

Consensus profiles and databasing of casework samples amplified with 34 PCR cycles: an empirical approach

V. Castella*, N. Dimo-Simonin, C. Brandt-Casadevall, P. Mangin

Institut universitaire de médecine légale, Rue du Bugnon 21, CH-1005 Lausanne, Switzerland

Abstract. Forensic laboratories commonly have to analyze samples that contain minute amounts of DNA (Low Copy Number DNA). LCN samples can be amplified with increased numbers of PCR cycles, but stochastic variations of their profiles are generally observed. Here, we compare some characteristics of the AmpliSTR SGM Plus profiles from casework samples amplified with 28 and 34 cycles in order to evaluate the interpretation guidelines used in our laboratory to genotype LCN samples. A first striking result was that 60% of the 563 casework samples considered yielded DNA concentrations < 200 pg/ μ l DNA and were considered as LCN. Conclusive profiles were recovered for 142 of them (42%). At the end, 142 profiles amplified with 28 cycles and 119 profiles amplified with 34 cycles were sent to the Swiss data base. Respectively, 43.66% and 43.70% of them made a hit. This suggests that reliable LCN profiles can be obtained when using an adapted strategy. © 2003 Elsevier B.V. All rights reserved.

Keywords: STR; LCN; Consensus profile; Multi-tube; Data base

1. Introduction

Forensic laboratories commonly have to analyze samples that contain minute amounts of DNA (Low Copy Number DNA). Furthermore, part of this DNA may be degraded and/or some PCR inhibitors may be present in the extract. Consequently, although it is theoretically feasible to amplify the genetic content of a single cell (~ 6 pg DNA), it may be problematic to obtain reliable AmpliSTR SGM Plus (Applied Biosystems) profiles when concentrations of template DNA are < 200 pg/ μ l. Increasing the number of PCR cycles enables to enhance the sensitivity of the PCR. Nevertheless, the benefit of increased sensitivity has to be balanced against a stochastic variation of the profiles (heterozygous imbalance, allelic dropout and false alleles) [1]. Here, we compare some characteristics of SGM Plus profiles from casework samples amplified with 28 or 34

* Corresponding author. Tel.: +41-21-314-70-58; fax: +41-21-314-70-90.

E-mail address: Vincent.Castella@hospvd.ch (V. Castella).

Table 1
Information concerning the 563 casework samples considered

Categories of samples	Number of samples	Extraction protocol	[DNA] ≥ 200 pg/ μ l	CODIS Profiles ^a
Blood stains	100	Chelex	86%	94%
Semen stains	8	Chelex	88%	88%
Saliva stains	238	Chelex	65%	84%
Epidermal stains on clothes	56	Phenol/chloroform	54%	55%
Epidermal stains on swabs	155	Chelex	6%	19%
Hairs	6	Phenol/chloroform	17%	17%
Overall	563		40%	64%

^a ≥ 6 and 8 loci scored for profiles of respectively one and two persons.

cycles in order to evaluate the interpretation guidelines used in our laboratory to genotype LCN samples.

2. Materials and methods

A total of 563 casework samples analyzed during the first semester of 2002 were considered. Their DNA was extracted using chelex or phenol/chloroform protocols (Table 1). One microliter of each extract was used to estimate the DNA concentration of the samples with the Quantiblot method (Applied Biosystems). Samples with DNA concentrations ≥ 200 and <200 pg/ μ l were amplified with the SGM Plus kit in 25 μ l reaction volume with 28 or 34 PCR cycles, respectively. One microliter of the amplicons was analyzed with an ABI Prism 310 Gene Sequencer using standard protocols.

Profiles obtained with 28 cycles were confirmed with a second amplification, generally performed with the same PCR conditions. When DNA concentrations were <200 pg/ μ l, the first 34 cycles amplification was always performed using 1 μ l extracted DNA. The PCR was then replicated, sometimes with different amount of template DNA, in order to apply a multi-tube approach [2,3]. The interpretation guidelines adopted in our laboratory were the following. Each locus was evaluated independently and the heterozygous loci were checked first. The two alleles present at one heterozygous locus were considered only when they appeared together at least three times (minimum three positive amplifications per locus) or when they appeared together twice and the two alleles were further detected at least once each (minimum four positive amplifications per locus). The allele present at one homozygous locus was considered only when no other allele was detected more than once over all the amplifications available.

3. Results

Overall, DNA concentrations ≥ 200 pg/ μ l were obtained for 226 out of the 563 casework samples considered (40%, Table 1). The vast majority (97%) of the stains with DNA concentrations ≥ 200 pg/ μ l enabled the obtainment of profiles suitable for the Swiss data base. Contrastingly, 42% of the stains with <200 pg/ μ l DNA enabled to recover such profiles. Negative profiles were generally not reproducible over amplifications or contained more than four alleles at some loci.

For each category of stains, the percentages of profiles suitable for the Swiss data base (overall 64%) were always \geq to the proportions of stains with substantial amounts of DNA (overall 40%; [Table 1](#)). Interestingly, several samples with DNA concentrations ≥ 200 pg/ μ l could not be amplified with 28 cycles. Once these extracts were diluted and amplified with 34 cycles, full SGM Plus profiles were obtained.

A total of 142 profiles amplified with 28 cycles and 119 profiles amplified with 34 cycles were sent to the Swiss data base. At the end of July 2002, respectively 43.66% and 43.70% of these profiles have made a hit in this data base.

4. Discussion

Samples that contain < 200 pg/ μ l DNA may be difficult to genotype with the SGM plus kit using 28 PCR cycles. In fact, with such conditions, conclusive profiles would have been expected for about 40% of the 563 stains considered here (i.e. those with DNA concentrations ≥ 200 pg/ μ l). Thanks to the enhanced sensitivity of the 34 cycles, this percentage was increased to 64%. No difference between the data base hits' rates of the casework samples amplified with 28 or 34 cycles was observed. This suggests that reliable LCN profiles were obtained with our interpretation guidelines.

In some cases, the use of smaller volumes of template DNA with enhanced number of PCR cycles improved the PCR reaction. In fact, the amount of PCR inhibitors present in the reaction mix is also diminished when less extract is used for the amplification. The PCR with 34 cycles may thus represent a strategy to analyze inhibited samples, even when they contain substantial amounts of DNA.

Acknowledgements

The authors gratefully acknowledge, Catherine Jan Cabrita, Syndie Delessert, Marie-Laure Morerod, Annelise Grini and Carmelo Simili for their assistance and contributions to this work.

References

- [1] J.P. Whitaker, E.A. Cotton, P. Gill, A comparison of the characteristics of profiles produced with the AMPFISTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis, *Forensic Sci. Int.* 123 (2001) 215–223.
- [2] P. Taberlet, et al, Reliable genotyping of samples with very low DNA quantities using PCR, *Nucleic Acids Res.* 24 (16) (1996) 3189–3194.
- [3] P. Gill, et al, An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, *Forensic Sci. Int.* 112 (2000) 17–40.