International Congress Series 1288 (2006) 759 – 761

ELSEVIER

www.ics-elsevier.com

# Comparison of six DNA quantification methods

K. Nielsen [a,*], H.S. Mogensen [a], B. Eriksen [a], J. Hedman [b], W. Parson [c], N. Morling [a]

[a] *Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Denmark*
[b] *Swedish National Laboratory of Forensic Science, Linköping, Sweden*
[c] *Institute of Legal Medicine, Innsbruck Medical University, Austria*

**Abstract.** Six commercial preparations of human genomic DNA were quantified using six quantification methods including UV spectrometry, SYBR-green dye staining, slotblot hybridization with the probe D17Z1, and three TaqMan real-time PCR assays: Quantifiler™ Human DNA Quantification kit, Quantifiler™ Y DNA Quantification kit, and RB1 rt-PCR. In general, all methods measured higher DNA concentrations than expected based on the information by the suppliers of the human DNA preparations. The Quantifiler™ Human DNA Quantification kit gave the highest measures of the DNA concentrations of five of the six human DNA preparations compared to the other five quantification methods. When the Quantifiler™ human DNA standard was replaced by a different commercial human DNA preparation (G147A, Promega) to generate the DNA standard curve in the Quantifiler™ Human DNA Quantification kit, the DNA quantification results of the human DNA preparations were comparable to those of the other DNA quantification methods. The results indicate a calibration problem with the Quantifiler™ human DNA standard for its use with the Quantifiler™ Human DNA Quantification kit. The possible reasons of the problem are discussed, and a solution is suggested. The results emphasise the need for standard reference DNA material and standard methods for DNA quantification. © 2005 Elsevier B.V. All rights reserved.

*Keywords:* DNA quantification; Human DNA; Quantifiler; Real-time PCR; TaqMan; Slotblot; D17Z1; SYBR-green; UV spectrometry

## 1. Introduction

For sensitive and reliable quantification of human DNA in forensic samples, real-time PCR seems to offer a good alternative to other methods, e.g. hybridization methods based on the D17Z1 DNA probe. A reliable estimate of the DNA concentration of a forensic

* Corresponding author. Tel.: +45 35326110; fax: +45 35326120.
*E-mail address:* karsten.nielsen@forensic.ku.dk (K. Nielsen).

0531-5131/ © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ics.2005.09.095

Table 1
Commercial DNA preparations used in this study

| DNA preparation | Supplier | Source | Sex | DNA concentration[a] (ng/μl) |
|---|---|---|---|---|
| D3160 | Sigma | Placenta | Male | 100 |
| D3035 | Sigma | Placenta | Female | 140 |
| Human genomic DNA | Roche | Blood (buffy coat) | Mix | 200 |
| G147A | Promega | Blood from multiple anonymous donors | Male | 177 |
| G152A | Promega | Blood from multiple anonymous donors | Female | 109 |
| Quantifiler™ DNA | ABI | Raji cell-line | Male | 200 |

[a] Suppliers' information.

sample ensures that a proper amount of DNA can be used for the STR analysis, thereby increasing the quality of the test and avoiding unnecessary use of limited amounts of DNA from samples.

As part of the validation of a real-time PCR quantification method at our department, we compared six different DNA quantification methods using six commercial human DNA preparations.

## 2. Material and methods

Six commercial human DNA preparations (Table 1) were diluted to 3 ng/μl, 5 ng/μl and 10 ng/μl before quantification with UV spectrometry, dye staining with SYBR-green and RB1 rt-PCR (Table 2). The DNA was diluted from 50 ng/μl to 0.023 ng/μl before quantification with slotblot hybridization with the D17Z1 DNA probe, Quantifiler™ Human DNA Quantification kit and Quantifiler™ Y DNA Quantification kit (Table 2). The DNA was diluted to 5 ng/μl before quantification with Quantifiler™ using G147A as DNA reference. Measurements of the DNA concentration were repeated at least twice. Quantification of the DNA using RB1 rt-PCR was performed at the Institute of Legal Medicine, Innsbruck Medical University, Austria. The DNA was also quantified at the Swedish National Laboratory of Forensic Science, Linköping, Sweden using the Quantifiler™ Human DNA Quantification kit.

