No More Powdering:
DNA Extraction Pre-treatment Kit for Teeth and Bones

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Introduction

Successful DNA extraction from teeth and bones is crucial for identification of human remains. DNA extraction from hard tissues inevitably requires crushing and grinding of samples into powder, but such procedure is troublesome and prone to cross-contamination. Tbone EX Kit is a DNA extraction pre-treatment reagents, which enable extraction of DNA directly from whole tooth or bone, bypassing the step of powdering (Fig.1C). Extracted DNA can be used for downstream assays such as mitochondrial DNA sequencing and STR genotyping.

Fig. 1 Appearance of Tbone EX Kit. (A) Kit container. (B) Contents of the kit. (C) Example of DNA extraction from a whole tooth.

Tbone EX Kit is widely used by Japanese forensic laboratories in police force and universities. However, its compatibility with downstream DNA extraction systems other than QIAamp® columns from QIAGEN has not been examined extensively. At Department of Chemistry Malaysia, we have evaluated applicability of Tbone EX Kit combined with downstream Applied Biosystems PrepFiler™ BTA and AutoMate Express™ DNA Extraction System.

Experimental Procedure

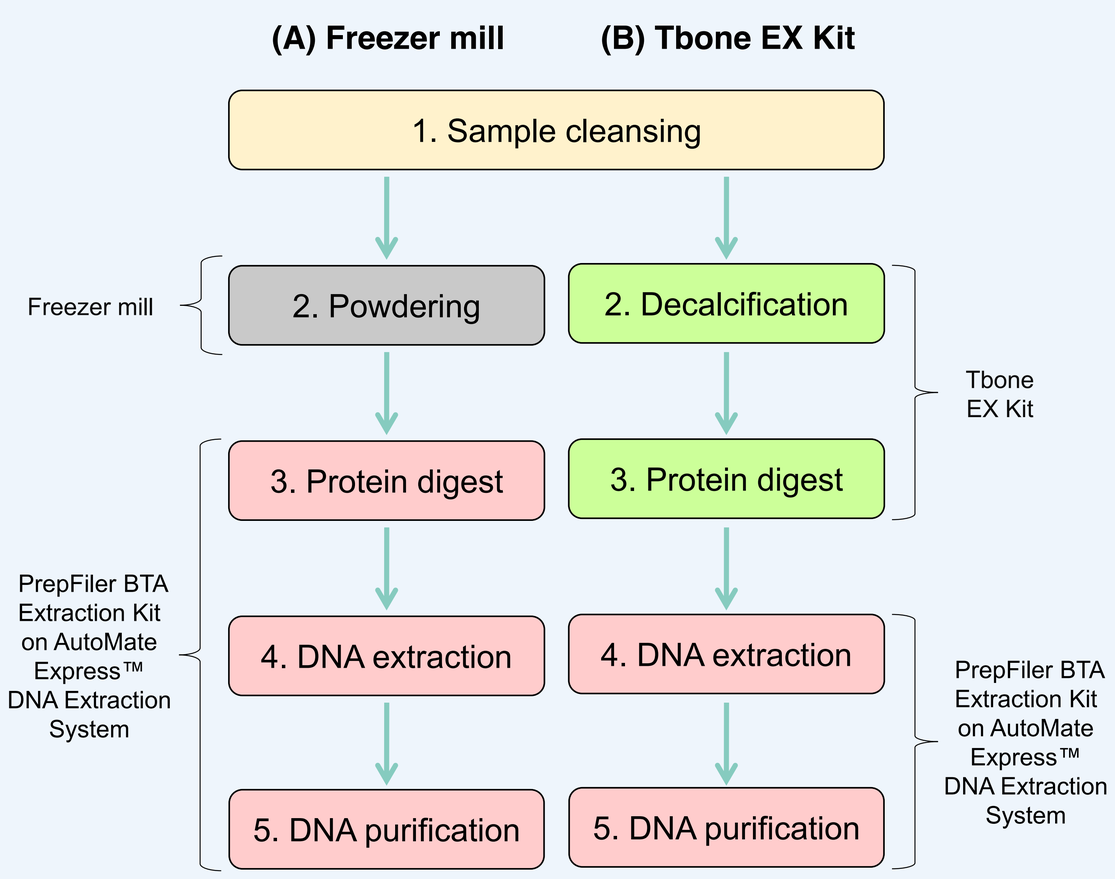

Fig. 2. Experimental flow of two sample treatment methods. (A) Powdering with freezer mill. (B) Direct incubation of bone piece with Tbone EX Kit.

