

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.

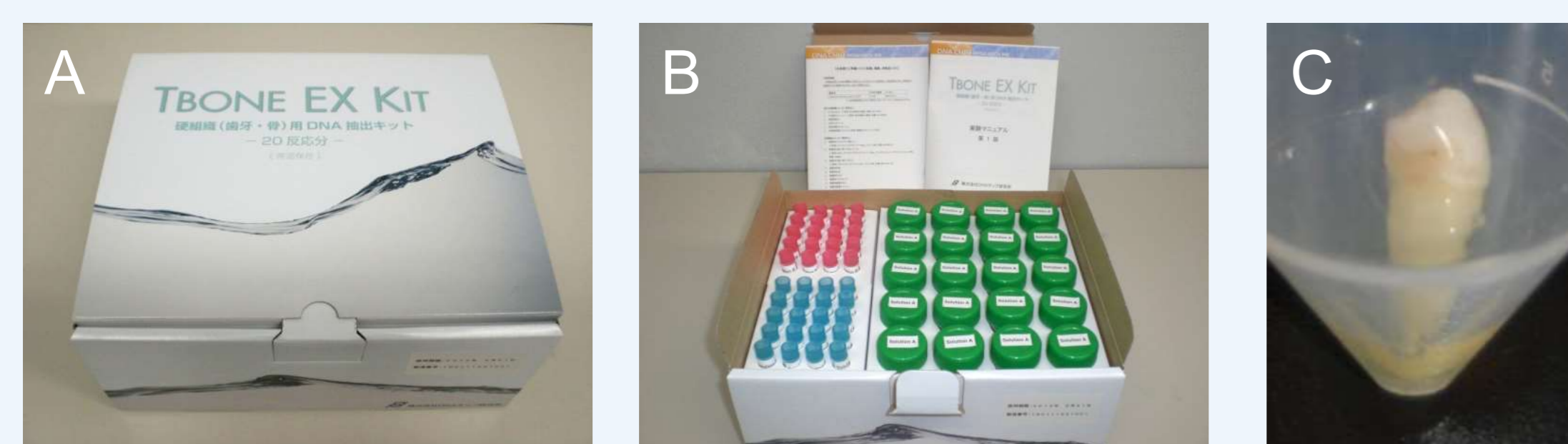


Fig. 1 Appearance of Tbone EX Kit. (A) Kit container. (B) Contents of the kit. (C) Example of DNA extraction from a whole tooth.

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

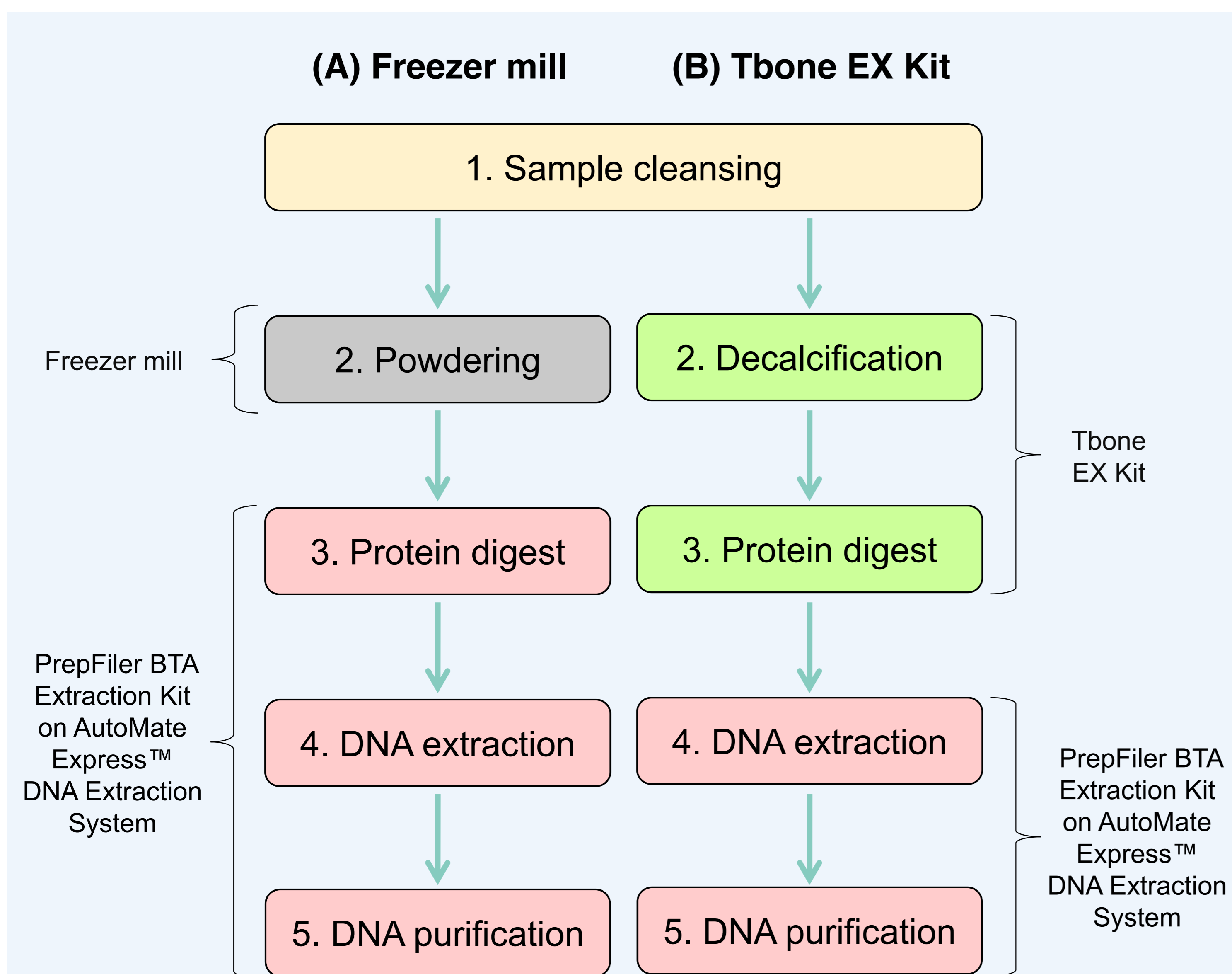


Fig. 2. Experimental flow of two sample treatment methods. (A) Powdering with freezer mill. (B) Direct incubation of bone piece with Tbone EX Kit.

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	
	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.