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Research article

Internal validation of the 7500 Real Time PCR System for use in forensic casework in Hellenic Police

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Abstract

According to guidelines of the quality assurance standards for the forensic DNA testing laboratories, prior to introducing an existing DNA analysis procedure, reliability of the procedure has to be demonstrated by carrying out internal validation. In order to introduce a sensitive and accurate DNA quantification method in our laboratory, a validation study of the 7500 Real Time PCR System (Applied Biosystems) using the QUANTIFILER Human DNA Quantification Kit (Applied Biosystems) was performed. Here we report the results and the experience we have gained during this internal validation study.

Keywords: Internal validation; Real Time PCR

1. Introduction

Accurate DNA quantification in forensic casework samples is a crucial contributing factor to successful STR genotyping. Advances in forensic DNA analysis have resulted in the advent of Real Time PCR as a reliable and sensitive DNA quantification method using small sample amount. The ABI 7500 Real Time PCR Sequence Detection System is a robust, promising tool to perform DNA quantification, with high automation feasibility.

2. Materials and methods

2.1. Sources and preparation of genomic DNA used in testing

Human genomic DNA was extracted from single source and casework samples using a commercial purification kit (Nucleospin® by Macherey-Nagel). In total, 47 samples were studied in order to evaluate reproducibility, precision and accuracy, sensitivity and performance of mixture samples.

2.2. Quantifiler human kit assays

Assays were performed according to the protocol specified in the User's Manual. Duplicate reactions of each quantification standard and triplicate reactions of each analysis sample were run.

2.3. PowerPlex® (Promega Corp.) PCR Amplification Kits genotyping assays

STR analysis was performed using the standard protocol for the PowerPlex® 16 and PowerPlex® Y, PCR Amplification Kits. PowerPlex® reactions were run on an ABI PRISM 3100 Genetic Analyzer and analyzed with GeneMapper® v.3.1 (ABI).

2.4. DNA quantification of DNA samples of known concentrations

Standard DNA sample for Quantiblot, (ABI) and the 9948 male DNA sample (Promega), were tested in triplicate in three different sets using the Quantifiler system, in order to assess probable differences between given and experimentally determined quantities.

2.5. Reproducibility, precision and accuracy studies

Thirty-seven single source and casework samples and one standard DNA sample supplied by ABI, were tested in triplicate reactions in each plate. The assay was repeated twice.

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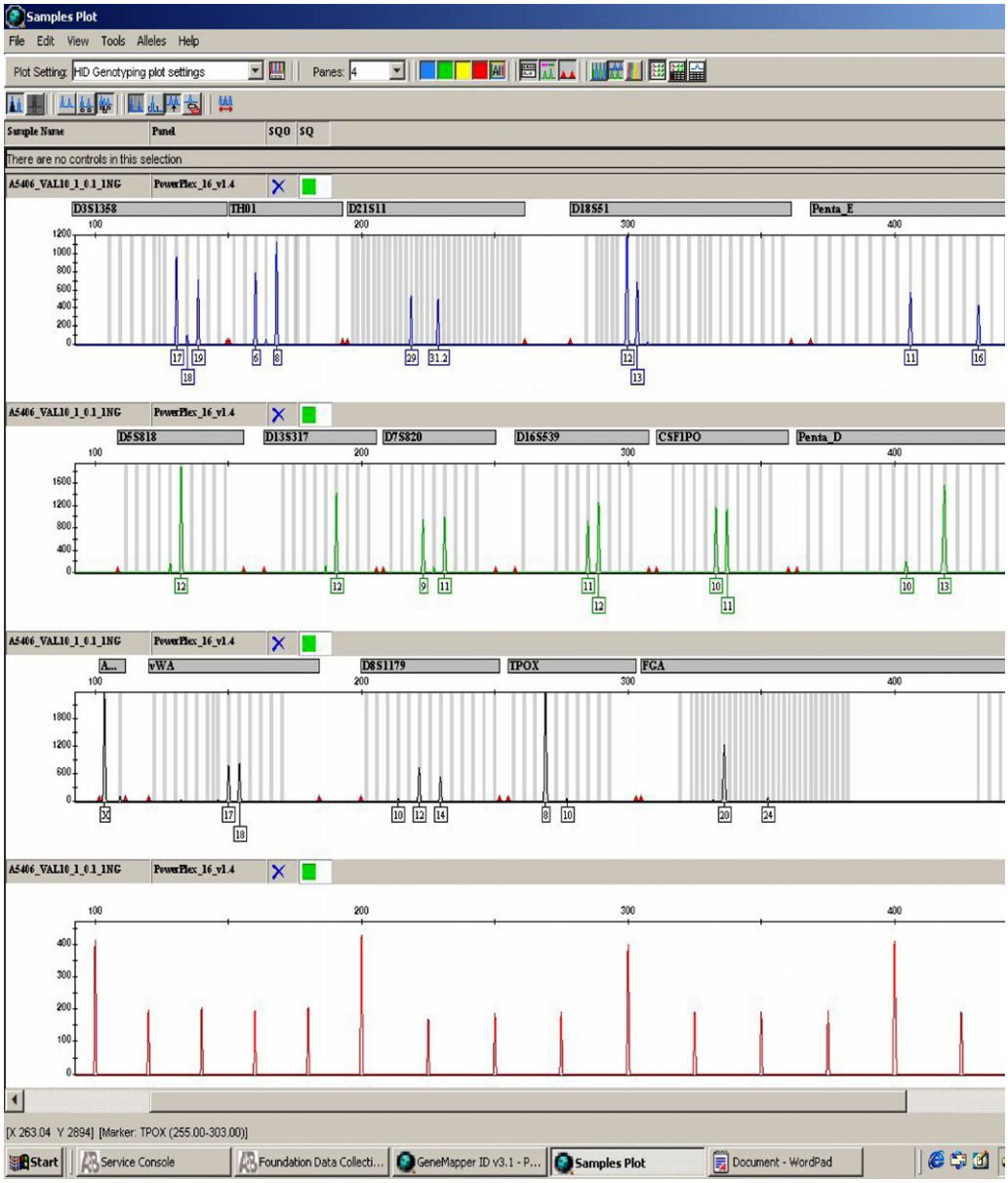

