THE ROLE OF DNA CONCENTRATIONS IN FORENSIC CASEWORK RESULTS REGRESSION MODELS APPLICATION

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INTRODUCTION

- In forensic DNA typing, short tandem repeats (STRs) are the most frequently genotyped markers in order to distinguish between individuals and to relate them to a crime or to exonerate the innocent. [1]
- In recent years, new controversies have arisen with the advent of more sensitive techniques, allowing profiles to be recovered from minimum amounts of DNA, hence, bringing challenges to weight of evidence evaluation for forensic DNA profiles obtained from low template DNA samples.
- Introduction of interpretation models, or even new weight of evidence software should be accompanied by a measure of uncertainty that is part of any biological analysis. Specially, due to stochastic effects, the reliability of the obtained profiles might differ between machinery, workflow and also PCR settings in use in different laboratories.
- In this work we try to understand the relation between Peak Area, DNA concentration and also size marker, as preparatory work to the construct adequate regression models in order to estimate peak area and peak height.

METHODS

- Buccal swabs from 180 individuals, with unknown identity, were selected for this study.
- DNA was extracted with prep-n-go™ buffer and quantified using Quantifiler® Trio DNA Quantification kit in a 7500 Real-Time PCR System (Applied Biosystems). [2]
- STR amplification was performed with Powerplex®Fusion 6C amplification kit (Promega). Amplified PCR products were separated and detected in an Applied Biosystems® 3500 Genetic Analyzer using manufacturer's conditions. Electrophoresis results were analysed with GeneMapper® ID-X v1.4. [3]
- Statistical analysis was performed with R Studio, with suitable packages.

RESULTS

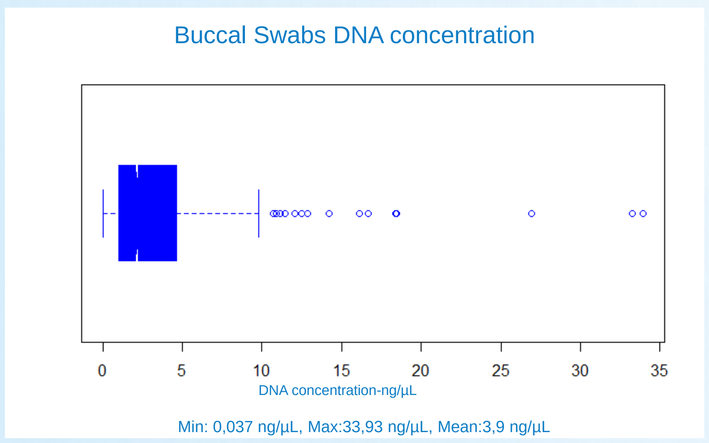

Fig. 1- Boxplot representing Buccal swabs DNA concentration in the studied samples

Fig. 1- represents Prep-n-go™ DNA concentration (ng/µL) in the studied samples. The minimum DNA concentration obtained was 0,037ng/µL and the maximum DNA concentration was 33,93 ng/µL. The mean value obtained was 3,9ng/µl. DNA profiles were obtained in all samples despite the variation in DNA amount.

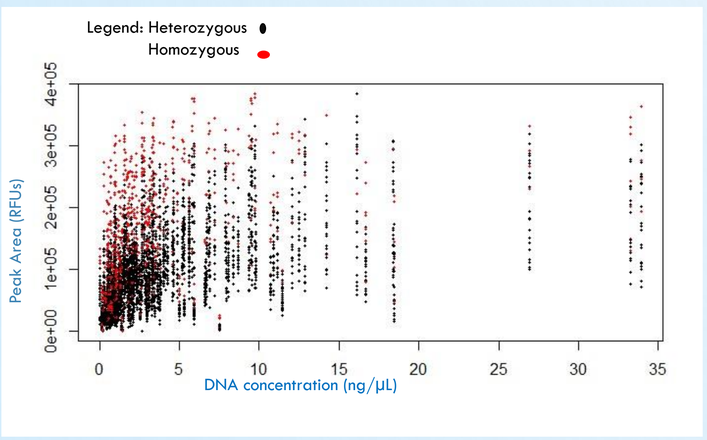

Fig 4 - Relation between DNA concentration and Peak Area (RFUS), in Heterozygous markers and homozygous markers.

