QUANTIFILER®TRIO DNA METHOD PERFORMANCE IN A COLLECTION OF ANCIENT SAMPLES

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INTRODUCTION

ÅDuring the past few years significant progress as been made in solving technical challenges associated with STR profiling including the ability to analyze degraded DNA and low amounts of DNA. The result of these changes is that useful STR profiles can now be obtained from previously untypeable forensic DNA samples.

ÅAnalysis of DNA from ancient material represents an important role in molecular anthropology, although there are many limitations concerning low DNA quantity and/or degraded DNA, and/or PCR inhibitors. These factors can make it difficult to decide whether to continue with STR analysis, which STR panel to use and how much DNA to add to PCR reaction. With all this constraints, DNA quantification represents an important tool to decide which method will follow in order to improve workflow and have good results in less time consuming.

ÅThe Quantifiler® Trio DNA method has a set of primers to amplify and detect two autosomal, multiple-copy target loci, known as the Small Autosomal (Small DNA) and Large autosomal (Large DNA), which provide a quality index (DI) to detect the presence of degraded DNA along with PCR inhibitors. Additionally it has a primer to detect human male specific target locus located on Y chromosome.These guides contribute to the selection of the optimal short tandem repeat (STR) analysis chemistry (autosomal, or miniSTR) and streamlines the workflow while increasing downstream analysis success rates.

MATERIAL AND METHODS

ÅIn order to compare DNA quality from different extraction methods, samples from 50 exhumed Middle Ages individuals were extracted with modified phenol chloroform method and also PrepFiler Express BTA™ method (Life Technologies)

ÅDNA quantification was performed with Quantifiler® Trio DNA Quantification Kit (Life Technologies) in a ABI PRISM® 7500 Real-Time PCR System, according to manufacturer's. DNA concentration was analyzed with HID Real Time PCR analysis Software v 1.2. (Life Technologies)

ÅStatistical analysis was performed with IBM SPSS Statistics 21

REFERENCES

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-Stefano Vernarecci, Enrica Ottaviani, Alessandro Agostino, Elisabetta Mei, Lisa Calandro, Paola Montagna, Quantifiler ® Trio Kit and forensic managment: A matter of degradation. Forensic Science International: Genetics 16 (2015) 77-85

-Jason Yingjie Liu, Direct qPCR quantification using Quantifiler ® Trio DNA quantificatin kit, Forensic Science International : Genetics 13(2014) 10-19.

RESULTS

Fig. 1,represents number of samples with DNA results. Concerning Large DNA (A), PrepFiler Express BTA™ method contributed for DNA detection in 10 samples and Phenol in 19 samples. Small DNA (B) was obtained in 26 DNA samples extracted with Phenol. Male DNA (C) was analysed in 14 phenol DNA samples, against only one DNA sample extracted with BTA® method.

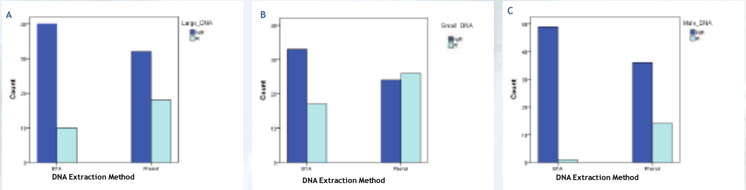

Fig.1-Number of samples with DNA results (R) and without DNA results (N/R) extracted simultaneously with PrepFiler Express BTA™ method (Life Technologies) and Modified Phenol Chloroform method.

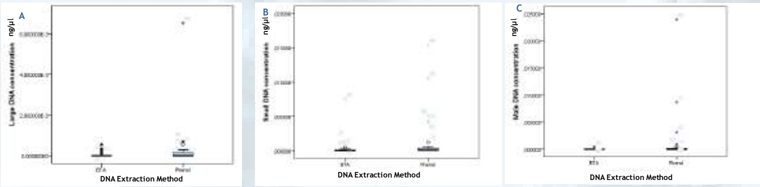

Fig.2-DNA concentration results obtained with PrepFiler Express BTA™ method (Life Technologies) and Modified Phenol Chloroform extraction methods.

Fig. 2, represents DNA concentration results with both extraction methods. Concerning Large DNA (A), with PrepFiler Express BTA™ maximum concentration value was 0.000549 (ng/µl) and with phenol, the maximum value was 0.006526 (ng/µl) .

Considering Small DNA (B), maximum concentration value with PrepFiler Express BTA™ was 0.007515 (ng/µl) and maximum concentration value with phenol chloroform was 0.015 (ng/µl).

Considering Male DNA (C) maximum concentration value with PrepFiler Express BTA™ was 0.000411 (ng/µl) and maximum concentration value with phenol chloroform was 0.0239 (ng/µl).

Male DNA results were also obtained in a sample with undetermined gender by anthropological means.

High levels of degradation index (DI), ex. 8.87, in one sample, indicates that this sample in particular should be studied with mini-STRs or mitochondrial DNA.

CONCLUSIONS

- Quantifiler Trio ® demonstrated that samples selected for this study were challenging and time consuming, using our existing protocols, requiring PCR input adjustment and also multiple amplifications to obtain a useful profile. Concerning DNA extraction methods, it's clear that in ancient samples, modified Phenol Chloroform extracts DNA more efficiently than PrepFiler Express BTA™ method (Life Technologies).

- DI parameter information is also effective in characterizing degraded samples and can be used to decide how to process the samples in order to maximize allele recovery.

- Analyzed results allow to point Quantifiler® Trio method as an important tool in pre-STR typing methods in ancient samples.

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