Thirteen bone samples were cleaned and cut into convenient size prior to the experiment. For protocol (A), the samples were crushed into powder by freezer mill, and DNA was extracted according to the standard procedure of Applied Biosystems PrepFiler™ BTA. For protocol (B), intact pieces of bones (0.5 g each) were incubated with reagents of Tbone EX Kit to carry out decalcification. Samples were treated by following the standard procedure of Tbone EX Kit up to the protein digest step, then by PrepFiler™ BTA procedure thereafter.

Results

Concentration of purified DNA obtained by two methods are shown in Table 1. Tbone EX Kit was compatible with Applied Biosystems PrepFiler™ BTA and AutoMate Express™ DNA Extraction System, facilitating successful DNA extraction from bones without the need of powdering. Tbone EX Kit yielded higher concentration of DNA than conventional protocol in all thirteen samples tested. Samples A201, A200 and A259 did not yield detectable DNA by freezer mill protocol, but all three resulted in measurable amount of DNA by pre-treatment with Tbone EX Kit combined with PrepFiler™ BTA and AutoMate Express™ DNA Extraction System.

Table 1. Amount of starting materials and concentration of purified DNA from two extraction methods. (A) Powdering with freezer mill. (B) Direct incubation of bone piece with Tbone EX Kit.

Treatment	(A) Freezer mill			(B) Tbone EX Kit	
Sample Marking	Starting amount	Input after powdering	Quant result (ng/ul)	Starting amount	Quant result (ng/ul)
A201	**0.5 g**	**70 mg**	**0.0000**	**0.5 g**	**0.0011**
A186C	0.5 g	70 mg	0.0002	0.5 g	0.0004
A200	**0.5 g**	**70 mg**	**0.0000**	**0.5 g**	**0.0014**
A192	0.5 g	70 mg	0.0005	0.5 g	0.0018
A198	0.5 g	70 mg	0.0016	0.5 g	0.0143
A184a	0.5 g	70 mg	0.0007	0.5 g	0.0097
A265	0.5 g	70 mg	0.0018	0.5 g	0.0121
A261	0.5 g	70 mg	0.0035	0.5 g	0.0227
A267	0.5 g	70 mg	0.0019	0.5 g	0.0102
A259	**0.5 g**	**70 mg**	**0.0000**	**0.5 g**	**0.0032**
A273	0.5 g	70 mg	0.0031	0.5 g	0.0049
A274	0.5 g	70 mg	0.0298	0.5 g	0.0367
A271	0.5 g	70 mg	0.1410	0.5 g	0.3150

Conclusion

Tbone EX Kit was developed for extraction of DNA from hard tissues, bypassing troublesome step of powdering. Here we demonstrated that the kit indeed enables extraction of DNA from non-powdered bone samples. We also evaluated compatibility of the reagent with Applied Biosystems DNA extraction system, and observed that it is not only compatible, but also increases the yield of DNA, comparing to the conventional method. Tbone EX Kit can be beneficial in reducing the handling time to minimize the risk of cross-contamination, while increasing the chance of obtaining DNA from previously challenging samples. Our data showed that Tbone EX Kit can be incorporated into existing forensic DNA automated extraction system. Tbone EX Kit also allows extraction of large number of samples in parallel by simply pretreating the samples by dipping them in the solution. This potentially saves time and effort, while increasing the overall efficiency and quality of DNA purification from hard tissues.

Acknowledgement

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Reference:
Evaluation of a new experimental kit for the extraction of DNA from bones and teeth using a non-powder method. Leg Med (Tokyo). 2010 Mar;12(2):84-9. Epub 2010 Jan 27.

DNA Chip Research Inc.

Application of Non-powdering Sample Pre-Treatment Method for Bone and Tooth

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Introduction

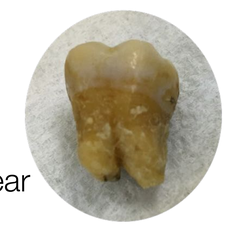
Identification of human remains using DNA extracted from tooth or bone is common in forensic science. Extraction of DNA from hard tissues requires pulverization in an extremely well controlled environment to prevent cross-contamination of powdered samples. To overcome this issue, we introduce a liquid-based sample pre-treatment reagent, Tbone EX Kit. Extraction of DNA directly from a whole tooth or a piece of bone just requires sequential treatment with three types of solutions in the kit, bypassing the problematic pulverization step. In this study, we have performed STR genotyping of a tooth sample, extracted using Tbone EX Kit coupled with different purification methods including PrepFiler™ BTA. We have also attempted to extract DNA of ancient cremated bone sample and would share the experience and knowhow.

Experiments and Results

1. Experiment Flow

One tooth and two ancient cremated bone samples were treated with Tbone EX Kit without pulverization respectively.

