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ScienceDirect
Forensic Science International: Genetics Supplement Series 1 (2008) 13–15
www.elsevier.com/locate/FSIGSS

Research article

A multiplexed system for quantification of human DNA and human male DNA and detection of PCR inhibitors in biological samples

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Received 26 September 2007; accepted 11 October 2007

Abstract

Forensic analysts routinely encounter samples containing DNA mixtures from male and female contributors. To obtain interpretable Short Tandem Repeat (STR) profiles and select the appropriate STR analysis methodology, it is desirable to determine relative quantities of male and female DNA, and detect PCR inhibitors. We describe a multiplex assay for simultaneous quantification of human and human male DNA using the ribonuclease P RNA component H1 (RPPH1) human target and the sex determining region Y (SRY) male-specific target. A synthetic oligonucleotide sequence was co-amplified as an internal PCR control. Standard curves were generated using human male genomic DNA. The SRY and RPPH1 assays demonstrated human specificity with minimal cross-reactivity to DNA from other species. Reproducible DNA concentrations were obtained within a range of 0.023–50 ng/μl. The assay was highly sensitive, detecting as little as 25 pg/μl of human male DNA in the presence of a thousand-fold excess of human female DNA. The ability of the assay to predict PCR inhibition was demonstrated by shifted IPC Ct values in the presence of increasing quantities of hematin and humic acid. We also demonstrate the correlation between the multiplex assay quantification results and the strength of STR profiles generated using the AmpFℓSTR®PCR Amplification kits.

Keywords: Human DNA quantification; Real-time PCR; PCR inhibitors; STR analysis for human identification

1. Introduction

Accurate quantification of human DNA in forensic samples is essential for defining the input DNA needed for obtaining interpretable Short Tandem Repeat (STR) profiles. Real-time PCR assays like Quantifiler® Human DNA Quantification kit and Quantifiler® Y Human Male DNA Quantification kit have proved very useful in STR profiling. Real-time quantification assays provide certain advantages over the traditional hybridization based assays: greater dynamic range, more rapid, increased limit of detection, ability to predict the presence of PCR inhibitors and ability to automate [1–4]. We describe a multiplex TaqMan® Real-Time PCR assay that the forensic scientist can use as a tool for quantitative and qualitative assessment of total human and human male DNA in forensic type biological samples. The described multiplex assay (Quantifiler® Duo DNA Quantification Kit) is designed to quantify total human DNA and human male DNA simultaneously, determine the ratio of human male and female DNA, detect PCR inhibitors, allow selection of the appropriate STR amplification kit, and predict success with downstream STR amplification.

2. Materials and methods

A multiplexed TaqMan® was assembled that amplifies SRY (FAM™-labeled probe), RPPH1 (VIC®-labeled probe) and an Internal Positive Control—IPC (NED™-labeled probe). Assays were designed using the Taqman® Gene Expression [5] assay (www.allgenes.com) design pipelines targeting genomic DNA. Multiplex reactions were optimized using factorial designs to optimize responses. Amplification reactions were performed on a 7500 Real-Time PCR System and the data were analyzed with the 7500 System SDS software v1.2.3 (Applied Biosystems, Foster City, CA). Assay species specificity was tested using a panel of mammalian and microbial DNAs. Non-human samples were obtained as purified DNA from Bios Laboratories, Inc., New Haven, CT and ATCC, Manassas, VA. Human genomic DNA used was

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1875-1768/$ – see front matter © 2008 Published by Elsevier Ireland Ltd.
doi:10.1016/j.fsigss.2007.10.197

Male/Female DNA ratio	SRYQuantity ng/µl	RPPH1 Quantity ng/µl	SRY/RPPH1 expected ratio	SRY/RPPH1 measured ratio
1:0	0.027	0.026	1:1	1:0.96
1:50	0.029	1.260	1:51	1:43.45
1:100	0.029	2.460	1:101	1:84.25
1:200	0.022	6.405	1:201	1:289.16
1:500	0.025	13.770	1:501	1:546.43
1:800	0.027	24.410	1:801	1:897.43
1:1000	0.020	28.210	1:1001	1:1389.65
0:1	Female	0.016	-----	-----

