Internal Validation of DNA Extraction from Bone and Tooth Samples Using Qiagen's BioRobot EZ1

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Abstract

The validation of a protocol for bone and tooth DNA extraction using the BioRobot EZ1 (Qiagen, Valencia, CA) was performed for use with casework samples. The protocol allows for an effective method of extracting DNA from these challenging sample types. In this validation multiple comparisons to an original protocol were performed, including the use of carrier RNA versus no carrier RNA, the EZ1 large volume protocol versus the trace protocol, a Chelex® 100 method and a North Louisiana Criminalistics Laboratory (NLCL) procedure. When all methods were compared, the NLCL method was chosen and validated for use at the Wyoming State Crime Laboratory (WSCL).

Introduction

Certain sample types, such as bone and tooth samples, are not often encountered at the Wyoming State Crime Lab but are common in the forensic realm. While these are not commonly encountered sample types, their importance is often magnified as DNA may not be available from more common sources such as blood or saliva. Due to this, the extraction of DNA contained in a bone or tooth sample may be crucial. For this reason the Wyoming State Crime Lab decided to develop and validate a DNA extraction procedure for bone and tooth samples using the BioRobot EZ1.

Materials and Methods

Decalcification and Lysis

175mg powdered bone/tooth sample
0.5M EDTA, Proteinase K, Qiagen Buffer ATL

EZ1 Extraction

Elution in 50µl TE Buffer

Quantitation

Quantifiler® Human DNA Quantification Kit
AB 7500 Fast Real-Time PCR System

Amplification

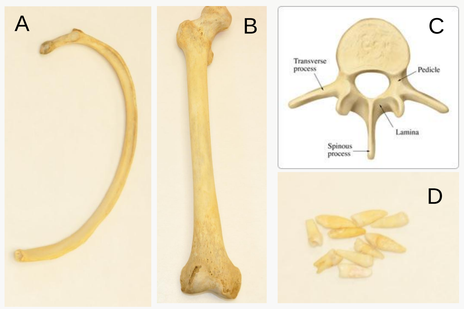
AmpFℓSTR® Profiler Plus® PCR Amplification Kit
AmpFℓSTR® COfiler® PCR Amplification Kit
96-Well GeneAmp® PCR System 9700

Capillary Electrophoresis

AB 3130 Genetic Analyzer
Data analyzed with GeneMapper® ID v.3.2.1

Sample Types Used

A. Rib
B. Femur
C. Vertebra
D. Tooth



Comparisons

Determination of the optimal decalcification, lysis and extraction method:

Original Protocol

Decalcification of 175mg bone/tooth powder was done with 700µL 0.5M EDTA at 37ºC for 24 hours (Vortexed often during incubation). Lysis was then performed using 20µl proteinase K and incubation of the sample at 56ºC for three hours with occasional vortexing. Extraction on the BioRobot EZ1 was performed using the trace protocol.

North Louisiana Crime Lab Protocol

Decalcification and cell lysis of the sample were done simultaneously utilizing 750µl 0.5M EDTA, 675µl Buffer ATL, and 75µl proteinase K and incubation at 56ºC for 24 hours with occasional mixing by inversion. EZ1 extraction was performed using the large volume protocol, 500 µl of sample lysate, 400µl Buffer MTL, and 30µl NaOAc.

EZ1 Large Volume Protocol

The original protocol was followed for this comparison. The large volume protocol was used on the EZ1 for extraction using 500µl sample lysate and 400µl of buffer MTL.

Chelex® 100 Protocol

Pre-extraction techniques employed the use of 400µl 5% Chelex® 100 solution and 40µl proteinase K added to each sample and incubation at 95ºC for 40 minutes.

Carrier RNA Addition

Samples with the lowest quantitation values from the original protocol were used to determine the necessity of carrier RNA addition prior to EZ1 extraction. One µl of carrier RNA (1ng/µl) was added to each sample prior to EZ1 extraction after pre-lysis and decalcification was performed using the original protocol.

Concentration Test

Two challenging samples (rib and femur) extracted using the NLCL protocol were concentrated using a Microcon® filter (rib) or EZ1 concentration (femur). Microcon® concentration was done on six rib samples after EZ1 extraction. EZ1 concentration was accomplished by combining six femur EZ1 extracts and re-extracting on the EZ1.

Results

Table 1: Summary of quantitation values from comparison techniques employed

	Original Protocol	Carrier RNA	Large Volume	Chelex® 100	NLCL: Lg-Vol, no carrier RNA
Sample:	**CONC (ng/µl)**	**CONC (ng/µl)**	**CONC (ng/µl)**	**CONC (ng/µl)**	**CONC (ng/µl)**
Rib	0.0029	0.0070	NR	NR	0.0473
Dremel Femur	0.0113	0.0125	0.0091	NR	0.0029
Vertebra	0.3340	-	0.1230	0.1260	-
Tooth 1	3.0267	-	2.4750	0.0010	7.9400
Tooth 2	0.0051	0.0056	NR	NR	-
Tooth - 72889A	15.2000	-	-	-	18.8000
Tooth - 10/02A	2.9200	-	-	-	7.9550
Rib - 91101	11.7300	-	-	-	20.6000
Vertebra - 122300	8.9000	-	-	-	19.8300
Extraction Negative (EN)	NR	NR	NR	NR	NR

Table 2: Summary of quantitation values from the concentration test compared to non-concentrated averages using the NLCL Protocol

	Pre-Concentration	Concentration
Sample:	**CONC (ng/µl)**	**CONC (ng/µl)**
Rib	0.0099	0.0178
Femur	0.0181	0.0943
Extraction Negative (Rib)	No Results	No Results
Extraction Negative (Femur)	No Results	No Results

Discussion

- Addition of carrier RNA prior to EZ1 extraction does not significantly enhance quantitation.
- The Chelex® protocol comparison yielded extremely low to no quantitation results and no genotype results.
- Large volume elution on the EZ1 yielded comparable quantitation data to the results obtained using the EZ1 trace protocol.
- The NLCL protocol resulted in quantitation and genotype data that exceed the results obtained with the original protocol.
- The NLCL protocol showed the best results when using the large volume protocol with no carrier RNA addition.
- Challenging bone samples can be concentrated using either a Microcon® apparatus or EZ1 concentration to obtain better quantitation values and more complete genetic profiles.

Conclusion

The validation of bone and tooth extraction was successfully completed using the BioRobot EZ1 and the appropriate protocol will be implemented. Although an original protocol was developed for the extraction of DNA from bone and tooth samples, the method developed by the North Louisiana Criminalistics Laboratory was determined to be the best extraction procedure and was chosen to complete the validation. The protocol has undergone a qualifying test and is now able to be used for casework samples at the Wyoming State Crime Laboratory.

References

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