Male individual, 40s
Sample weight 2.1g
Wisdom tooth, decayed, nerve removed
Kept at room temperature for about one year



Tooth sample

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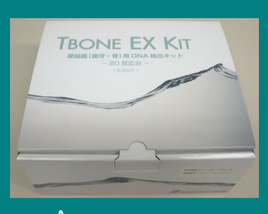
Tbone EX Kit



Appearance

Contents

Decalcification

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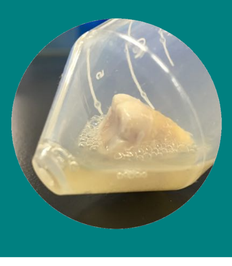
Protein Digest

The whole tooth/bone was dipped in solution A and processed according to the manual (Tbone)



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Phenol/Chloroform Treatment or Not

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PrepFiler™ BTA

DNA purification

2. Tooth

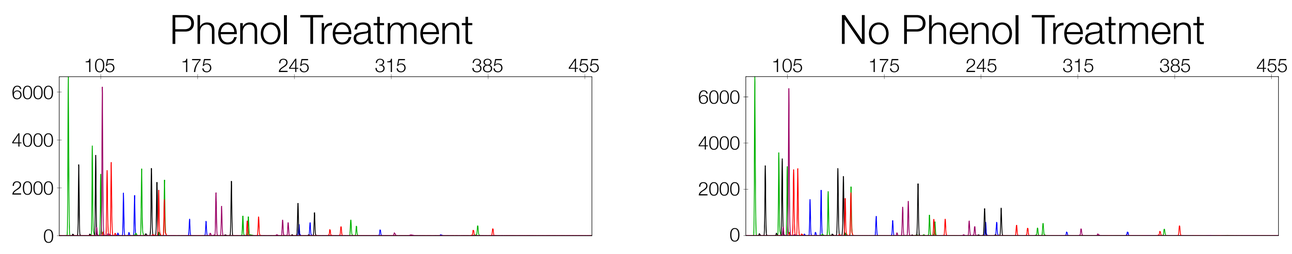

Fig. 1 STR genotyping graphs of tooth sample 2nd round extraction.

Table 1 DNA yield and STR Loci of tooth sample

	Phenol Treatment		No Phenol Treatment	
	DNA content (ng/ul)	Loci	DNA content (ng/ul)	Loci
1st Round	0.000186	9	0.000875	16
2nd Round	0.039	24	0.038	24

STR Amplification was performed with GlobalFiler™ PCR Amplification Kit, data was analyzed and visualized using GeneMapper™ *ID-X* v1.4. All 24 STR loci were detected from DNA extracted at second round.

3. Ancient Cremated Bones

Sample 1:
cremated male individual (~ 1915), 50s, sample weight 1.38g
Sample 2:
cremated male individual (~ 1867), 2 years old, sample weight 1.05g

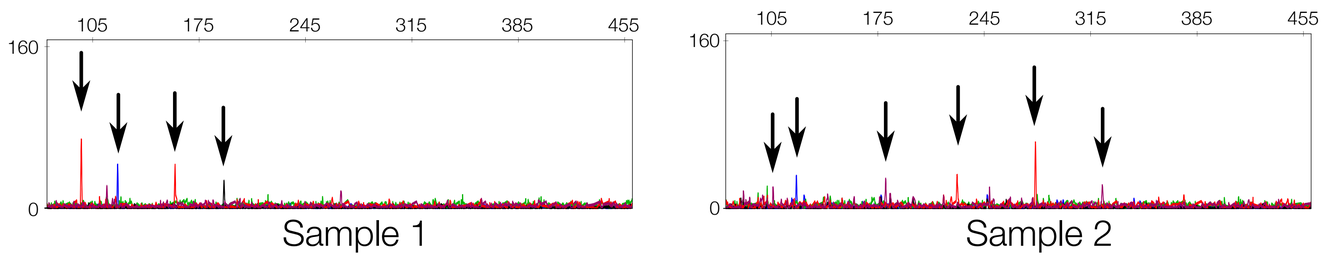

Fig. 2 STR genotyping graphs of ancient cremated bone samples.

Table 2 DNA yield and STR Loci of ancient cremated bone samples

	DNA content (ng/ul)	Loci
Sample 1	0.000494	4
Sample 2	0.000198	6

Conclusions

1. As pulverization of samples is not required, Tbone EX Kit reduced hands-on time required for extraction of DNA from bone and tooth.
2. Qualities of DNA extracted is good enough for down stream forensic analysis such as STR genotyping.
3. Extraction of DNA from cremated ancient sample is a difficult task especially when region containing DNA has been burnt. However, we could still able to extract from regions where cremation is partial or not complete.

Acknowledgements

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