Fig. 1. STR genotyping with PowerPlex 16 of a female to male 1:0.1 mixture (1 ng/μL).

2.6. Mixture studies

Mixture ratios of 1:1, 1:0.5, 1:0.1 and 1:0.05 female to male, male to female, and male to male were prepared up to a total DNA concentration of 2 ng/μL. Each specific ratio was also subjected in serial dilutions so that the total DNA concentration of the mixture would be 1 ng/μL, 0.5 ng/μL, 0.25 ng/μL, 0.125 ng/μL, 0.06 ng/μL and 0.03 ng/μL. Triplicate reactions were set for every sample of different ratio and concentration (112 samples in total).

2.7. Sensitivity studies and stochastic effects

Serial dilutions of eight samples were set so that various concentrations (0.25, 0.06, 0.03, 0.015) were tested down to 0.007 ng/μL. Each sample was tested in triplicate reactions. Eight reagent blanks, coming from various DNA purification protocols, were also tested to evaluate possible stochastic effects.

3. Results

3.1. DNA quantification of samples of known DNA concentration

Our data showed small to medium differences between the Quantiblot standard DNA concentrations given by suppliers and those experimentally determined with Quantifiler. Different concentrations – depending on the batch – were also determined for the second DNA standard used in our study.

3.2. Precision and accuracy

The accuracy and precision of the Quantifiler kit assays is a function of the measurement of C_T in amplification reactions. An assessment of this parameter measurement was made by running triplicate reactions of the quantification standard dilutions typically used in the Quantifiler kit assays, repeated in two different sets. The results of the C_T precision experiments are shown in Table 1. Our results provide a useful indication of C_T stability, which was consistent from reaction to reaction and day to day.

Table 1
Quantifiler kit C_T run to run precision results

DNA concentration (ng/μL)	Mean C_T	S.D. C_T
3.1400	27.062	0.036
1.4800	28.113	0.111
0.7300	29.117	0.248
0.3800	30.002	0.069
0.0330	33.483	0.290
0.0067	35.995	0.841

3.3. Reproducibility

Our results demonstrate stability of measured C_T values and consequent quantification for thirty-eight samples examined.

The following step of this study was the setting up of STR amplification reactions using the DNA amounts determined by Quantifiler kit assays. All samples tested, produced full, balanced profiles with no off scale allele peaks or ambiguous stutter bands (data not shown).

3.4. Mixture studies

The main goal of the mixture study validation was to determine the ability (limits) of the system to detect the minor contributor by STR genotyping of a mixed stain. Our autosomal

