

# 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

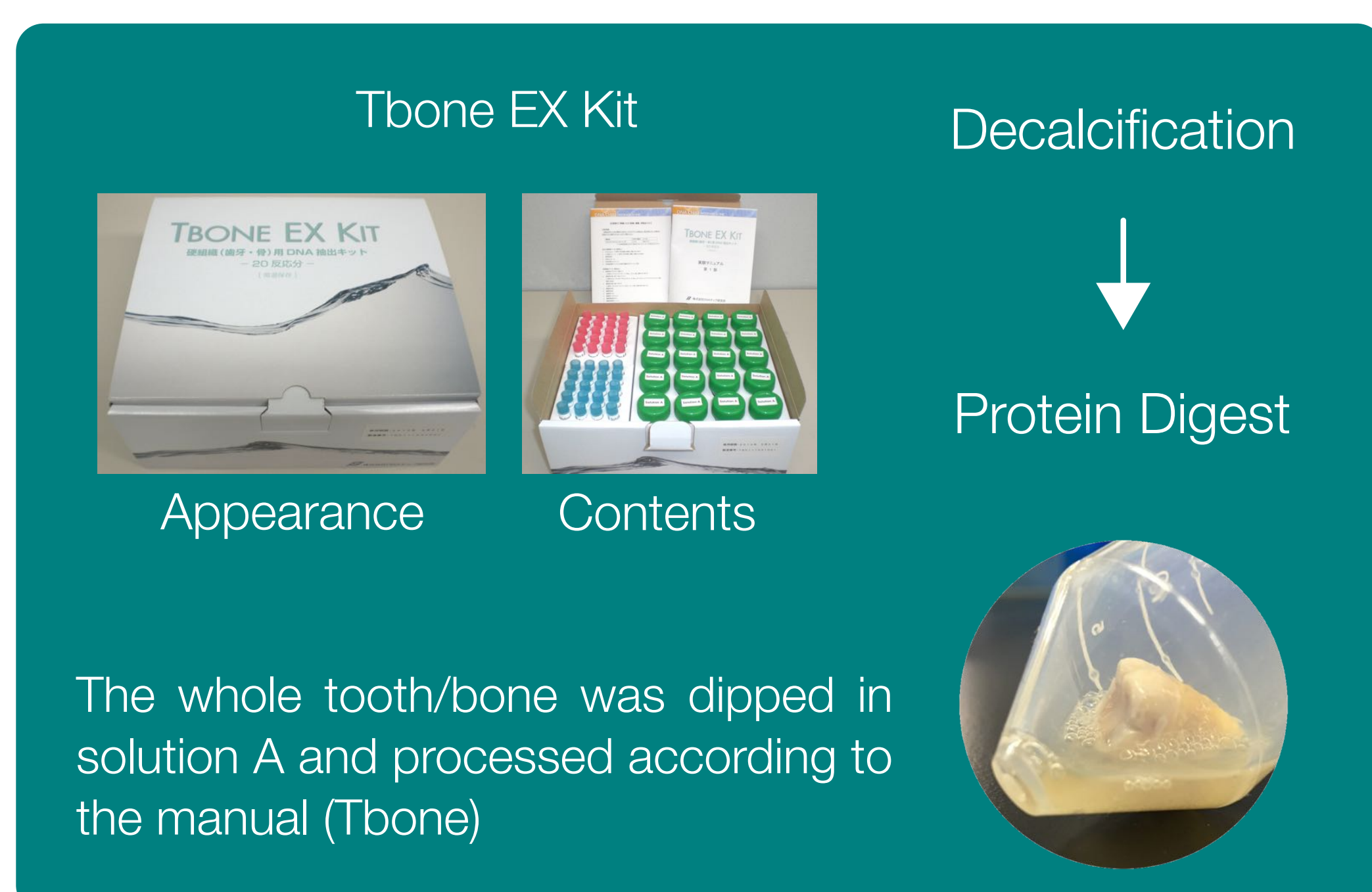
### 1. Experiment Flow

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



Phenol/Chloroform Treatment or Not



### 2. Tooth

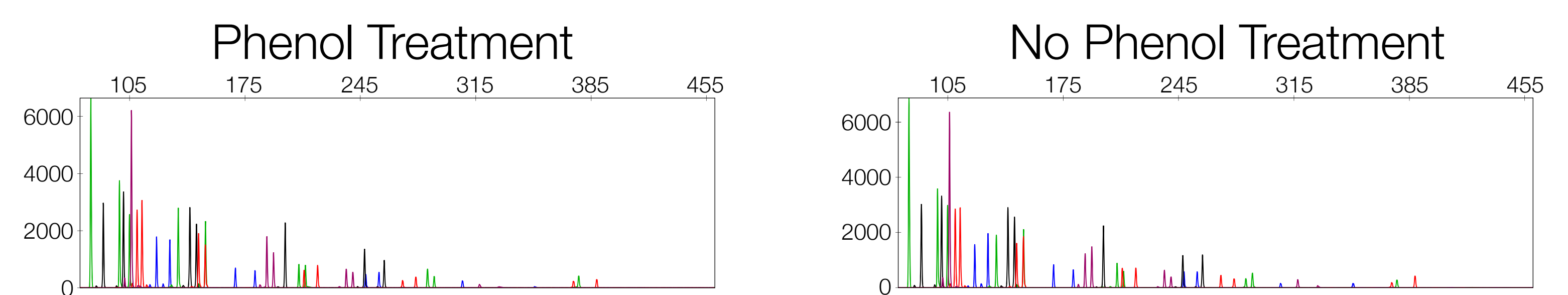


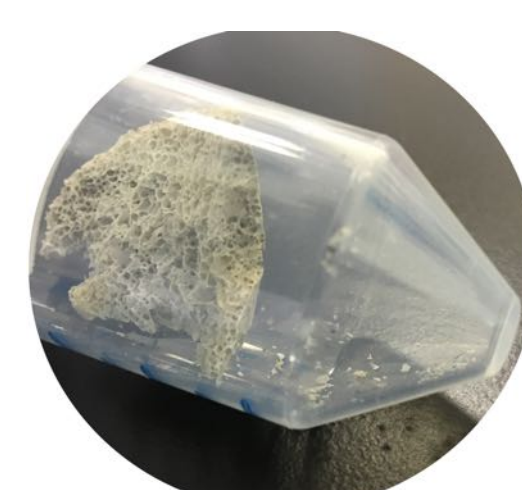
Fig. 1 STR genotyping graphs of tooth sample 2nd round extraction.

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

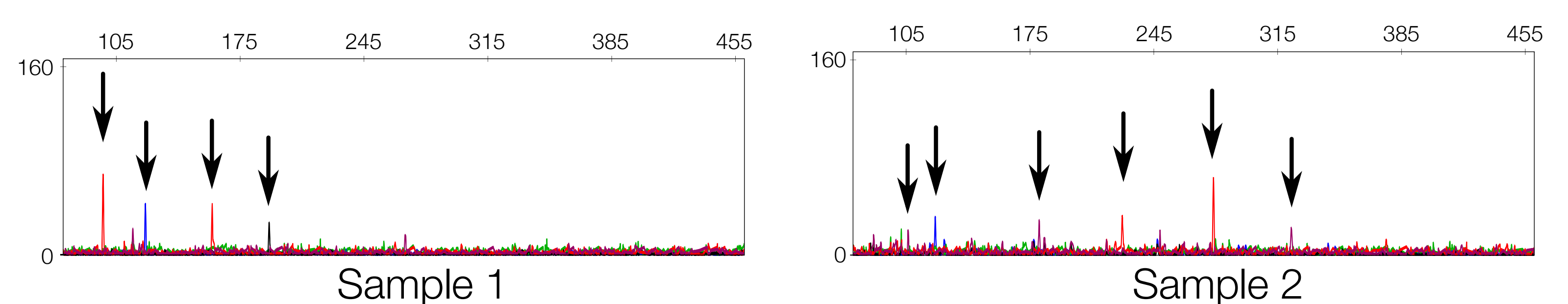


Fig. 2 STR genotyping graphs of ancient cremated bone samples.

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

- As pulverization of samples is not required, Tbone EX Kit reduced hands-on time required for extraction of DNA from bone and tooth.
- Qualities of DNA extracted is good enough for down stream forensic analysis such as STR genotyping.
- 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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