Fig. 4- represents the association between DNA concentration and Peak Area, in heterozygous and homozygous individuals for a given genetic marker.
Apparently low DNA concentration samples lead to peaks with lower areas, as expected, and allelic peak area distribution patterns of homozygous and heterozygous individuals are similar.
Correlation between DNA concentration and homozygosity 0.4747
Correlation between DNA concentration and heterozygosity 0.3729

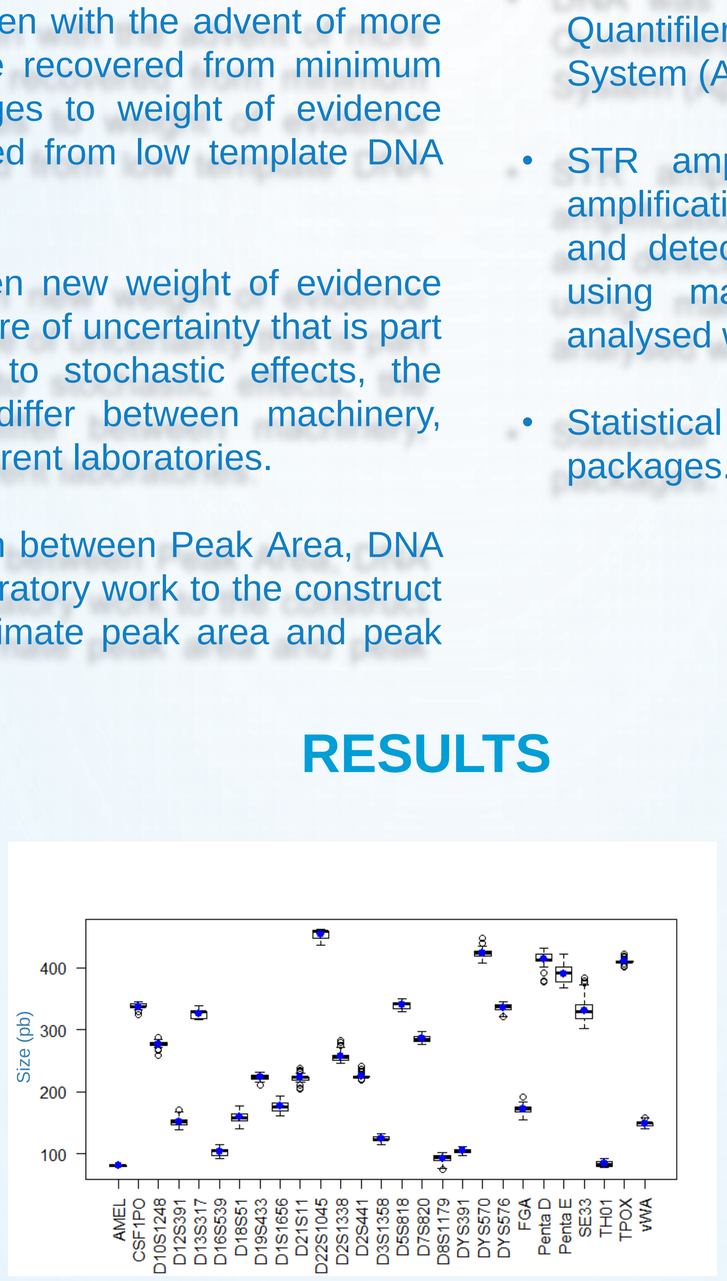

Fig. 2- representative boxplots of the size of the amplified fragments in each of the genetic markers