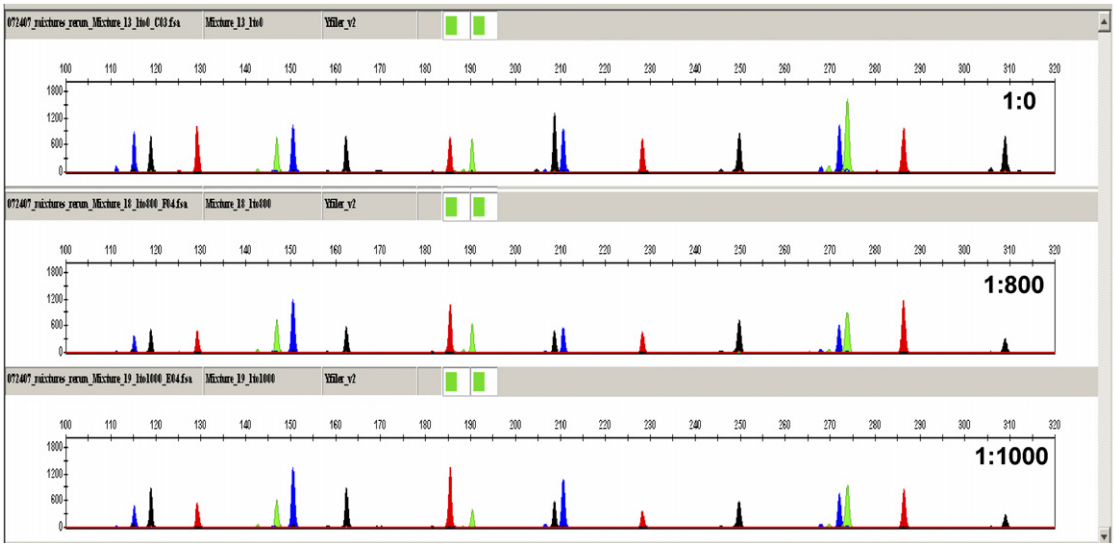

Fig. 1. Purified genomic DNA from a male and a female individual were combined according to various ratios (see table) to mimic sexual assault evidence samples. The male gDNA was added to the mixtures at a constant concentration of approximately 25 pg/µl. The mixtures were tested with the multiplex assay to determine the concentration of total and male DNA. The multiplex assay can quantify 25 pg/µl of male DNA in the presence of up to approximately 25 ng/µl of female DNA (1:1000 ratio). Electropherograms obtained by using the AmpFℓSTR® Yfiler® PCR Amplification kit for the samples are shown: the male STR profile is complete up to the 1:1000 mixture ratio.

