International Congress Series 1288 (2006) 753–755

www.ics-elsevier.com

A single assay for human-specific quantification of less than 1 pg DNA and detection of the presence of PCR inhibitors in forensic samples

Maura T. Costello, James W. Schumm *

The Bode Technology Group, Research and Development, 7364 Steel Mill Drive, Springfield, VA 22150, USA

Abstract. We describe the development, validation, and application of a duplex quantitative real-time PCR assay for human-specific quantification of DNA samples containing as little as 0.5 pg/μl of DNA with the presence of an internal positive control (IPC) allowing simultaneous detection of PCR inhibitors. The combination of human DNA quantity and quality data provides the forensic DNA analyst with substantial information prior to STR amplification, thereby reducing the number of samples needing re-evaluation following initial profiling. The features of this assay will allow us to apply it very effectively to evaluation of touch evidence and low copy number samples. © 2005 Elsevier B.V. All rights reserved.

Keywords: Real-time PCR; *Alu* sequence; Human DNA quantification; Forensic

1. Introduction

Human-specific quantification of DNA in casework sample analysis is necessary to ensure successful DNA amplification and genetic profiling. Real-time PCR is less labor intensive, more accurate, and lends itself to automation better than previous methods such as slot-blot hybridization. Our work builds on that described by Nicklas and Buel [1], Richard et al. [2], and the commercially available Quantifiler™ Kit (Applied Biosystems, Foster City, CA). We have combined the sensitivity and human specificity of *Alu*-based quantification with an internal positive control (IPC) allowing simultaneous detection of PCR inhibitors. *Alu* sequences are short, repeated elements interspersed throughout the primate genome. Because the *AluYb8* subfamily is specific to higher primates [3] we selected it to develop real-time primers and a fluorogenic probe [4]. The IPC system is

* Corresponding author. Tel.: +1 703 644 1200x303; fax: +1 703 644 2319.
E-mail address: james.schumm@bodetech.com (J.W. Schumm).

doi:10.1016/j.ics.2005.09.004

duplexed with the *Alu* quantification, and consists of a second set of primers and probe specific for an exogenous template. Combining highly sensitive DNA quantity data with quality data provides the analyst with considerable information that can reduce the number of samples that need re-evaluation following initial profiling.

2. Materials and methods

Primers and probes were designed using Primer Express (Applied Biosystems); the *Alu* set against *AluYb8* (FAM probe dye) and the IPC set against an exogenous rat sequence (VIC® probe dye). Control human DNA (9947, 9948) was obtained from Coriell Laboratories (Camden, NJ). The standard curve for the assay is a 1:10 dilution series of 9947 DNA (50 ng/μl–0.5 pg/μl). PCR inhibitors were obtained from Sigma (St. Louis, MO). Case-type samples were obtained from internal sources at The Bode Technology Group (Springfield, VA). Real-time PCR was performed on the ABI Prism® 7000 as described (Applied Biosystems).

3. Results and discussion

3.1. Sensitivity, reproducibility, and accuracy

We showed that the quantification assay is linear, sensitive, and reproducible in the range of 50 ng/μl to 0.5 pg/μl, or to less than one human genome equivalent (R^2: 0.997). A 9948 DNA dilution series was quantified in two separate trays of three replicates (Table 1). The assay was accurate and reproducible both within and across the two trays.

3.2. Species specificity

Twenty non-primate DNA extracts were quantified. No cross-reactivity was detected in 14 extracts, including *E. coli* and *C. albicans*. Six extracts (mink, deer, beaver, rabbit, elk, and raccoon) showed low level cross-reactivity; however, in each case the assay was at least 5000 times less sensitive than when using an equal quantity of human DNA (data not shown).

3.3. Internal positive control testing

The IPC feature consists of amplification of an exogenous rat DNA and primer/probe set added in equal quantity to each reaction. If no inhibition is present, the threshold cycle of IPC amplification will always be the same. If inhibitors are present in the test DNA extract, the IPC threshold cycle number increases. The IPC feature was tested with the known PCR inhibitors hematin (blood),

Table 1
Accuracy and reproducibility of quantification (DNA concentration in ng/μl)

Input DNA	Plate 1			Plate 2			Std. dev.	Std. dev./ % of input
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Rep. 6		
50	38.8	57.85	52.95	41.83	51.78	47.17	7.187	14.4%
5	5.24	4.34	7.03	4.92	5.34	4.31	0.998	20.0%
0.5	0.46	0.41	0.37	0.39	0.43	0.36	0.040	8.0%
0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.003	5.2%
0.005	0.0021	0.0025	0.0035	0.0012	0.0029	0.0024	0.0005	12.0%
0.0005	0.0006	0.0008	0.0005	0.0005	0.0004	0.0004	0.00015	30.0%

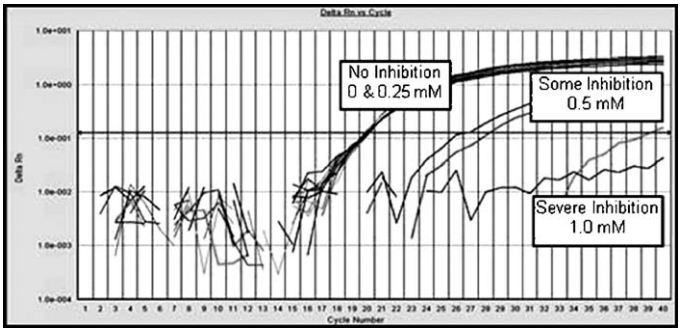

Fig. 1. Internal positive control inhibition detection with increasing amounts of hematin.

indigo (denim), and humic acid (soil) and was sensitive to the addition of varying amounts of each (Fig. 1; hematin data shown).

3.4. Case-type sample analysis

Seventy-five samples previously subjected to STR analysis were re-quantified using this assay. Many had required dilution and re-amplification to acquire a DNA profile; others had been successful with the first amplification. This quantification assay detected PCR inhibitors in the samples that had required multiple analyses or never provided STR profiles; some samples that previously showed no amplification were found to contain adequate DNA but also high levels of PCR inhibitors (data not shown). From these data, guidelines have been developed to predict sample performance in STR assays and requirements for dilution prior to amplification. Implementation of this approach will reduce the number of samples that require re-analysis and may increase the number of positive results.

4. Conclusions

This assay provides a valuable combination of extreme quantification sensitivity and DNA quality data that can help reduce the frequency of delays and increased cost of STR re-amplification and re-analysis. The features of this assay make it ideal for touch evidence and low copy number samples, in which picogram sensitivity is necessary and detection of inhibition before wasting valuable sample is extremely important.

References

[1] J.A. Nicklas, E. Buel, Development of an ALU-based, real-time PCR method for quantitation of human DNA in forensic samples, J. Forensic Sci. 48 (5) (2003) 935–944.
[2] M.L. Richard, R.H. Frappier, J.C. Newman, Developmental validation of real-time quantitative PCR assay for automated quantification of human DNA, J. Forensic Sci. 48 (5) (2003) 1041–1046.
[3] M.L. Carroll, et al., Large-scale analysis of the Alu Ya5 and Yb8 subfamilies and their contribution to human genomic diversity, J. Mol. Biol. 311 (2000) 17–40.
[4] P.M. Holland, et al., Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of *Thermus aquaticus* DNA polymerase, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 7276–7280.