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Forensic Genetics as a Tool for Peace and Justice: An Overview on DNA Quantification

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Abstract

In Forensic Genetics, DNA analysis is performed to obtain a Short Tandem Repeat (STR) profile from an evidence sample, which is then compared with the victim and suspect(s) reference sample STR profile, to determine their contribution for that evidence sample. However, forensic biological samples can be present in low quantities, and be exposed to different environmental insults leading to DNA degradation and contamination by inhibitor compounds. Thus, it is desirable for the forensic scientist to have useful information about the forensic sample quantity and quality prior to STR amplification.

New methods in Forensic DNA analysis for detecting, preserving and quantifying DNA, as well as its recover from different biological material are continually being developed. Real Time PCR (RT-PCR) assays for DNA quantification, like the recent Quantifiler® Duo DNA quantification kit (Applied Biosystems) proved to be very useful in forensic samples. Since many samples, mainly those resulted from sexual assault cases are often composed by unbalanced male/female DNA mixtures, the knew RT-PCR quantification assay, developed to quantify relative male/female DNA ratio, contributes not only for total DNA determination, but also to ascertain the presence and quantity of enough male DNA in the sample. These results are important to guide for the optimal STR analysis selection, such as autossomal STR, Y-STR or mini-STR, increasing downstream analysis success rates. In this work we present real forensic caseworks where the DNA amount and quality was important to guide for the selection of the appropriate STR amplification kit in order to increase the success of profiling in the first attempt, reducing the number of samples that need to be reprocessed and thereby decreasing the turn around time in a forensic laboratory.

Keywords: Forensic genetics; DNA quantification; RT-PCR; Short tandem repeat

Introduction

The human DNA amount estimation in forensic casework samples is a critical step in the overall DNA typing process, since many extraction procedures can recover not only human DNA but also other exogenous DNA. The Federal Burier Investigation, FBI's quality assurance standards (Standard 9.4, QAS2009) for forensic DNA testing laboratories recommend quantification procedures in forensic evidence samples prior to amplification, in order to determine the appropriate amount of DNA template to include in short tandem repeat (STR) loci amplification [1].

A number of quantification procedures have been developed and are traditionally used to provide human specific DNA quantification, such as hybridization techniques as for example QuantiBlot® Human DNA Quantitation Kit (Applied Biosystems, Foster City, CA). These are generally considered time-consuming, labour intensive, not suitable for automation and also low sensitive for STR genotyping systems [2]. In the last years, Real Time PCR (RT-PCR) methods based on Taqman probes were developed in a single multiplex reaction, allowing the use of an additional internal template control to detect PCR inhibitors and ensure quality results. RT-PCR, due to its easy automation, specificity for a certain target and sensitivity has become the most commonly used method for these trials.

Several kits were developed in order to use a RT-PCR quantitative assay, such as Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit (Applied Biosystems, foster City, CA) and most recently Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, foster City, CA), for simultaneous quantification of total human and human male DNA, proved to be very useful not only for human DNA quantification, but also for detection of DNA degradation and inhibitor's [2,3]. These assays can accurately reflect both the quality and the quantity of the DNA template that was extracted from samples, which allows an unerring decision about how to proceed in laboratory work flow with those quantified samples.

Since many forensic casework samples, mainly those resulting from alleged sexual assault consist in a mixture of unbalanced proportions of male/female DNA, the Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA), permit to quantify the relative male/female DNA ratio contributing not only for the determination of the total amount of DNA, but also to ascertain the amount of amplifiable autosomal DNA and the presence of male DNA to produce an Y-STR profile. The detection of the male component in sexual assault samples can be very valuable in deciding how to follow up the case [3]. In male/female mixtures the comparison between male and female concentrations suggested which type of STR analysis might provide more useful male genotype information. The Quantifiler® Duo

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DNA Quantification Kit (Applied Biosystems, Foster City, CA) is very sensitive and detects quantities as little as 3.1 pg of total input DNA.

Sexual violence has a significant negative impact on victim's life with numerous physical and psychological consequences which can be severe and long-lasting. In most of these cases, the DNA evidence is the only proof that can contribute to condemn or dismiss a suspect.

The DNA evidence as testimony is playing a larger role than ever to solve criminal cases all over the world. In this paper, are displayed three cases of sexual assault investigated in the Forensic Genetics Service of the National Institute of Legal Medicine and Forensic Sciences, South Branch, where quantification methods before STR typing played a major role.

