International Congress Series 1288 (2006) 750–752

www.ics-elsevier.com

Quantification of human DNA by real-time PCR in forensic casework

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Abstract. We extensively employed a real-time quantitative PCR system, together with a commercially available kit (Quantifiler™ Human DNA Quantification Kit, Applied Biosystems), for the quantification of human DNA in a large variety of samples. The results we obtained were reliable, with a low deviation standard for the same sample; however, when the inhibitors in a sample were in high concentration and/or DNA degradation was present (like in postmortem matrices), we observed variable results of quantification in the same extract. Here, we report our experience using this real-time based method for quantification and typing of samples recovered in forensic casework.

Keywords: Real-time PCR; DNA quantification; Forensic analysis

1. Introduction

The estimation of the amount of human DNA is a recommended procedure in forensic genetics [1]. To establish the DNA concentration and to evaluate the PCR inhibition in a sample represents a necessary step before developing a PCR reaction. Real-time PCR is based on the 5-nuclease activity of the *Taq* DNA polymerase to cleave a target-specific fluorogenic probe. With this technique, it is possible to use a limited amount of DNA to obtain information about DNA degradation [2] and to reveal the presence of PCR inhibitors [3]. The Quantifiler™ Human DNA Quantification Kit uses two *Taq*Man MGB probes containing two different labelled reporter dyes. FAM™ dye detects the amplified sequence of the human telomerase reverse transcriptase gene (hTERT) on chromosome 5p15.33 (Quantifiler Human) and VIC™ dye detects a synthetic sequence not found in nature (IPC

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0531-5131/
doi:10.1016/j.ics.2005.11.135

template). Because the IPC system template DNA is added to the reaction at a fixed concentration, the threshold cycle ($C_{T(VIC)}$) value should be constant if no inhibition occurs. No amplification of Quantifiler Human and IPC leads to invalid results, while no amplification of Quantifiler Human and amplification of IPC should occur when no human DNA is present ("true negative"). However, using this kit in forensic casework requires attention, because false results of quantification can occur.

2. Materials and methods

Fifty DNA extracts from blood, thirty from bone marrow, ten from fresh bone, twenty from saliva and various swabs from gloves used by a known donor were extracted with a Qiagen Kit (Germany). These samples were dosed with the spectrophotometric determination at 260 nm, before being analysed with this real-time system. Forensic samples including bloodstains, sperm, vaginal swabs, hairs, old bones and teeth were extracted as suggested [4]. In many cases, duplicated analyses were performed to evaluate the reliability of the system. Depending on the obtained results, these samples were amplified with AmpF*l*STR® Profiler Plus™ (Applied Biosystems) and/or the Y-specific amplification kit PowerPlex® Y System (Promega).

3. Results

Spectrophotometric determination of DNA gave reliable results as expected only with samples extracted from blood and bone marrow; these results were approximately concordant with real-time. In the other undegraded samples, reliable results were obtained with this quantification system. In forensic samples, positive and reliable quantification was observed when no contamination was present in an extract (i.e. touched objects and hair). For samples like bloodstains, bone, saliva, vaginal swabs, dilution of the extracts was necessary to obtain positive quantification results. The probable inhibition in a sample could be predicted by age, conservation, contaminant, colouring, etc. For example, the intensity of the colour due to heme in bloodstains samples is a good indicator of probable inhibition of PCR. Using four extracts from 3 mm^3 of bloodstains, no results or very diverging quantification were obtained with repression of $C_{T(VIC)}$ (Table 1). When dilution was used with these samples, reproducible results were obtained. In twelve replicates from

Table 1
Estimation of quantity of DNA from bloodstain and bone extracts before and after dilution

Sample	Quantifiler Human	IPC	Quantity	$\sigma_{CT\ FAM}$
G1	22.61	37.38	160.14	–
G1 1:10 (three replicates)	25.10	25.89	7.53	0.86
G2	39.04	Undetected	0.0024	–
G2 1:10 (three replicates)	25.40	26.42	12.80	0.68
S1	Undetected	Undetected	–	–
S1 1:10 (three replicates)	26.15	25.92	5.81	0.81
S4	Undetected	Undetected	–	–
S4 1:10 (three replicates)	27.57	26.60	1.69	0.13
A1	37.07	36.02	0.0086	–
A2	35.23	30.51	0.0383	–
A1 1:10 (three replicates)	33.50	27.40	0.018	0.90
B1	31.73	33.12	0.0662	–
B2	32.51	38.30	0.0351	–
B1 1:10 (three replicates)	31.70	26.50	0.0790	0.81

G1, G2, S1, S4=different undiluted bloodstains on cotton; G1 1:10, G2 1:10, S1 1:10, S4 1:10=diluted bloodstains on cotton. A=extracts from femur; B=extracts from humerus; A 1:10=diluted extracts from femur; B 1:10=diluted extracts from humerus.

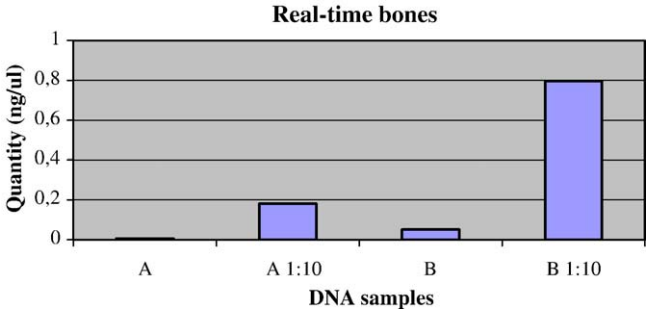

Fig. 1. Estimation of quantity of DNA from 2-year-old bone samples before and after dilution: A – femur; B – humerus.

four different bloodstains, the $C_{T(VIC)}$ value was approximately 26.3 cycles ($\sigma = 0.37$) and the final concentration of the samples was approximately constant (Table 1).

Similar results were observed in replicate analyses of two bone samples (femur and humerus) in which both degradation and contamination with soil were present, taken from a man deceased 2 years earlier and recovered in a wood. Inconsistency results were observed analysing two replicates from both original extracts (Table 1), but when a dilution 1 : 10 of these samples was analysed with real-time in triplicate, the results were concordant. However, the final DNA concentration resulted to about 500% higher than the evaluation with original samples (Fig. 1). Similar results were also obtained from vaginal swabs. Negative results in a sample, also after dilution, predict a probably complete failure of typing via PCR. Despite these variable results in quantification, using the appropriate amount of DNA suggested by these real-time-based kits for performing amplification reactions with AmpF*l*STR® Profiler Plus™ [5] and with PowerPlex® Y System [6], it is possible to obtain balanced DNA profiles with a fluorescence intensity in the linear dynamic range of the instrument. According to our experience, the use of these kits permitted to set the amplification reactions in a rational way, adding the appropriate amount of DNA template to perform a balanced PCR reaction. However, when inhibitors are present, the results should always be considered with caution in a report; absolute quantification of a forensic human DNA should be confirmed with hybridization procedure analysis. In fact, a negative PCR-based DNA quantification could be due to the absence of human DNA, but also to the presence of *Taq* inhibitor compounds on the extracts. Thus, the occurrence of "false negative" regarding the presence of human DNA in real forensic samples could be very high.

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