DNA CHIP RESEARCH INC.

TBONE EX KIT

facilitates

DNA extraction from hard tissues (teeth/bones)

- 20 Preps -

[Stable at Room Temperature]

Protocol

4th Edition (Version.3.4)

DNA Chip Research Inc.

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1. About「TBONE EX KIT」

This product facilitates DNA extraction from hard tissues such as tooth and bone.

You can extract DNA from samples of poor quality, such as corpses buried in the soil for a long period of time and victims of disaster (fire, flood, hurricane, earthquake, plane crush, etc.) to perform mitochondrial DNA sequencing and Short Tandem Repeat (STR) analysis.

With this kit, you will no longer need to grind samples into powder. You can use a whole tooth or small piece of bone as they are.

Users have to procure additional reagents and consumables for a complete extraction procedure. Please refer to [3. Equipment and Reagents to be Procured by Users]. Please contact each manufacturer if you have question about those recommended reagents.

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2. Kit Contents and Storage Condition

●TBONE EX KIT（20 preps）

【Contents】

- □ Solution A（30 mL, Green cap） : 20 tubes
- □ Solution B（1.9 mL, Red cap） : 20 tubes
- □ Solution C（600 μL, Blue cap） : 20 tubes

【Storage】

All reagents should be stored at room temperature (15℃～25℃), avoiding direct sunlight.

【Shelf life】

Please use the product before the expiration date printed on the package. Once you open the tube, please use it as soon as possible. You can store unopened tubes at room temperature.

【Safety information】

Solution A is hazardous if it comes into direct contact to one's eyes or skin. It can cause inflammation. Please refer to Material Safety Data Sheet (MSDS) included in this kit, and handle with caution.

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3. Equipment and Reagents to be Procured by Users

① Specified reagent

During DNA extraction procedure, you will need a part of the following kit or similar product.

【Product】	【Manufacturer】
QIAamp® DNA Mini and Blood Mini	QIAGEN
PrepFiler® BTA Forensic DNA Extraction Kit	Thermo Fisher Scientific

*In case the PrepFiler BTA Forensic DNA Extraction Kit is used, the step「6-4. DNA extraction」can be skipped (see also「6-4. DNA extraction」) .

② Other reagent (Manufacturer unspecified)

- □ Proteinase K
- □ TE-saturated Phenol
- □ Pure water
- □ 100% ethanol
- □ Sodium hypochlorite solution (to clean bench top)

③ Consumables (Manufacturer unspecified)

- □ Neutral detergent (kitchen dish soap)
- □ Tooth brush
- □ Dental explorer (optional)
- □ 1.5 mL microcentrifuge tube

④ Equipment (Manufacturer unspecified)

- □ Hair dryer
- □ Centrifuge
- □ Incubator

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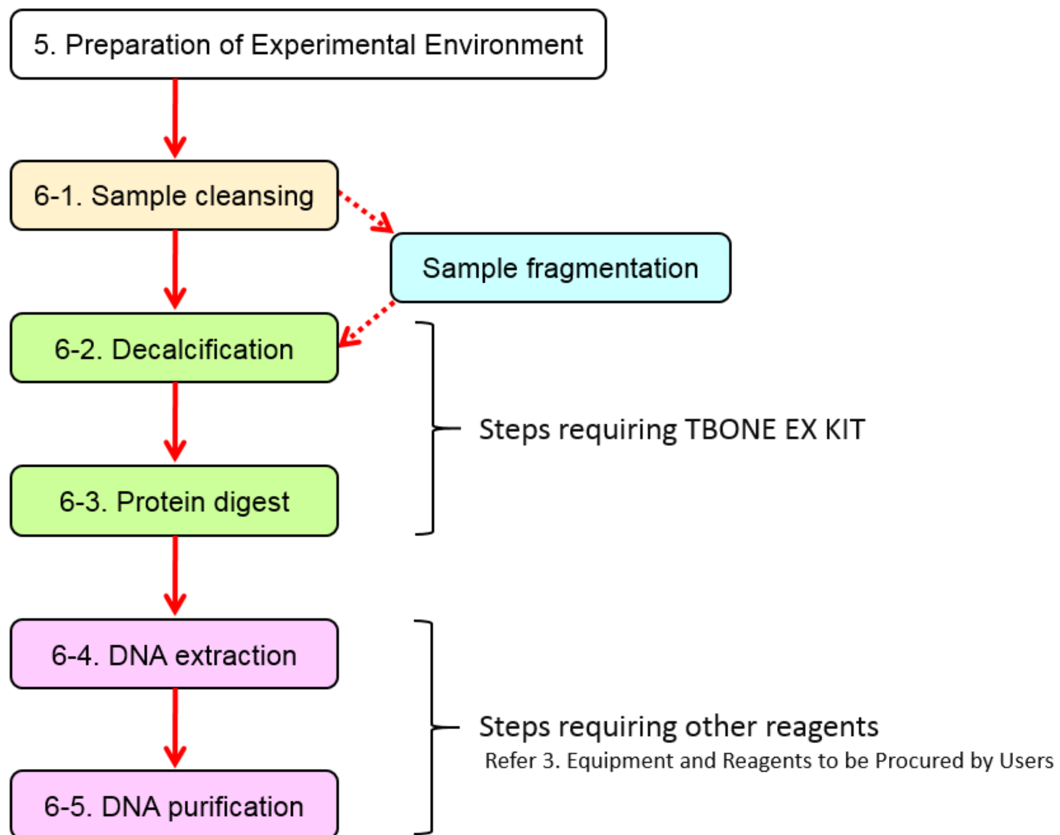
4. Workflow of DNA Extraction

The following is the overview of DNA extraction protocol using TBONE EX KIT.



