22nd Congress of the International Society for Forensic Genetics

A Multiplexed System for Quantification of Human DNA and Human Male DNA and Detection of PCR Inhibitors in Biological Samples

Maura Barbisin, Rixun Fang, Cristin E. O'Shea, Pius M. Brzoska, Lisa M. Calandro, Jaiprakash G. Shewale and Manohar R. Furtado
Applied Biosystems, 850 Lincoln Centre Drive, Foster City CA 94404, USA

ABSTRACT

Forensic analysts routinely encounter samples containing DNA mixtures from male and female contributors. To obtain interpretable 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 to 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 AmpF*t*STR® PCR Amplification Kits.

INTRODUCTION

Accurate quantification of human DNA in forensic samples is essential for defining the input DNA needed for obtaining interpretable STR (Short Tandem Repeat) profiles. Hybridization-based quantification methods that are traditionally used for the quantification of DNA in forensic samples are generally considered time-consuming, labor-intensive and not suitable for automation. Further, it is difficult to predict the amplitude of the STR profile because of the difference in the sensitivity of quantification methods and STR genotyping systems. Real-time PCR assays like Quantifiler® Human DNA Quantification kit and Quantifiler ® Y Human Male DNA Quantification kit have proved very useful. Real-time quantification assays provide certain advantages over the hybridization based assays: Greater dynamic range, more rapid, increased limit of detection, ability to predict the presence of PCR inhibitors and ability to automate. 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.

RESULTS

Figure 1. Assay configuration

Target	Marker	Dye
Human DNA	RPPH1 (Ribonuclease P RNA component H1)	VIC®
Human Male DNA	SRY (Sex determining region Y)	FAM™
IPC	Artificial Template	NED™

Fig. 1. A multiplex real-time PCR assay was assembled that amplifies SRY (FAM™-labeled probe), RPPH1 (VIC ®-labeled probe) and an Internal Positive Control-IPC (NED™-labeled probe). 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).

Table 2. Assay specificity

Tested Species	RPPH1 Average Ct	SRY Average Ct
Orangutan	40.0	40.0
Chimpanzee A	40.0	32.3
Chimpanzee B	40.0	31.1
Gorilla A	40.0	40.0
Gorilla B	40.0	40.0
Macaque	40.0	40.0
Dog	40.0	40.0
Cow	40.0	40.0
Pig	40.0	40.0
Cat	40.0	40.0
Horse	40.0	40.0
Sheep	40.0	40.0
Chicken	40.0	40.0
Fish	40.0	40.0
Rabbit	40.0	40.0
Mouse	40.0	40.0
Rat	40.0	40.0
Hamster	40.0	40.0
Human Male	27.0	27.9
Human Female	27.5	40.0
E. coli	40.0	40.0
Pseudomonas	40.0	40.0
Neisseria	40.0	40.0
Staphylococcus	40.0	40.0
Saccharomyces	40.0	40.0
Candida	40.0	40.0

Fig. 2. The RPPH1 and SRY assays were designed to be human specific with minimal cross-reactivity to higher primate DNA. The two human control samples (Biochain, Hayward, CA) showed expected results. Only chimpanzee DNA was detected by the SRY assay. The assays didn't detect DNA from the remaining species included in the specificity panel (5 ng of DNA/rxn). A and B indicate DNA samples from two separate animals. Non-human samples were obtained as purified DNA from BIOS Laboratories, Inc., New Haven, CT and ATCC, Manassas, VA.

Figure 3. Standard Curve and IPC

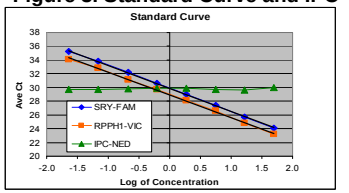


Fig. 3. Human genomic DNA from a pool of male donors (EMD Chemicals Inc., Madison, WI) was used to generate in a single reaction two standard curves for the human and the human male-specific targets with the DNA concentration ranging from 50 ng/µl to 23 pg/µl (LOD) in three-fold increments. The eight concentration points are 50, 16.7, 5.56, 1.85, 0.62, 0.21, 0.068 and 0.023 ng/µl and 2.0 µl of each sample were tested with the multiplex assay. IPC artificial template was included in each reaction to obtain the CT value of about 30 across the whole standard curve.

