template (9947A) as provided in the PowerPlex® 16 System. Template amounts in 10 µL volume in conventional tubes were amplified in comparison to 10 parallel LV-reactions of 1 µL each on the AmpliGrid. The 10 LV-reactions were pooled in one reaction tube after PCR, analysed by Capillary Electrophoresis (CE) and compared with CE results of the conventional 10 µL PCRs. Analysis of redundant LV-PCRs identified significantly more of the expected 25 alleles than the single tube amplification reactions (fig. 3). Furthermore the signal intensities of the CE electropherogram were significantly higher for the LV-PCRs in comparison to the single tube reactions (fig. 4).



Percentage of expected STR profile using the PowerPlex16 Kit (Promega, Germany) on the AmpliGrid slide (redundant PCR in 1 µL) compared to a standard single tube PCR (25 µL volume) after filtering with peak height cut-off values. 100% is equivalent to 25 alleles.

## Discussion

Comparison of the CE electropherograms of the LV-PCRs to the single tube PCRs shows significantly higher detected signal intensity when amplifying the same amount of template using the AmpliGrid platform (fig. 3 and 4). LV-PCR using the AmpliGrid technology increases PCR efficiency by a factor of 10, therefore enabling researchers to get a full profile from as little as 60 pg of template DNA compared to the 500 pg required for conventional methods. This is due to the higher amplification efficiency within the smaller reaction volumes used on the AmpliGrid LV-PCR system.



A part of electropherogram with allelic profile signals of LV-amplification on the AmpliGrid (bottom) and conventional PCR in 10 µL volume in tube (top). Note: Attend the change in scale when comparing peak heights.

## REFERENCES

Proff C, Rothschild MA, Schneider PM. Low volume PCR (LV-PCR) for STR typing of forensic casework samples. In: Amorium A, Corte-Real F, Morling N (Eds.) Progress in Forensic Genetics (2006), Vol 11. Elsevier Amsterdam, pp. 645-647

Lutz-Bonengel S, Sanger T, Heinrich M, Schon U, Schmidt U. Low volume amplification and subsequent sequencing of mitochondrial DNA on a chemically structured chip. Int J Legal Med (2006), submitted

<sup>1</sup> „LV-PCR“ was first used by: Proff *et al.* (2006)

<sup>2</sup> CE analysis of amplified PCR fragments was done at Dr. Lauk & Dr. Breitling GmbH, Wildberg

Power Plex® System is a trademark of Promega GmbH, Germany

Polymerase Chain Reaction (PCR) process is covered by patents which are owned by Hoffmann-La-Roche Inc. and F. Hoffmann-La Roche Ltd.