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Efficacy and limits of genotyping low copy number DNA samples by multiplex PCR of STR loci

A.D. Kloosterman*, P. Kersbergen

Netherlands Forensic Institute, Volmerlaan 17, 2288 GD Rijswijk, The Netherlands

Abstract

In this validation study, we have evaluated the efficacy and the validity of the SGM Plus test using an amplification regime of 34 cycles. We obtained valid DNA typing results from pristine extracts with an extremely low DNA content. In this context, the aspects of single cell PCR typing were also evaluated. In these experiments, the allele dropout phenomenon was clearly demonstrated. From actual casework samples, we obtained conclusive DNA profiles from highly purified extracts of bone and teeth that failed to demonstrate typing results using the standard PCR protocol of 28 cycles. Moreover, low copy number (LCN) DNA typing offered us the possibility to reanalyse crime samples that failed to produce a conclusive profile after 28 cycles. Unfortunately, several complications accompany ultrasensitive PCR amplification. During our validation studies, we have observed increased risk of contamination, allelic dropout, locus dropout and heightened stutters. Analyses of heterozygote balance, between-loci balance and stutter heights, show that the 34-cycle PCR has its own characteristic features. We finally show that reamplification of SGM Plus PCR products by an extra 6 PCR cycles offers a promising new alternative if too little of the original sample extract is left for a complete reanalysis.
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1. Introduction

STR analysis by multiplex PCR has enabled the forensic scientist to derive DNA profiles from minute amounts of biological sample. Using the commercially available SGM Plus kit, reliable typing results can be obtained [1]. Complete and accurate STR profiles can be obtained from as little as 100 pg of DNA template. This amount of DNA is

* Corresponding author.
E-mail address: a.kloosterman@nfi.minjus.nl (A.D. Kloosterman).

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contained in the nuclei of no more than 18 diploid cells. By further increasing the sensitivity of the typing system, DNA profiling from minute traces of evidence such as residues left in a fingerprint or palm mark comes within reach. One way to increase the sensitivity of PCR amplification is by simply extending the number of PCR cycles. Increased sensitivity of the SGM Plus typing system has been achieved by extending the number of cycles from 28 to 34. Unfortunately, several complications accompany ultrasensitive PCR amplification. Gill et al. [2] observed increased risk of contamination, allelic dropout, locus dropout and heightened stutters.

In this study, we assessed the validity of the 34-cycle PCR method and made an inventory of the complications that accompany ultrasensitive DNA typing of forensic samples with low copy number (LCN) of DNA template molecules. The sensitivity and the characteristics of the 34-cycle PCR were examined by typing single-diploid cells and extremely diluted pristine DNA samples. The efficacy in the actual forensic setting was taken from experiments with selected samples from human remains and forensic casework. Most of these samples had failed to generate a complete profile using standard PCR conditions. We have also investigated the possibility of reamplification. PCR products from LCN samples that failed to generate a complete profile after 28 cycles were subjected to a further 6 PCR cycles. This approach might be relevant in those cases where too little original extract is left for renewed amplification.

2. Materials and methods

2.1. Multiplex PCR

During all our experiments, the AM*Fl*STR® SGM Plus™ primer mix and AmpliTaq Gold® DNA polymerase were used for amplification. PCR products were analysed on an ABI Prism 310 Genetic Analyzer. DNA from swabs was extracted using the Chelex extraction method. DNA from pulverised teeth and bones was extracted using the phenol/chloroform extraction. The PCR reaction components and cycling conditions were according to the manufacturers' instructions [3]. The cycling conditions of the 34-cycle PCR were unchanged. During all our experiments, we maintained a reaction volume of 25 μl.

2.2. Single cell PCR

Single cells from the white cell pellet of a freshly obtained blood sample were picked out by micromanipulation and placed directly into a PCR reaction tube already containing the PCR reaction components.

2.3. "28+6 cycles"

AmpliTaq Gold® DNA polymerase of 2.5 U was added to the PCR reaction mix that had already been subjected to 28 PCR cycles. The cycling conditions for the "extra" 6 cycles were identical.

3. Results

3.1. LCN profile characteristics

Seventy-five different profiles from LCN samples that were obtained from skin swabs, touched objects and highly diluted pristine DNA samples were evaluated for stutter, between-loci balance and heterozygous balance. The increased number of PCR cycles had no dramatic effect on the incidence and proportion of stutter. All 10 STR loci showed median stutter values of 0.1 or less. The loci that showed the largest amount of stutter were D3 (median stutter proportion 0.08 and maximum observed stutter 0.15) and D19 (median 0.10; maximum 0.16). The evaluation of the between-loci balance showed a major tendency for the short STR loci to amplify preferentially. This effect appears to be inherent to the amplification of LCN samples. In addition, DNA degradation and the presence of PCR inhibitors may add to this effect. The effect of preferential amplification of the shorter fragments was also demonstrated within heterozygote loci. Especially the FGA system was prone to heterozygote imbalance with the long FGA fragments tending to allele dropout.

3.2. One-cell experiments

The 34-cycle PCR amplification was sufficient to generate SGM Plus profiles from single-diploid cells. However, in almost every one-cell profile, we observed allele dropout and sporadically locus dropout. One of the three artefacts in the profiles of seven cells tested was of special interest because it was probably due to a somatic mutation (D8 $8 > 9$). The other two artefacts concerned the observation of spurious alleles that could not be put down to sample contamination.

3.3. Casework samples

During this study, we reanalysed a variety of casework samples that produced no or only partial SGM Plus profiles using the standard 28-cycle PCR protocol. DNA extracts were submitted to 34-cycle PCR amplification. In a number of cases, Microcon ultrafiltration was used to further purify and concentrate DNA extracts. The most striking results were obtained when extracts of bone and teeth were submitted to 34-cycle PCR. In a major exercise, we analysed over 20 human remains from a 10-year-old mass grave. In this experiment, we obtained complete profiles from all remains tested. In addition, the profiling of biological samples with minute amounts of DNA extract from a specific and discrete origin (blood, hair-root and saliva) benefits from the 34-cycle amplification. LCN crime samples containing cells from unknown origin (touched objects, undefined biological stains, swabs from the skin of strangulation victims and potentially containing cells from the assailant) gave varying results. Often a non-interpretable or inconclusive profile was obtained. However, in a limited number of instances, we were able to produce profiles with a high evidential value. In the course of these experiments, it was noticed that the template efficiency of the genomic template DNA can be enhanced through ultrafiltration of the DNA extract.

3.4. "28+6 cycles"

Twenty-eight-cycle PCR products from various casework mentioned above were subjected to reamplification with 6 extra PCR cycles. It appeared that this approach was at least as effective as the 34-cycle PCR amplification for genotyping LCN samples.

4. Discussion

The efficacy of genotyping LCN DNA samples by multiplex PCR of STR loci can be improved by increasing the number of PCR cycles. We propose that 34-cycle PCR is a valuable tool when samples that could not be sufficiently analysed after the standard PCR procedure have to be reanalysed. The potential of this delicate new application of the PCR technology can only show to be an advantage if the analysis is executed under rigorously clean laboratory conditions with proper negative and positive controls and if the guidelines for the evaluation of the profiles are fully taken into account [2].

Finally, in the course of this study, it appeared that reamplification of PCR products from LCN samples with an extra 6 PCR cycles seems to offer an alternative and promising new approach for typing LCN samples in the forensic DNA practice.

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