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Research article

Implementation of a robotized real-time PCR setup for the use of the Quantifiler™ Human DNA Quantification Kit

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Abstract

Human DNA quantification occupies a central role within the DNA analytic process of forensic casework samples as DNA quantification results have an important impact on the quality of the short tandem repeat data. Manual processing for the setup of quantification reactions can be time consuming and labor intensive. Therefore automation of quantitative real-time PCR setup was an important component of our DNA-analysis automation concept. Here we show the implementation of a robotized setup for the Quantifiler™ Human DNA Quantification Kit.

Keywords: Automation; DNA quantification; Forensic casework samples; Real-time PCR; Quantifiler™ Human DNA Quantification Kit

1. Introduction

To enhance both the efficiency and the quality standards of the DNA analytic process of forensic casework samples a partial automation and an associated LIMS concept was developed at the Landeskriminalamt (Office of Criminal Investigation) Baden-Württemberg, Stuttgart, Germany, as shown in our contribution ''Implementation of a semi-automated processing system for DNA profiling of forensic casework samples'' in this issue [1].

Human DNA quantification occupies a central role within the analytic process as DNA quantification results have an important impact on the quality of the short tandem repeat (STR) data. Performing an STR multiplex amplification reaction with a suboptimal target DNA concentration can result in substrate excess inhibition or insufficient signal intensities even up to signal loss. If human DNA quantification is used as a screening method in order to identify samples with sufficient DNA for STR analysis validation of the assay with respect to sensitivity, accuracy and reproducibility is critical.

In our laboratory the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems) used with the AB 7500 Real-Time PCR System has been validated as a DNA-quantification and screening system performing all forensic evidence DNA-samples in the non-automated/manual DNA-analysis process. Since this requires a high effort regarding staff and time, the implementation of the real-time PCR setup was an important task within our automation concept.

2. Material and methods

2.1. Hardware components and software-tools

Tecan Freedom EVO 150 liquid handling workstation (LHW), Applied Biosystems 7500 Real-Time PCR System with Sequence Detection Software v1.2.3 and a software tool for normalization and negative selection, Tecan.

2.2. Quantifiler™ Human DNA Quantification Kit

The mastermix is prepared manually (8.5 μl primer mix and 12.5 μl reaction mix per reaction), 21 μl are distributed by the liquid handling workstation using multipipetting mode into the microtiterplate with 5 μl of each sample previously added by an LHW (i.e. 1/14 of the total DNA-extract volume). The sample volume was increased to 5 μl instead of 2 μl according to the manufacturer's protocol [2] in order to optimize the system regarding inhibitory and stochastic effects. A three-fold dilution series of the standard DNA is performed by the LHW according to the manufacturer's protocol. The standard curve comprises the concentration

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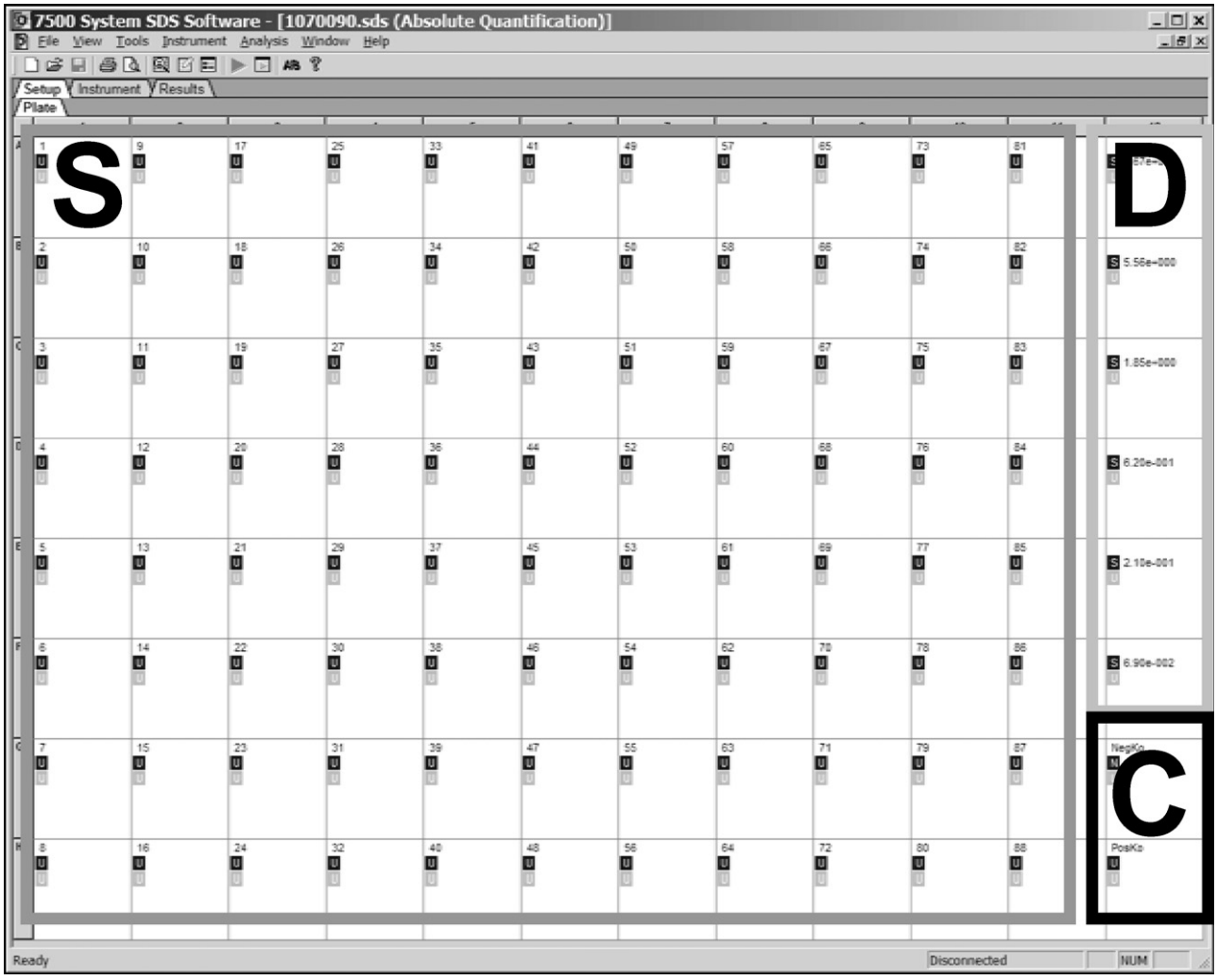