Figure 4. Reproducibility

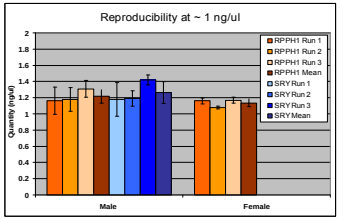


Fig. 4. Five different human DNA samples (Biochain, Hayward, CA; EMD Chemicals Inc., Madison, WI) were diluted to approximately 1 ng/µl and tested for reproducibility of the quantification results in three successive runs. One sample was from a female individual, the other samples were from male sources. For each sample, three replicates per run were quantified with the multiplex assay on the 7500 Real-Time PCR System. Averages and standard deviations for each run for two out of five samples are shown. The results from the human and the human male specific assay are in concordance for the male sample and are reproducible across runs for both male and female samples. The same study was done for 20, 10, 0.1 and 0.05 ng/µl DNA samples with comparable performance (data not shown).

Figure 5. Mixture study

Male/Female DNA ratio	SRYQuantity ng/ul	RPPH1 Quantity ng/ul	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	-----	-----

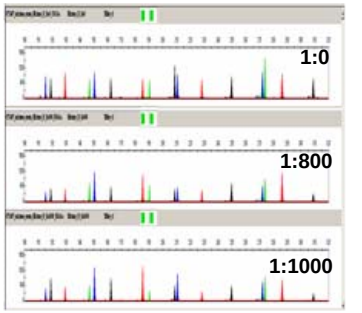
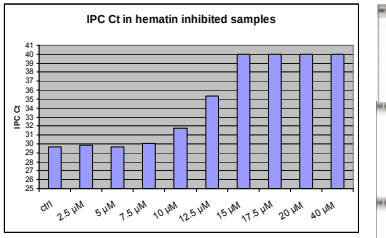


Fig. 5. Purified genomic DNA from a male and a female individual (Biochain, Hayward, CA) were combined according to various ratios (see table) to mimic sexual assault evidence samples. The male gDNA was added to the mixtures at a constant concentration of approximately 25 pg/µl. The mixtures were tested 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 AmpF*t*STR® Yfiler ® PCR Amplification kit for the 1:0, 1:800 and 1:1000 ratio samples are shown: the male STR profile is conclusive up to the 1:1000 mixture ratio. GeneAmp® PCR System 9700 and the ABI PRISM® 3100 Genetic Analyzer were used as described in the instruction manual.

Figure 6. Performance with hematin inhibited samples



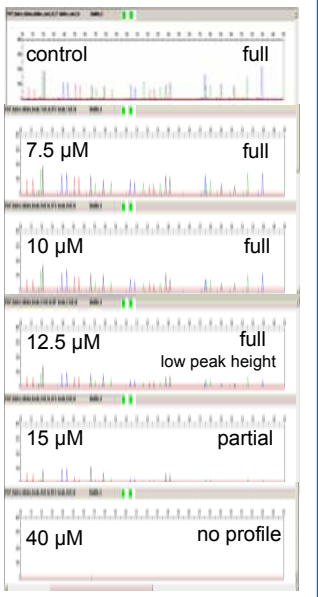


Fig. 6. Human male genomic DNA extracted from whole blood was mixed with varying final concentrations of hematin: 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, and 40 µM. 2.0 µl of each DNA/hematin mix, containing 1.0 ng of DNA, was quantified using the multiplex assay. The IPC C_T values were monitored (see 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 AmpF*t*STR® Identifiler™ kit reactions. 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 C_T values in the presence of increasing quantities of hematin. Similar results were obtained with samples containing increasing quantities of humic acid (data not shown).

CONCLUSIONS

Quantification of human DNA in forensic samples is essential for defining 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 AmpF*t*STR® 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.

ACKNOWLEDGEMENTS

The authors thank Genevieve Tang for technical support and the staff members of the Applied Markets Division at Applied Biosystems for the helpful discussions.

TRADEMARKS/LICENSING