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5. Preparation of Experimental Environment

① Physical separation

We recommend separating a bench for DNA extraction from that for PCR. You should avoid coming back to the extraction bench once DNA is amplified. Also, place incubator and centrifuge near appropriate bench, so that you do not need to carry around the samples.

② Outfit of workers

If contaminating DNA gets into the sample, unwanted DNA will be amplified together with your actual sample. Also, some substances can inhibit PCR reaction. For those reasons, workers should be careful not to introduce any contaminants. Wash hands and wear lab coat, gloves (change frequently), hair cap, and surgical mask.

③ Preparation of bench top

Put away any unnecessary items to make a clean working space. Wet paper towel with sodium hypochlorite solution and wipe the bench top, then wipe it away with pure water.

④ Preparation of tools and equipment

Wipe pipette, centrifuge and incubator with sodium hypochlorite solution and pure water in the same manner as ③. Wash sample cleansing tools, such as tooth brush and dental explorer, with sterilized distilled water. Tubes and pipette tips must be disposable and should not be reused.

⑤ Others

Please follow rules of individual institution and laboratory. Dispose samples and reagent according to MSDS and local regulations.

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6. Protocol for DNA Extraction from Tooth and Bone

6-1. Sample cleansing

（1） Cleaning with neutral detergent

Clean surface of the sample with diluted neutral detergent using tooth brush. Use dental explorer to remove dirt where necessary. Rinse with sterile distilled water until all the detergent is removed from the sample.

（2） Cleaning with ethanol

Rinse the sample with 100% ethanol, and place it on a sterile plastic tray. Use a hair dryer to remove moisture from the surface and inside of the sample.

If the starting material is a large piece of bone, use an electric knife to cut it into an appropriate size as long as the sample could be immersed in the following solutions「6-3. Protein Digest」as much as possible. For reference, sample could weigh about 0.5 gram or a size of about 1cm x 0.5cm x 0.5cm.

Attention ：
＊Extract DNA immediately ⇒ Proceed to「6-2. Decalcification」
＊Store for later extraction ⇒ Proceed to「（3） Storage」below.

（3） Storage

If you do not use the sample right away, keep it in a clean 50 mL tube and store at room temperature (23℃) in a dark place.

6-2. Decalcification

（1） Incubation

Place each sample into individual tubes of **Solution A,** and incubate at 23℃ for 12 hours. Mix three to four times by inversion during the incubation period.

After 12 hours of incubation, switch the temperature to 37℃. Also, place a tube of **Solution B** in the incubator to pre-warm the reagent for about 15 minutes.

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（2） Addition of **Solution B**

Add 1.8 mL of pre-warmed **Solution B** into the 50 mL sample tube, tighten the cap, and mix three to four times by inversion. Incubate at 37℃ for 2 hours. Mix three to four times by inversion during the incubation period.

Attention ： Make sure the incubator is warmed up to 37℃ before adding **Solution B**.

（3） Setting up an incubator

Take out the sample from incubator. Switch the temperature to 56℃ for「6-3. Protein digest」.

（4） Removing **Solution A** and **B**

Using disposable 50 mL serological pipette, remove solution (**A** and **B**) from the sample. Spin down remaining liquid by brief centrifugation, and completely remove the solution.

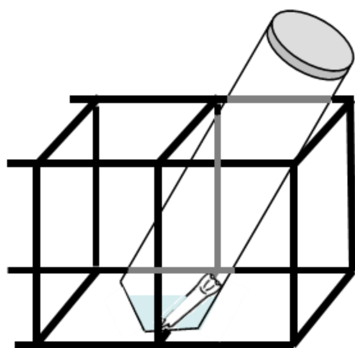
6-3. Protein digest

（1） Addition of **Solution C**

Add 400 μL of **Solution C** into the 50 mL tube from「6-2(4)」, trying not to touch wall of the tube.

（2） Addition of Proteinase K

Add 50 μL of Proteinase K（20 mg/mL）and tilt the tube to completely immerse the sample【Figure 1】. Incubate at 56℃ for 3 hours. Mix three to four times during the incubation period by circular motion, keeping the solution at bottom of the tube【Figure 2】.



