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Absolute quantification of forensic casework samples using quantitative real-time PCR (qPCR) methods

I. Schulz *, P.M. Schneider, M.A. Rothschild

Department of Legal Medicine, University of Cologne, Melatengürtel 60-62, 50823 Cologne, Germany

Abstract. DNA quantification of forensic samples was carried out using two quantitative real-time PCRs (qPCRs) that amplify the same target sequence but differ in their amplicon length. In a retrospective survey STR profiling results have been compared to qPCR results for the degree of agreement. © 2006 Published by Elsevier B.V.

Keywords: Quantitative real-time PCR; Forensic casework; Amplicon size differences; Quantifiler™ Human DNA kit; Telomerase assay

1. Introduction

A typical problem in forensic casework is to determine the optimal DNA amount for successful STR amplification. Deviations from the optimal template range leads to DNA profiles with e.g. imbalanced peaks, allelic or locus drop-outs, or to no DNA profile at all. The strong impact of DNA concentration on the subsequent PCR amplification success makes a reliable, sensitive and human-specific quantification essential. Therefore, two quantitative real-time PCRs (qPCRs) were optimized with respect to reaction volume, within- and between-run precision, sensitivity and species specificity. Both methods, the Quantifiler™ Human DNA quantification Kit and the homemade Telomerase assay amplify the same target sequence but differ in the amplicon length with 62 bp for the QTF assay and 98 bp for the TEL assay. A total of 1084 samples which were previously genotyped in the period from 2003 to 2004 were quantified with both qPCRs and compared with previously obtained genotyping results from STR analysis.

* Corresponding author. Tel.: +49 221 4784287; fax: +49 221 4783496.
E-mail address: iris.schulz@uk-koeln.de (I. Schulz).

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2. Material and methods

DNA from routine casework samples ($n = 1084$) was extracted using conventional PCI method, M48 BioRobot and QIAmp DNA Mini Kit (QIAGEN), respectively. DNA profiling was performed using commercially available multiplex kits (SEfiler, Identifiler, AB; PowerPlex 16 and Y, Promega) in a total reaction volume of 12.5 μL. 1 μL PCR product was electrophoresed using the ABI PRISM™ 3100 Avant Genetic Analyser. For qPCR a human control DNA (10 ng/μL, AB) was twofold serially diluted to construct a standard curve ranging from 5 ng/μL to 0.078 ng/μL and quantified in triplicates. Quantification of all extracts was done with 2 μL of template in a total of 10 μL reaction volume with 5 μL 1 × TaqMan® Universal PCR Mastermix for both assays. 3 μL primer probe mix was used for the QTF assay and 200/600/600 nM of probe and primers for the TEL assay. qPCR conditions followed recommendations of AB. Runs were accepted with a slope of the standard curve between −3.1 and 3.4, correlation factor >0.98 and no signal for NTC.

3. Results and discussion

A reliable, sensitive and human-specific DNA quantification is essential for optimal amplification of STR systems and enables to exclude the large group of "DNA negative" samples from further analysis (Fig. 1A). Validation work indicated a precise, reproducible and sensitive quantification of human DNA for both qPCR methods (data not shown). Besides the very helpful indication of PCR inhibitors, the QTF assay was also found to be more efficient and more sensitive than the TEL assay (Fig. 1B). However, for quantifying DNA in heavily degraded samples the length of the qPCR target size plays an important role since the overall (degraded) DNA quantity is not necessarily the amount of amplifiable DNA via STRs [1,2]. Thus, the smaller sized amplicon of the QTF assay yielded more false-positive results. Due to similar sizes of the TEL and STR amplicons the TEL assay had a better predictive value for STR amplification success.

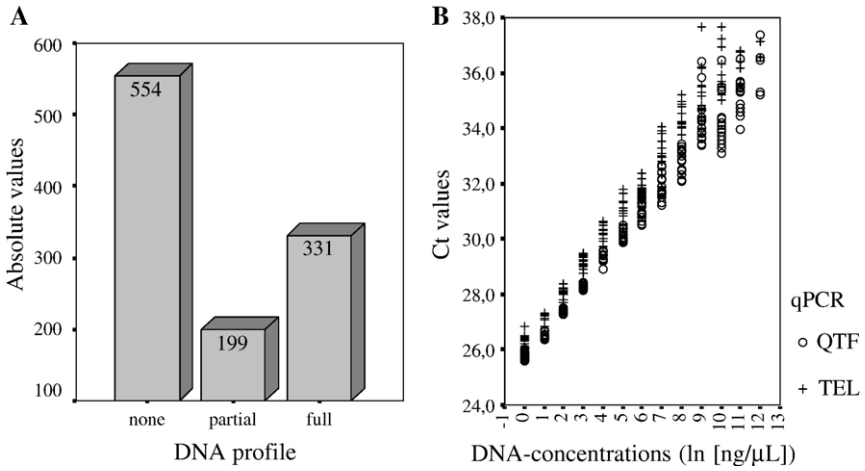

Fig. 1. (A) Overall distribution of DNA profiles. (B) DNA concentrations (ln) and Ct values.

Table 1
Degree of agreement for qPCR results and STR profiles

Expertise	Method	DNA detected	0	<500 pg	≥500 pg	Total
No profile	TEL assay	Abs. values	357	194	3	554
		% of method	64.4	35.0	0.5	100.0
	QTF assay	Abs. values	218	329	7	554
		% of method	39.4	59.4	1.3	100.0
	Total	Abs. values	575	523	10	554
		% of method	51.9	42.2	9.0	100.0
Partial profile	TEL assay	Abs. values	57	114	28	199
		% of method	28.6	57.3	14.1	100.0
	QTF assay	Abs. values	11	125	63	199
		% of method	5.5	62.8	31.7	100.0
	Total	Abs. values	68	239	91	398
		% of method	17.1	60.1	22.9	100.0
Partial profile	TEL assay	Abs. values	49	97	185	331
		% of method	14.8	29.3	55.9	100.0
	QTF assay	Abs. values	21	70	240	331
		% of method	6.3	21.1	72.5	100.0
	Total	Abs. values	70	167	425	662
		% of method	10.6	25.2	64.2	100.0

However, both qPCRs did only agree fair with STR profiling results (measure of degree of agreement with kappa: $\kappa_{TEL}=0.395$; $\kappa_{QTF}=0.345$; $n=1084$). On the one hand, there were samples in which no DNA could be detected by both qPCRs but which previously produced an STR profile (Table 1). The inability to quantify DNA could be due to two reasons: first, the amount of available molecules had been completely consumed by PCR repetition for STR analysis; second, due to the long time span between STR amplification and qPCR quantification (>2 years) the former high-quality DNA had been gradually degraded into smaller fragments than the qPCR amplicons can detect. The other scenario was also observed in which no STR profile could be retrieved but the recent quantification results suggest that there is a reasonable DNA amount to enable genotyping (Table 1). This discrepancy could be caused by e.g. contaminants which previously inhibited STR profiling. Further analysis will clarify whether a profile can be retrieved for the inhibited samples as it was found for some of these (data not shown). The other reason for deviations between STR and qPCR results could be due to degradation and different amplicon lengths. The smaller sized qPCRs will still detect more degraded fragments whereas the STR amplicons will fail to amplify these. Thus, the amount of amplifiable DNA is overestimated by qPCR, which was especially true for the QTF assay. Even though the degree of agreement was rather fair for both assays, quantification via qPCR provides a reliable and robust tool to determine specifically and sensitively human DNA including the advantages of saving time, labour and precious DNA.

References

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