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Measuring human DNA degradation and gender detection in forensic DNA samples by q-PCR/HRM analysis

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ABSTRACT

Human DNA quantification, DNA degradation assessment and gender determination are key aspects in most field of human DNA analysis. The assay reported here is a tri-plex Real Time quantitative PCR reaction followed by high resolution melting (HRM) using Syto9 as intercalating dye. The system produces three amplicons: 1-transducin (beta)-like 1, Y-linked −TBL1Y (84 bp), 2- DeGraded small target DNA–DGst- (152 bp) and 3-DeGraded large target DNA-DGlt- (244 bp). DNA quantitation is based on total fluorescence; TBL1Y amplicon allows detecting male DNA and the ratio DGst/DGlt to assess DNA degradation level. q-PCR quantitation proved good linearity in triplicates among 3.2 pg/ul–50 ng/ul DNA concentration range. Amplification efficiency (E) and reaction slope (m) mean values were 1.04 and 3.23 respectively. Upon HRM analysis, three melting peaks are detected in a male DNA sample and two if only female DNA is present. We define the parameter D as the ratio DGst/DGlt that reflects the extent of DNA degradation in a given sample. A direct correlation has been demonstrated between DNA damage and increased value of parameter D. This q-PCR approach is rapid, sensitive, and a cost-effective method suitable for detecting degraded DNA samples and applicable to any field where human DNA quantitation-qualification is required.

1. Introduction

Ancient remains as well as evidentiary material collected at crime scenes are usually exposed to environmental factors that could alter DNA integrity [1,2]. Its effects are usually reflected in partial STRs profiles where stochastic effects are produced [3]. The importance of determining DNA degradation level is a crucial factor for defining the experimental strategy to be adopted (e.g. mini-STRs, SNPs, Indel or mtDNA analysis).

2. Materials and methods

2.1. DNA samples

Plexor® HY Male Genomic DNA Standard was used to set up PCR conditions and to test the triplex sensitivity and accuracy.

DNA derived from a human male donor was used to make the DNA degradation assay. Additionally, crime scene DNA samples (deposited on swabs) were tested by this triplex q-PCR system.

2.2. Primer design

Optimal primers were designed using Primer Tool software from IDT website. In silico amplification of designed primers was performed in https://genome.ucsc.edu/cgi-bin/hgPcr. Melting products behavior was tested with the Oligocalc program.

2.3. PCR conditions

Reagents: 0.3 nM Syto9 (Invitrogen), 0.05 U/μL GoTaq DNA Polymerase (Promega), 5× Colorless GoTaq Reaction Buffer (Promega), 15 pmol Y chromosome-specific primers, 10 pmol short target primers, 15 pmol long target primers, 0.15 mM dNTPs, 0.2 mM Mg^{2+} (1.7 mM final concentration) and 2 μL DNA sample (25 uL final volume). DNA amplification reactions were carried out in a Rotor Gene 6000 (Corbett) Real Time PCR equipment. Cycling conditions: 94 °C for 2 min, followed by 37 cycles of 94 °C for 30s, 57 °C for 30s, 72 °C for 30s. High resolution melting analysis was performed from 69 °C to 85 °C at 0.2 °C/s. Degraded DNA assessment was determined by D parameter (D = Short segment melting peak height/long segment melting peak height) for each aliquot.

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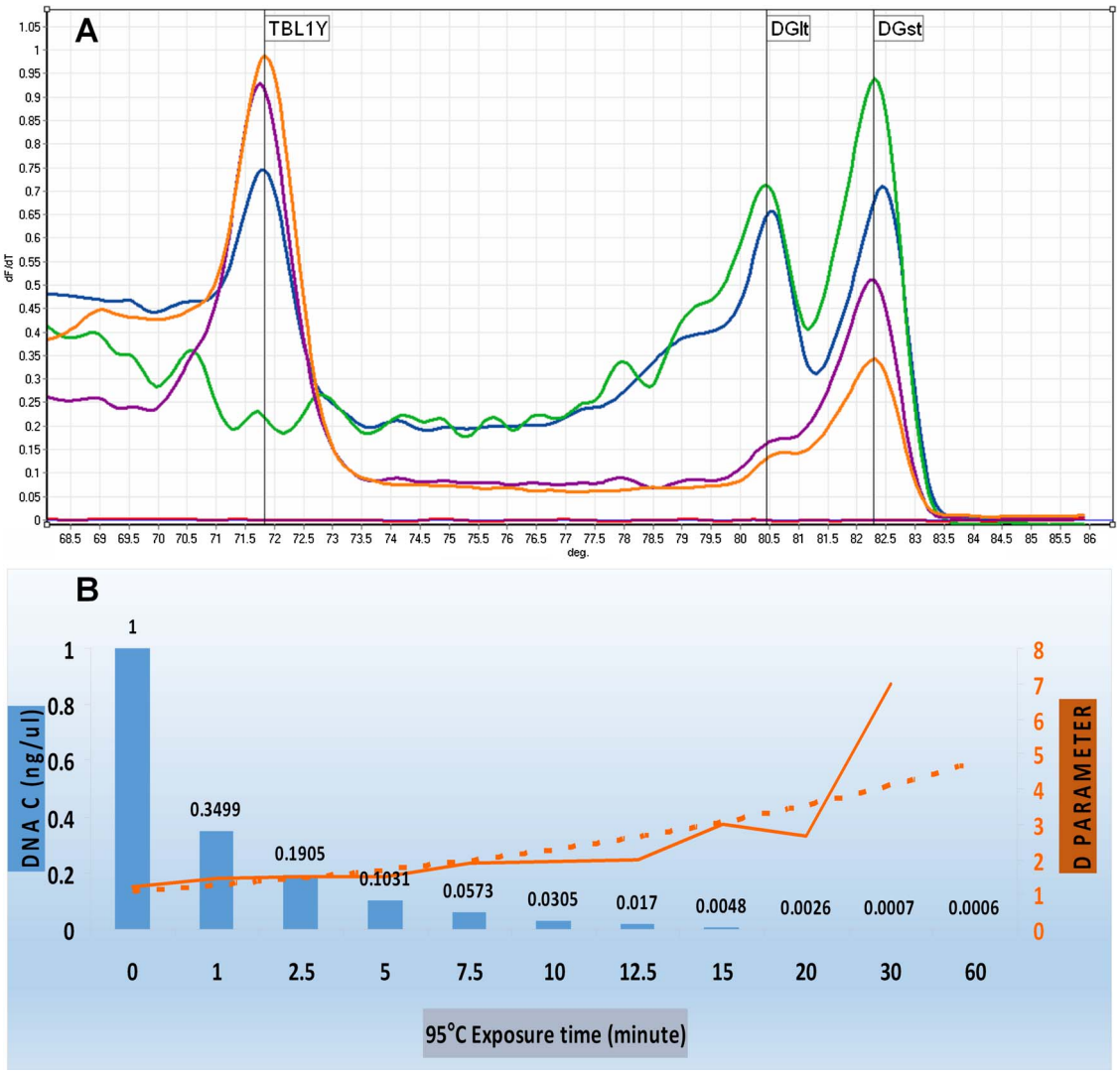