Fig. 1. Robotized setup for the Quantifiler™ Human DNA Quantification Kit: (S) Samples 1–88, (D) standard DNA dilution series, one reaction for each value ranging from 16.7 to 0.069 ng/μl, and (C) positive and negative amplification controls.

values from 16.7 to 0.069 ng/μl. The lowest standard value in the manufacturer's protocol (0.023 ng/μl) was excluded from the standard curve because of the high stochastic variance due to the low target DNA concentration. This improves the reproducibility of the sloping characteristics of the standard curves.

3. Results

With respect to the operational organization and the efficiency of the automation concept the sample number has been set to 88. One positive and one negative amplification control and one standard series with six different DNA

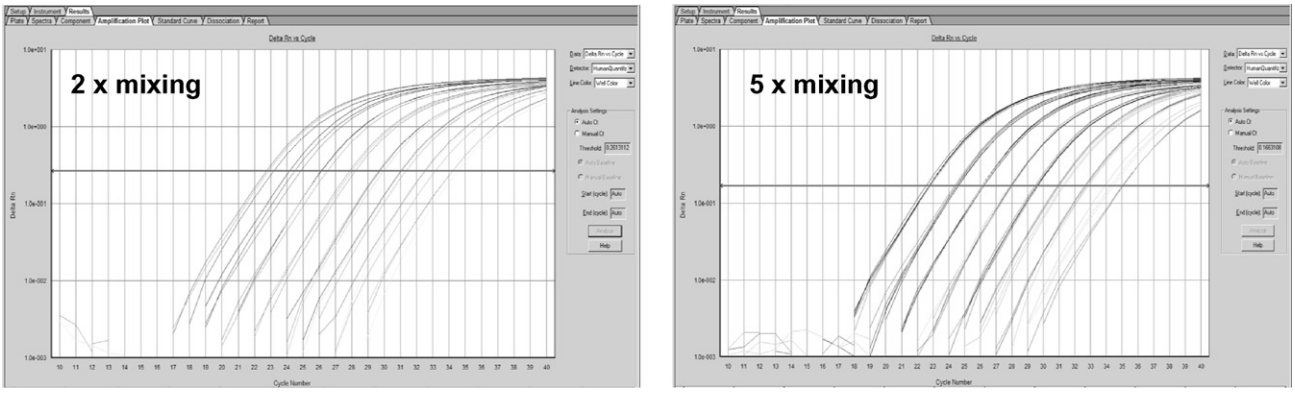

Fig. 2. Influence of mixing frequency on the reproducibility of the standard values.