【Figure 1. Tilt the tube to completely immerse the sample】

【Figure 2. Mix by circular motion】

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6-4. DNA extraction

*If you are using 'PrepFiler BTA Forensic DNA Extraction Kit「6-4」, DNA extraction (Phenol-Chloroform)」can be skipped. However, please note that it may take a longer time for DNA binding if you skip Phenol-Chloroform extraction step.

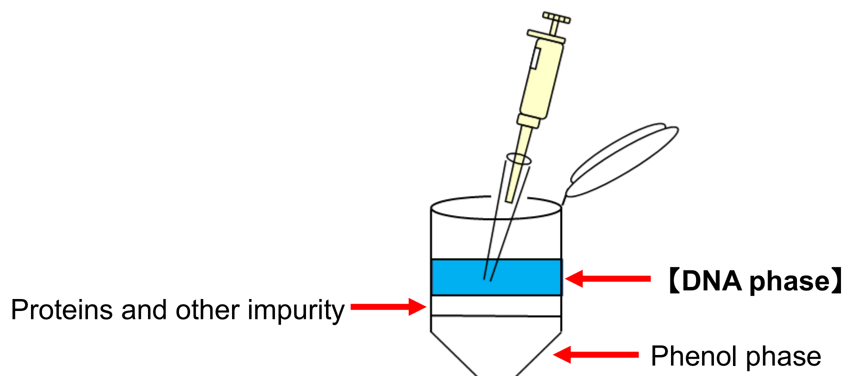
(1) Addition of TE-saturated phenol

Transfer the reaction mix from「6-3(2)」(about 500 µL) into a 1.5 mL microcentrifuge tube. Add 500 µL of TE-saturated phenol and mix by inversion 15 times.

> Attention : TE-saturated phenol is deleterious and corrosive. It is harmful if directly contacted by skin and eyes. It can cause poisoning if absorbed through skin, and depresses central nervous system. Please wear gloves and handle with caution

(2) Phase separation

Centrifuge for 5 minutes at 13,000 rpm. Transfer upper aqueous phase containing DNA (about 400 µL) into a new clean microcentrifuge tube.



【Figure 3 Phase separation and sample transfer】

> Attention : As shown in【Figure 3】, remove the upper layer slowly without touching the protein interphase. Protein and other impurities may not be visible. Be careful not to disturb the layer.

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6-5. DNA purification

＊Continue DNA purification, using one of the following DNA extraction kits, which have been tested for their compatibility to TBONE EX KIT.

【Product】	【Manufacturer】
QIAamp® DNA Mini and Blood Mini	QIAGEN
PrepFiler® BTA Forensic DNA Extraction Kit	Thermo Fisher Scientific

Alternatively, you can proceed using your usual DNA purification procedure.

＜ Example ＞

If you are using「QIAamp® DNA Blood Mini Kit」;

（1） Add 1 volume (about 400 μL) of Buffer AL (from「QIAamp® DNA Blood Mini Kit」) to the sample from「6-4（2）」.

（2） Add 1 volume (about 400 μL) of 100% ethanol, and vortex.

（3） Apply to the Mini Column, and follow wash steps of「QIAamp® DNA Blood Mini Kit」.

（4） Elute DNA from the column, and use the eluate for downstream application, such as PCR for mitochondrial DNA sequencing and STR analysis.

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7. Reference and Troubleshooting

	Question	Answer
【1】	Do you have a scientific literature for this product?	Please refer to「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.」
【2】	I see some precipitations in Solution B.	Incubate at 37℃ until it dissolves.
【3】	The solution volume in step「6-4（1）」became too much to fit into a 1.5 mL tube.	Probably the solution from decalcification step「6-2（4）」was not removed completely. You can transfer the sample into 2 mL tube and proceed with experiment, or concentrate the sample down to about 500 μL with commercial ultrafiltration column.
【4】	Can I have an electronic copy of this handbook?	Please contact our support center at the following e-mail address. dnachip-support@dna-chip.co.jp
【5】	My sample does not dissolve completely at protein digest step「6-3」.	Extent of dissolution varies depending on condition and size of the sample. Incomplete cleansing or decalcification can affect the efficiency of protein digest. Complete dissolution is not necessary to obtain DNA.
【6】	I could not extract enough DNA.	Fragmentation of the sample into small pieces helps to facilitate DNA extraction. Alternatively, you can try re-extraction of the same sample after washing away the previous solutions (Refer to the literature in【1】).

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8. Notifications

① Terms of Use

Do not re-aliquot each solution before use to avoid introduction of unspecified DNA into the reagent. Solutions were prepared by designated personnel whose genomic information has been determined.

② Copyrights

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③ Disclaimer

1. DNA Chip Research Inc. does not indemnify damages (any damages including direct or indirect damage, lost profit. etc.) caused by usage of this product.
2. The specification of the kit may be changed without any advanced notice.
3. Information last updated: December 2015.

④ Reference

QIAamp® DNA Mini and Blood Mini protocol and troubleshooting.

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DNA extraction kit for hard tissues (teeth/bones)

- 20 Preps -

Protocol

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