Fig. 1. (a) High resolution melting profiles. None degraded male DNA in blue (D = 1.10; full STR profile was obtained); lowly degraded female DNA in green (D = 1.30; full STR profile was obtained); significantly degraded DNA from biological stains in orange (D = 2.45) and in violet (D = 3.0) where partial STR profile was obtained since drop out occurred in three high weight molecular markers respectively. Negative control in red. (b) Combined graph for DNA Degradation Assay. DNA concentration is represented in blue bars and D parameter in orange continuous line (orange dot line represents D parameter trend line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. DNA degradation assay

10 aliquots of human male DNA (1 ng/μl final concentration) were exposed to 95 °C for different times: 0, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 30 and 60 min in a *GeneAmp*® PCR System *9700* (Applied Biosystems) for obtaining degraded DNA [4].

3. Results

Three melting peaks are present in the male sample due to the presence of Y chromosome segment while two are detected in female DNA sample (Fig. 1a).

DNA samples of known concentration ranging from 3.2 pg/μL to 50 ng/μL were tested in triplicate. The system was able to amplify as low as 3.2 pg/μL DNA and no significant dispersion was evidenced in their triplicates (%CV = 1.99). Amplification efficiency (E) and reaction slope (m) mean values were 1.04 and 3.23 respectively.

Degraded DNA was tested by melting profiles obtained after HRM by comparison of melting peak heights of DGlt and DGst amplicons. DNA amplification of long segment is under-represented compared to the short one and this is reflected in the D parameter values obtained. Thus, after heat treatment, more degraded DNA (exposed to longer times at 95 °C) greater D parameter was observed (Fig. 1b).

By last, forty biological stains collected from crime scene were quantified by this triplex assay. After HRM analysis male DNA detection and DNA degraded assessment was successful (Fig. 1a). D parameter values greater than 2.4 are associated with low RFU (relative fluorescence unit) values in high molecular weight STR markers denoting a ski slope effect and/or allelic drop out.

4. Discussion

The triplex qPCR approach is a useful tool for simultaneously assessing the quantity and quality of human DNA and human male DNA detection consuming a minimal amount of sample. Amplification efficiency and reaction slope values obtained from sensitivity assays reflect that this triplex system is able to produce reliable quantification results.

The ability to gather such information during the quantification step enables to make the best decision concerning the most suited markers to be selected according to the sample condition: DNA quantity, quality and sex contributor. It also reduces the time and cost of analysis and allows redefining DNA extraction strategies.

Additionally, employing intercalating dyes, such as Syto9, considerably reduces the analysis costs.

5. Conclusion

We have developed a useful qPCR system that suitable for a wide range of field where DNA quantitation-qualification is required, such as forensic field, ancient-degraded DNA or DNA molecular diagnosis.

Conflict of interest

The authors report no declarations of interest.

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References

[1] W. Bär, A. Kratzer, M. Machler, et al., Post mortem stability of DNA, Forensic Sci. Int. 39 (1988) 59–70.
[2] R. Alaedini, S.J. Walsh, A. Abbas, Forensic implications of genetic analysis from degraded DNA – a review, Forensic Sci. Int. Genet. 4 (2010) 149–157.
[3] J.M. Butler, Advanced Topics in Forensic DNA Typing: Interpretation, Elsevier Academic Press, San Diego, 2015.
[4] M. Neumaier, et al., Method for the Quantitative Analysis of the Nucleic Acid Fragmentation and Amplificability, Patent Application Publication, 2016.