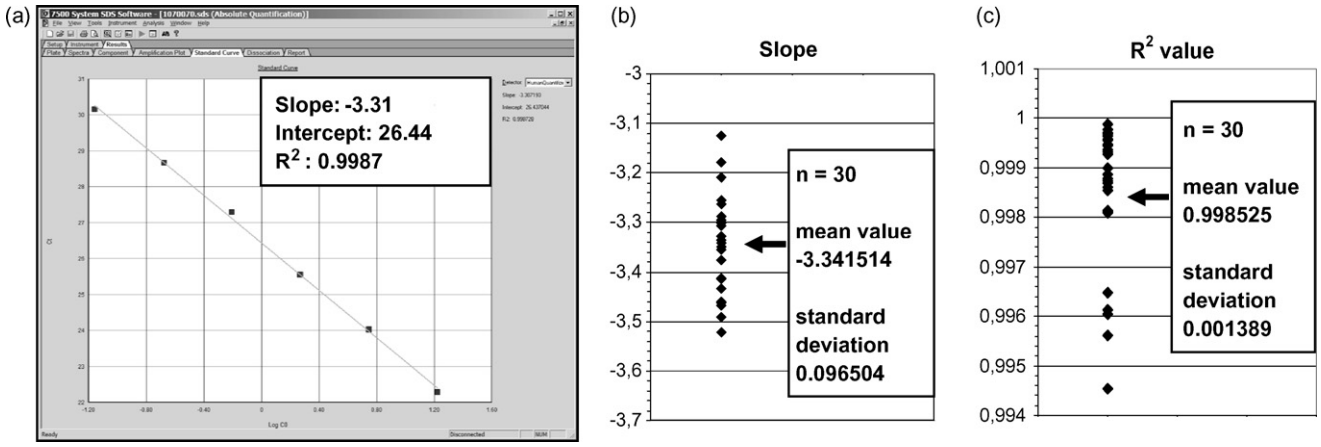

Fig. 3. Standard curve: representative example (a), reproducibility and compliance with the defined evaluation criteria of slope (b) and R^2 value (c).

Table 1
Example of a report of the quantification data for evaluation and modification

S.P.N.	Barcode (mod.)	Comment 1	Comment 2	C_T	QTY	CtIPC	TARGET-DNA (ng)	PCR	DNA (μl)	Dilution
85_E11	07012345_029_001	C_A.B._1951_Susp	Homicide	23.36	6.59	25.58	0.9835	Yes	0.1492	1:67
86_F11	07012345_030_001	B_Blade	Homicide	0	0	0	0	Yes	10	1:1
13_E2	07009876_001_002	Z_Sock	Property crime	0	0	25.96	0	No	10	1:1
15_G2	07009876_001_004	Z_Sock	Property crime	36.31	0.0008	25.94	0.0086	No	10	1:1
16_H2	07009876_001_005	Z_Sock	Property crime	31.92	0.0179	26.06	0.179	Yes	10	1:1
71_G9	07008642_003_006	B_Wooden_bar	Bodily injury	29.78	0.0786	0	0.786	Yes	10	1:1
74_B10	07008642_006_001	B_Bloodstain	Bodily injury	28.16	0.24	26.09	1	Yes	4.1666	1:2.4
75_C10	07008642_007_001	C_C.D._1967_Vict	Bodily injury	25.92	1.12	25.94	1.0011	Yes	0.8938	1:11.18

concentration values in the range of 16.7–0.069 ng/μl are used for absolute quantification (Fig. 1).

An important factor regarding reproducibility of the standard curve is the mixing function during pipetting of the serial dilution by the liquid handling system. Although the evaluation criteria for the standard curve could be fulfilled within the corresponding tolerable range by mixing the respective standard dilutions twice (V_{asp} = 15 μl, V_{dis} = 15 μl, V_{tot} = 40 or 30 μl, respectively) dramatic deviations can be observed between individual serial dilutions. An increase of the mixing frequency to five times leads to an efficient mixing of the individual standards and thus to a uniform and reproducible appearance of the standard curve (Fig. 2).

If one reaction of each standard value within a single standard series is used for an automated quantification setup reproducibility and compliance of the standard curve with the defined evaluation criteria of slope (a slope close to −3.3 indicates optimal PCR amplification efficiency) and R^2 value (an R^2 value ≥0.99 indicates a close fit between the regression line and the individual C_T data points of the quantification standard reactions) are important provisos. Fig. 3a shows a representative example of a standard curve generated by the robotized setup during lab routine application. Fig. 3b and c shows the reproducibility of slope and R^2 value respectively over 30 independent experiments.

After real-time PCR a report of the quantification data is generated allowing the scientist to review and decide if a sample will be excluded from further processing according to an experimentally validated cut-off value. Concerning the optimal amplification volume of the sample inhibitory effects indicated by the C_T value of the internal positive control can be taken into account (Table 1). From the evaluated and normalized data an input file for the LHW is generated for automated pipetting of STR PCR.

4. Conclusion

In this report we show the implementation of a robotized setup for the Quantifiler™ Human DNA Quantification Kit, which we use for the analysis of casework samples since June 2006. We optimized the efficiency and device utilization by using a single standard series per plate with six standard values. Our validation data for this setup confirms the reproducibility and the compliance of the standard curve with the defined evaluation criteria.

Conflict of interest

None.

References

[1] K. Vollack, B. Haak, R. Schwenzer, W. Pflug, Implementation of a semi-automated processing system for DNA profiling of forensic casework samples, Forensic Sci. Int. 1 (2008) 83–85.
[2] Quantifiler™ Kits User's Manual, Applied Biosystems, 2003.