

INVESTIGATIONS ON COMBINED SINGLE CELL, LCN AND DEGRADED DNA PROFILING BY REDUNDANT ON-CHIP LOW VOLUME PCR (LV-PCR)

Proff, Carsten¹, Mann, W. ², Rothschild, M.A.¹, Schneider P.M.¹

¹*Institute of Legal Medicine, University Clinic Cologne, Germany*

²*Advalytix AG, Brunnthal, Germany*

Commercial multiplex STR typing kits are often used with reduced PCR volumes as a volume reduction of 30-50% normally does not result in a significant loss of typing quality [1, 2]. In this study commercial PCR chips (AmpliGrid™ AG480, Advalytix, Brunnthal) have been used where multiplex PCR can be performed in a 1 µL-PCR volume on a 48 well glass chip in microscopic slide format. Circular hydrophilic wells are separated by hydrophobic regions to prevent cross contamination. Using this technology, it is possible to obtain a full DNA profile in a 1 µl volume consisting of 0.5 µL DNA sample and 0.5 µL PCR reaction master mix. After successful testing of routine casework samples [3], tests on sensitivity, reproducibility, allele balance, allelic dropout and signal intensities, and selected forensic casework samples (mixture, low copy number and degraded DNA) were carried out by dispensing 10 µL of the DNA sample into 20 LV-PCRs of 0.5 µl each. To compare the results from the redundant reactions with those from a standard reaction, 10 µL of the same DNA sample were amplified in a single 25 µL-PCR. The results of the 20 LV-PCRs were combined which, in many cases, led to a much more representative and reliable STR profile of the sample compared to the standard 25 µL-PCR, especially as doubtful allele calls for one DNA sample are often confirmed by one or more of the remaining 19 LV-PCRs. In the present study our primary goal was to check out experimentally how many redundant LV-PCRs are necessary to obtain an optimal DNA profile from various difficult DNA sample types, and in particular from LCN samples. For this objective a large number of different DNA samples were redundantly amplified using the PowerPlex® ES (Promega) and the AmpFISTR® SEfiler™ (AB) STR typing kits by up to 46 LV-PCR for each DNA sample. Serial DNA dilutions down to 5-6 pg, single cells obtained by a fluorescence activated cell sorter (FACS) and artificially degraded samples (DNA and single cells) were chosen to obtain sufficient data to evaluate the redundant LV-PCR typing strategy. Taking this data into account, further LCN, mixture and degraded routine casework samples were amplified using the now improved typing strategy. Furthermore, results combining mini-STR typing with redundant LV-PCRs on chip will be presented. Our findings comprising several thousand LV-PCRs demonstrate that combining the results of redundant LV-PCRs into a single DNA profile is a serious option in the investigator's toolbox to get reliable and reproducible results out of difficult DNA samples.

[1] Gaines ML, Wojtkiewicz PW, Valentine JA et al. (2002) Reduced volume PCR amplification reactions using the AmpFISTR® Profiler Plus™ Kit. *J Forensic Sci* 47:1224-1237 [2] Leclair B, Sgueglia JB, Wojtowicz PC, et al. (2003) STR DNA typing: increased sensitivity and efficient sample consumption using reduced PCR reaction volumes. *J Forensic Sci* 48:1001-1013 [3] Proff C, Rothschild MA, Schneider PM (2006) Low volume PCR (LV-PCR) for STR typing of forensic casework samples. In: Amorim A, Corte-Real F, Morling N (Eds.) *Progress in Forensic Genetics*, Vol. 11. Elsevier, Amsterdam, pp. 645-647