## 3. Results

All methods measured higher DNA concentrations than expected based on the information by the suppliers of the human DNA preparations (Fig. 1). The Quantifiler™ Human kit gave the highest measures of the DNA concentrations of five of the six human DNA preparations compared to the other five quantification methods. When the Quantifiler™ human DNA standard was replaced by a different commercial human DNA preparation (G147A, Promega) to generate the DNA standard curve in the Quantifiler™ Human kit, the DNA quantification results of the human DNA preparations were comparable to those of the other DNA quantification methods (Fig. 1).

Table 2
DNA quantification methods used in this study

| Quantification method | Instrument | Principle | Supplier | References |
|---|---|---|---|---|
| UV spectrometry | Perkin Elmer Lambda 16 | OD-260[a] | – | [1] |
| Dye staining | Lightcycler (Roche) | SYBR-green I | Bie & Berntsen | [2] |
| Slotblot hybridization | – | D17Z1 DNA probe | DNA-technology | [3,4] |
| TaqMan real-time PCR | ABI 7000 SDS, ABI 7300 SDS | Quantifiler™ Human | ABI | [5] |
| TaqMan real-time PCR | ABI 7000 SDS | Quantifiler™ Y | ABI | [5] |
| TaqMan real-time PCR | ABI 7700 SDS | RB1 rt-PCR | ABI | [6] |

[a] Samples were scanned from 200 nm to 350 nm.

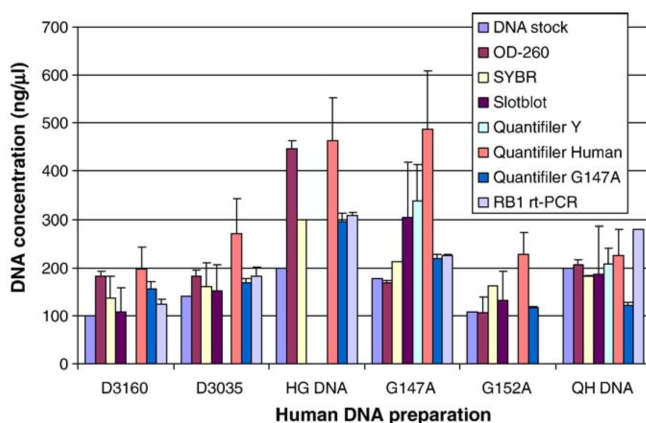


Fig. 1. DNA concentration of six human DNA preparations measured by six methods.

The Quantifiler™ results obtained in Copenhagen were confirmed by the Swedish National Laboratory of Forensic Science in Linköping (results not shown).

## 4. Discussion

The high DNA concentration estimates of the Quantifiler™ human kit indicated a calibration problem with the Quantifiler™ DNA standard for its use with the Quantifiler™ kit. Quantification of the Quantifiler™ DNA standard with the other methods supported that the DNA concentration was 200 ng/µl. When DNA samples were quantified with Quantifiler™ using the DNA preparation G147A as reference DNA, the quantification results were comparable to those obtained with the other methods. The results show that there is a problem with the Quantifiler™ DNA standard, causing overestimation of the DNA concentration in the samples. This could be caused by a mutation in the primer or probe site of the hTERT gene in the Quantifiler™ DNA standard. The hTERT gene encodes the catalytic component of the human telemorase that is inactivated in most normal cells, but is activated in 85% of all tested immortalized cells (e.g. cell lines). The results emphasise the need for internationally agreed standard reference DNA materials and standard methods for DNA quantification.

## References

[1] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, 2001, pp. 6.11–6.12.
[2] K. Rengarajan, et al., Quantifying DNA concentrations using fluorometry: a comparison of fluorophores, Mol. Vision 8 (2002) 416–421.
[3] J.S. Waye, H.F. Willard, Structure, organization, and sequence of alpha satellite DNA from human chromosome 17: evidence for evolution by unequal crossing-over and an ancestral pentamer repeat sharing with the human X chromosome, Mol. Cell. Biol. 6 (1986) 3156–3165.
[4] P.S. Walsh, J. Varlaro, R. Reynolds, A rapid chemiluminiscent method for quantification of human DNA, Nucleic Acids Res. 20 (1992) 5061–5065.
[5] Quantifiler kits user manual (2003) Applied Biosystems.
[6] S. Köchl, H. Niederstätter, W. Parson, DNA extraction and quantification of forensic samples using the phenol-chloroform method and real-time PCR, Forensic DNA Typing Protocols: Methods in Molecular Biology, Humana Press, 2005, pp. 13–29.