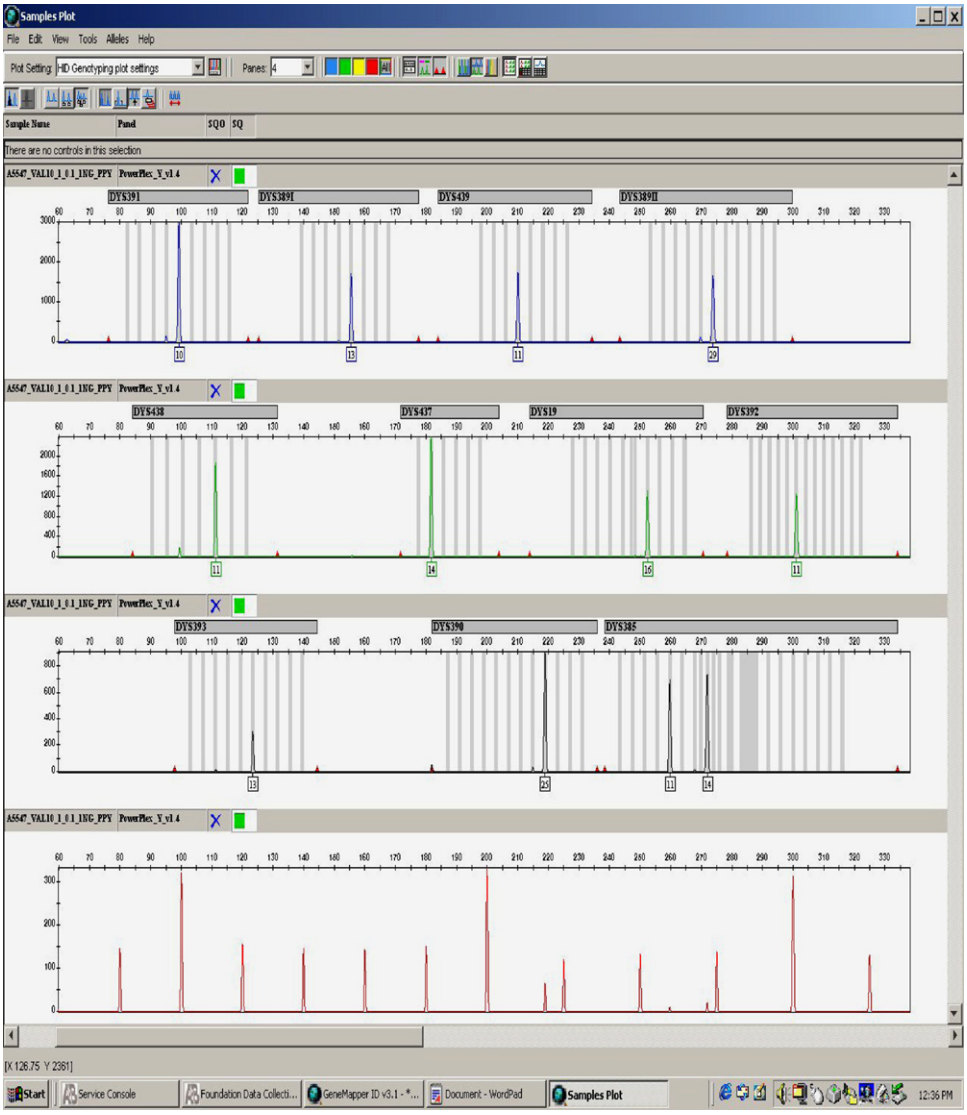

Fig. 2. STR genotyping with PowerPlex Y of a female to male 1:0.1 mixture (1 ng/μL).

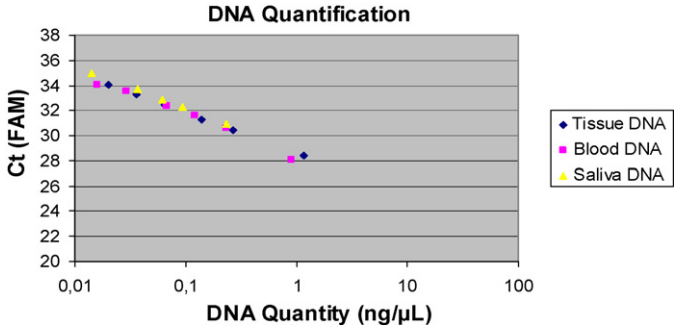

Fig. 3.

STR results claim that mixed stains with ratio equal to or less than 1:0.1 could be considered as single source DNA stains with stutter bands or increased background depending on the initial DNA concentration (see Fig. 1). STR amplification of the Y chromosome, in samples containing male DNA as a minor contributor, succeeded in producing Y STR male profile in samples of DNA concentration 2–0.25 ng/μL (see Fig. 2). Samples with less DNA failed to produce full Y STR profile.

3.5. Sensitivity

The results of sensitivity testing showed that all concentrations of each DNA tested gave positive assay results and the resulting plots of C_T vs. DNA concentration showed all data points to be located along a straight line, indicating valid results (Fig. 3). Samples of 16 pg/μL DNA concentration gave correct STR genotype for all samples tested. Samples with less than 16 pg/μL DNA concentration failed to give full DNA profiles and drop outs were also observed.

Quantification of all the reagent blanks showed that they did not contain detectable DNA.

4. Conclusions

The internal validation study that was conducted for the evaluation of Quantifiler Human DNA Quantification Kit (Applied Biosystems) has demonstrated that it is a reliable system with high sensitivity, which gives accurate and reproducible results.

Further reading

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