Material and Methods

Casework 1, female victim, age 20, from who were collected two vaginal swabs and an oral swab (reference sample). Casework 2, female victim, age 43, from whom were collected one vaginal swab, one anal swab, a pair of panties (undergarment) and an oral swab (reference sample). Casework 3, female victim, age 15, from whom were collected two vaginal swabs, a pair of panties (undergarments), a blanket, one pair of denim jeans, and an oral swab (reference sample).

The DNA from forensic samples was extracted with PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA), in the AutoMate *Express*™ Forensic DNA Extraction System (Applied Biosystems, Foster City, CA).

Before application in routine casework samples, the Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA) was previously validated with DNA controls 9947A and 007. After DNA quantification, was determined which STR method to be applied to the extracted samples, autosomal and/or Y-STR amplification. The quantity of DNA extracted from samples was determined by Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA) in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) following the manufacturer's instructions with conditions as follows: 50°C, 2 min; 95°C, 10 min; 40 cycles of 95°C, 15 sec and 60°C, 1.0 min. The data were analysed using 7500 system HID Real Time PCR analysis software v 1.1, with a threshold value of 0.2 [4].

Samples were typed with AmpFℓSTR® Identifiler® Plus PCR Amplification Kit (Applied Biosystems, Foster City, CA) and with AmpFℓSTR® Yfiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA), using the procedures described in the user manual accompanying the kit.

The amplified products were analysed on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA), with GeneMapper® ID Software version 3.2 (Applied Biosystems, Foster City, CA).

Results and Discussion

The interpretation of an STR profile that features a mixture, often found in a sample resulting from an alleged case of sexual assault, creates great challenges to forensic scientists. In this type of samples, the DNA of the aggressor is often present in small amount compared with the quantity of DNA from the victim.

Forensic samples from caseworks 1, 2 and 3 were tested for the presence of male DNA and also total human DNA with the Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA) assay. In Table 1 given are the total human DNA and human male DNA in samples when both were detected. Considering casework 1, even with small concentrations of male DNA it was possible to obtain an incomplete Y-STR profile, without results in DYS389II, DYS19 and DYS439. The maximum scale for Relative Fluorescence Unit (RFU) obtained was 100 RFUs and the minimum 50 RFUs. The quantification of this sample showed a total human DNA amount much higher than the amount of male DNA. By studying the autosomal STRs the result obtained was already expected, the victim´s profile.

Casework	Sample Type	Total Human (ng/µl)	Male DNA (ng/µl)
1	vaginal swab	44.08	0.02
2	vaginal swab	93.08	11.94
3	undergarment	0.28	0.01

Table 1: DNA concentration results by casework.

In casework 2, we obtained a huge amount of total human DNA as well as a large amount of male DNA. The ratio female: male DNA was about 8:1. Based on these results, it was necessary to dilute the extracted sample to obtain the required amount of DNA for the PCR amplification with both kits (0.1 ng/µl) In this case it was obtained a complete Y-STR profile, with approximately 8000 RFUs. In autosomal STR a mixture of female and male contributor's (victim and suspect) was obtained with 6000 RFUs for major contributor, the female, and about 2000 RFUs for minor contributor, the male.

In casework 3 despite of small concentrations of male DNA it was possible to obtain a complete Y-STR profile with peaks about 400 RFU. In total DNA, the female proportion was, in this casework, 28 times higher than the male part. Due to the female/male ratio and the low concentration of male DNA, when studying autosomal STRs only the victim's profile was obtained.

These analyzes showed that caseworks wherein large amounts of victim´s DNA compared to male DNA, the amplification of the minor component may be suppressed or allelic drop can arise, giving difficult results to interpret.

Conclusions

In the RT-PCR analysis it is possible to detect the presence of a male minor component in the presence of excess female DNA. Our results in forensic casework samples demonstrated that estimation of DNA amount and quality is important to guide the selection of the appropriate STR amplification kit. With these quantification assays there is an increased success of typing on the first attempt, which reduces the number of samples to be reprocessed and thus reduces costs and lab time with each forensic sample.

The development of new methods and the wide variety of samples challenges forensic analysts to assess those methods in their current workflows in an attempt to achieve the best tool available. Data obtained at the quantification step, along with the knowledge and experience of the analyst; provide ideal conditions for the study of each evidence.

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