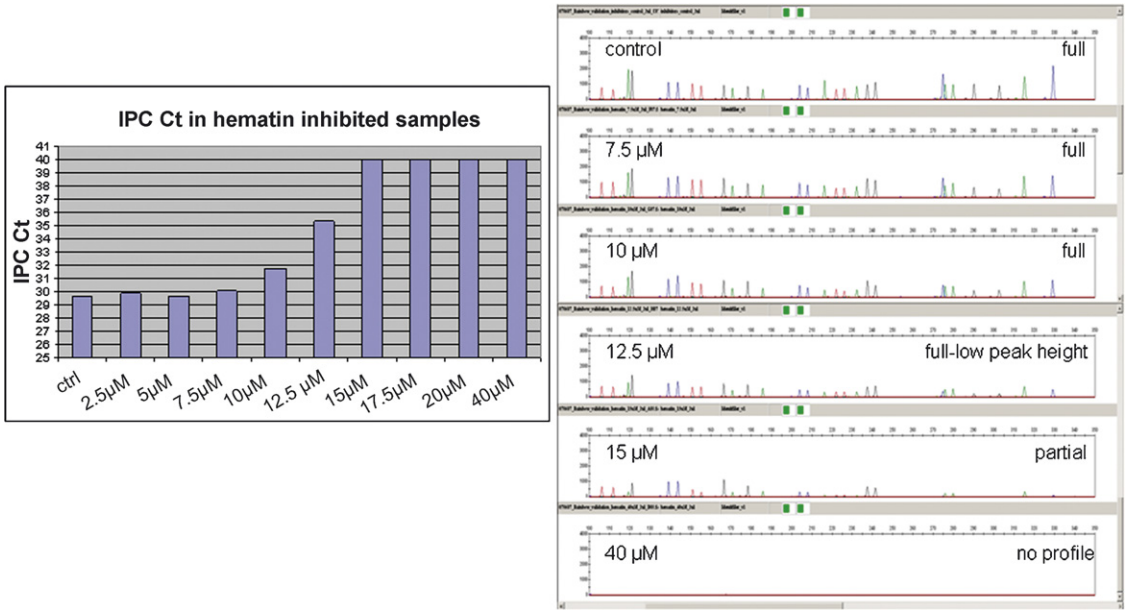

Fig. 2. Human male genomic DNA extracted from whole blood was mixed with varying final concentrations of hematin: 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, and 40 µM. 2.0 µl of each DNA/hematin mix, containing 1.0 ng of DNA, was quantified using the multiplex assay. The IPC Ct values were monitored (see bar graph). Partial inhibition was detected between 10 and 12.5 µM hematin; total inhibition at 15 µM hematin. 2.0 µl of each DNA/hematin mix was also added to the AmpFℓSTR® Identifiler™ kit reactions. STR profiles from a subset of samples are shown. The results from the quantification assay provided reasonable predictions of samples that would fail STR analysis because of the presence of the PCR inhibitor (e.g., sample containing 15 µM hematin). The ability of the assay to predict PCR inhibition was demonstrated by shifted IPC Ct values in the presence of increasing quantities of hematin. Similar results were obtained with samples containing increasing quantities of humic acid (data not shown).

from a pool of male donors (EMD Chemicals Inc., Madison, WI). GeneAmp® PCR System 9700 and the ABI PRISM® 3130 *xl* Genetic Analyzer were used as described in the instruction manual.

3. Results and discussion

DNA from several mammalian and microbial sources was tested to determine species specificity. The SRY assay detected chimpanzee DNA exhibiting some cross-reactivity. The multiplexed assay performed well across a large dynamic range and is well suited for quantification of human samples. Five different human DNA samples were diluted to approximately 20, 10, 0.1, 0.05 and 1 ng/μl and tested for reproducibility of the quantification results in three successive runs. One sample was from a female individual, the other four samples were from male sources. Averages and standard deviations for all experiments were within acceptable limits and the results from the human and the human male specific assay were in concordance (data not shown). Purified genomic DNA from a male and a female individual were combined according to various ratios (Fig. 1) to mimic forensic evidence samples. The mixtures were tested with the multiplex assay to determine the concentration of total and male DNA. The multiplex assay can quantify 25 pg/μl of male DNA in the presence of up to approximately 25 ng/μl of female DNA (1:1000 ratio). Further, to determine the effect of common inhibitors DNA samples were mixed with inhibitors (Fig. 2) and the IPC Ct shifts were monitored to determine the ability to predict failure of STR analysis.

4. Conclusions

Quantification of human DNA in forensic samples is essential for defining the input DNA needed for obtaining interpretable STR profiles. The most accurate method of choice for forensic DNA quantification is Real-Time PCR. We have developed a multiplex Real-Time PCR assay for the simultaneous quantification of human and human male DNA with IPC in forensic samples. The assay is efficient, specific, sensitive and robust. The results correlate well with the AmpFℓSTR® Identifiler® and Yfiler® kit performance in terms of predicting the generation of interpretable STR profiles for inhibited DNA samples and male/female DNA mixtures. The described multiplex assay (Quantifiler® Duo DNA Quantification Kit) is a useful tool for the quantitative and qualitative assessment of DNA in forensic type biological samples.

Funding source

Applied Biosystems R; D. The sponsor was involved in the study design; collection, analysis and interpretation of data; the writing of the manuscript and the decision to submit the manuscript for publication.

Conflict of interest

Employment at Applied Biosystems, the company that will market these kits.

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