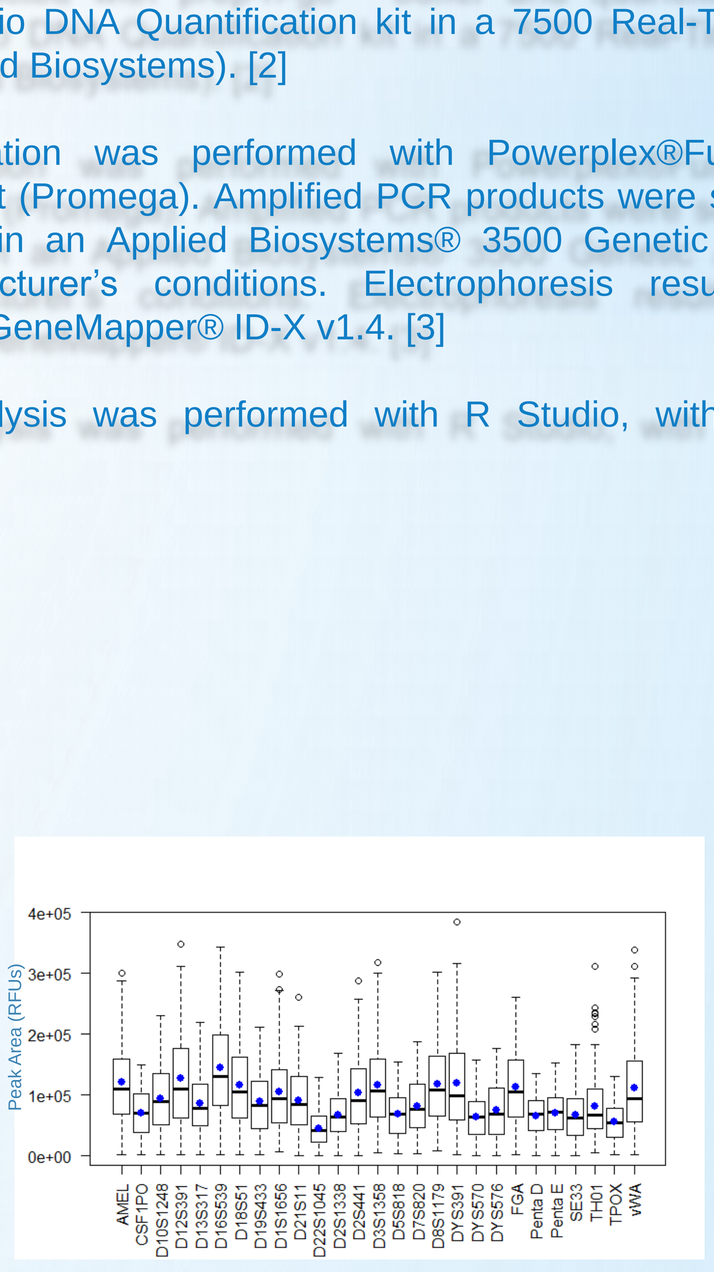

Fig. 3- representative boxplots of the peak area of the amplified fragments in each of the genetic markers

	Size (pb)	Height (RFUs)	Área (RFUs)
Min.	74,83	65	541
Median	227,02	9586	80065
Mean	245,36	11103	91585
Max.	462,66	41506	384704

Table 1- summary of statistical measures of locationof Size (bp), Height (RFUs) and Peak Area (RFUs) variables

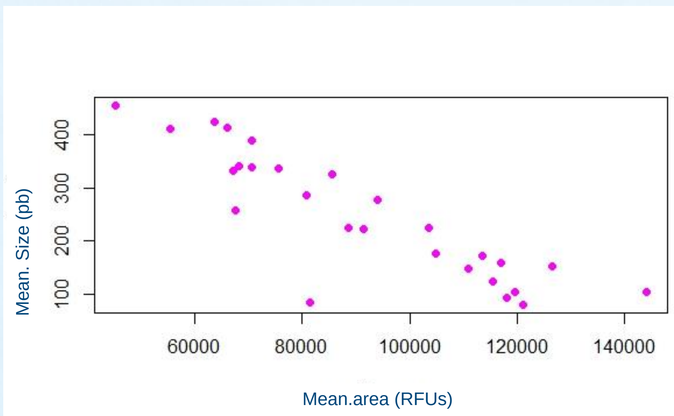

Fig.5- Relation between DNA concentration and Peak Area (RFUS), in Heterozygous markers and homozygous markers.

Fig. 5- Correlation between allele Size (pb) and Peak Area (RFUs) is - 0,82142. This value indicates that theres a strong negative correlation between Size in bp and Peak Area.

Discussion

These results are preliminary and part of a much larger study on this subject.

The results demonstrated that even with very low DNA concentrations it is possible to obtain a complete genetic profile, which demonstrates the sensitivity level of this kit.

The correlation between DNA concentration and peak area does not seem so strong, perhaps due to the characteristics of the kit itself.

We can see from the graphs that there is a strong negative correlation between fragment size / genetic marker and peak area in each of the genetic markers.

Even with preliminary results it is possible to determine a trend among the variables under study. Other studies will be performed to better adjust the statistical models in these variables.

REFERENCES

[1] Martin G. Ensenbergera, Kristy A. Lenza, Learden K. Matthiesb, Gregory M. Hadinotob, et al Developmental validation of the PowerPlex® Fusion 6C System, . Forensic Sci Int Genet, March 2016, Volume 21, Pages 134–144
[2] Prep-n-Go™ Buffer User Guide, ThermoFisher™ Scientific, Foster City, USA, 2012.
[3] PowerPlex® Fusion 6C System for Use on the Applied Biosystems® Genetic Analyzers

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