Bone-Teeth DNA Purification

Bones and teeth are frequently the only DNA samples for identifying human remains. The effective extraction of DNA from challenging samples such as bones and teeth is significantly more difficult than analyzing fresh tissues. Bone is a highly organized, sophisticated, and specialized connective tissue with significant calcium levels. The majority of the DNA in bone is found in osteocytes. Because hard connective tissue has a high calcium content, DNA in bones and teeth is often better conserved than in soft tissues. Enamel, dentin, cementum, and pulp tissue make up teeth. Dentine and pulp both contain DNA. Because of the extensive mineralization, selecting an efficient DNA extraction process is critical to eliminate PCR inhibitors and avoid excessive sampling quantities of minerals.

The ability to extract DNA sequence and STR data from bones and teeth exposed to various environmental conditions over time has become a powerful tool for identifying missing people and unknown remains. Due to mineralization, low amounts of endogenous DNA, environmental, bacterial, and postmortem DNA damage, as well as the existence of environment-born inhibitors that co-extract with DNA, recovering DNA data from damaged specimens can still be challenging. Despite the availability of commercial technologies and procedures, bone extraction for forensic operations offers problems. Analysts struggle with low template DNA, significant degradation, and generally suppressed genetic material, which limits STR profile development for identifying reasons. Because of the above-mentioned considerable factors, selecting an efficient DNA extraction process is critical to minimizing mineral sampling and removing polymerase chain reaction (PCR) inhibitors.

BcMag™ Bone and Teeth DNA Purification Kit are designed to extract total nucleic acids from bone and teeth samples efficiently and sequentially. The kit uses our unique proprietary magnetic beads in combination with an optimized demineralization buffer to higher yield and super quality of DNA. Purified genomic DNA has the highest integrity and can be used in various downstream applications such as qPCR, STR, etc. The procedure employs mild lysis conditions, avoiding harsh conditions such as alkaline lysis and toxic chemicals for lysing cells to maintain DNA integrity and the time-consuming cleanup of organic solvent from the sample.

Workflow (Fig.1)

Fig.1 Workflow of Bone and Teeth DNA Purification Kit

1. Add lysis buffer and proteinase K to the sample to lyse the bone and incubate at 65°C.
2. Add functional magnetic beads and vortex/pipette the beads with the sample to the DNA.
3. Wash the beads
4. Separate the beads from the sample using a magnet.
5. Elute DNA from the beads

Handling and Storage: Store the kit components according to the table below on arrival.

Products

Components	Storage	Cat #: AB-101 (50 Preps)	Cat #: AB-102 (100 Preps)
BcMag™ HO-DNA Beads	4°C	0.75 ml	1.5 ml
1x Lysis Buffer	4°C	5 ml	10 ml
1x Elution Buffer	4°C	1.5ml	3ml
Proteinase K (20mg/ml)	-20°C	10 mg	20 mg
Proteinase K Suspension Buffer	4°C	0.5 ml	1.0 ml
1 M DTT	-20°C	77 mg	154 mg

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

Notes

- DNA yield: Varies (depends on sample size and type)
- DNA size: Varies (depends on the quality of starting material
- For long-term storage, store the extracted nucleic acids at -20°C.
- Proteinase K preparation: Provide protease K as lyophilized powder and dissolve at a 20 mg/ml concentration in Proteinase K Suspension Buffer. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded.
- DTT solution preparation: Provide DTT as powder and dissolve at a concentration of 1M in d_2H_2O. For example, 77 mg dissolved in 500µl d_2H_2O. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.

A. Materials Required by the User

- 95–100% ethanol
- 80% isopropyl alcohol
- 65°C Incubator chamber
- Microcentrifuge tubes, 1.5ml
- Aerosol-resistant micropipette tip
- Magnetic rack: Based on sample volume, the user can choose one of the following magnetic Separators:
  BcMag™ separator-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat.# MS-01)
  BcMag™ separator-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat.# MS-02)
  BcMag™ separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat.# MS-03)
  BcMag™separator-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat.# MS-04)

B. Sample collection

The quality of an STR profile derived from a bone sample is determined by the bone's type, age, and environmental storage state. The quality of DNA is greatly influenced by soil and humidity conditions. The success of purifying nuclear DNA from bone is also dependent on DNA integrity.

Finding good sampling locations in bones is exceptionally problematic. When working with cremated remains, materials must be handled to reduce contaminants while maintaining adequate material for DNA extraction. Incorrect extraction handling and sample storage might result in cross-contamination of target DNA with non-target DNA. It has been proposed that bone density influences DNA yield because denser tissues give higher physical protection from harm. As a result, DNA is frequently extracted from teeth protected by strong enamel or dense, weight-bearing long bones (tibia or femur). The petrous (or petrosal) part of the temporal bone is denser than many other skeletal sites and has been demonstrated to give much more usable DNA, sometimes four to sixteen times more than teeth.

To effectively extract DNA from the calcium matrix, bone must be preprocessed by removing and discarding the bone or teeth surfaces using scalpels and forceps. The extraction process's success depends on the degree of grinding, which can be performed through physical grinding or using a low-speed drill to reduce heat buildup. The extraction procedure works best with finely ground bone because cells dispersed in the bone matrix are more accessible for lysis.

C. Purification

1. Add 10mg of pulverized bone powder into 1.5ml tubes.
2. Make bone lysis cocktail according to the instructions below, allowing for an excess of n+2 samples:

Component	100 µL reaction volume for 5 mg bone
1x Lysis Buffer	85 µL
Proteinase K (20mg/ml)	5 µL
1M DTT	10 µL
Total	100 µL

3. Add 100 µL of lysis cocktail to each 1.5ml tube containing the bone powder.
4. Mix the sample by pipetting.
5. Incubate the tubes in the Incubator chamber with a gentle shake at 65°C for 24 hours.
6. Remove the tubes from the chamber and mix by vortex or pipetting.
7. Centrifuge the tubes at 12,000 × g for 5 minutes.
8. Carefully transfer supernatant to a new 1.5ml centrifuge tube.
9. Transfer 20 µL of supernatant to a new 1.5ml centrifuge tube.
10. Add 97 µL of 80% isopropyl alcohol and mix by vortex or pipetting.
11. Add 15 µLof BcMag™ HO-DNA Beads and mix by vortex or pipetting.
    Note:
    - Vigorously shake the bottle until the magnetic beads become homogeneous before dispensing. Do not allow the beads to sit for more than 2 minutes before dispensing. Resuspend the magnetic beads every 2 minutes.
12. Incubate at room temperature for 15 minutes with gentle rotation.
13. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator. Add 200µLof 85% Ethanol and mix by pipetting 10-15 times to wash the beads. Place the tube on the magnetic separator for 1-3 minutes and remove the supernatant completely while the tube remains on the separator.
14. Repeat step (13) two times.
15. Remove the tube from the magnetic separator and let the beads air dry for 10-30 minutes to evaporate the ethanol completely.
16. Add 15µL to 30µLof 1x Elution buffer and mix by pipetting 30 times to elute the DNA from the beads. Place the tube on the magnetic separator for 1-3 minutes and transfer the supernatant to a new centrifuge tube.
17. The eluted DNA should be stored at -20°C.

D. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA Recovery	Poor starting sample material	• Use better quality of the sample. • Add more samples

E. Related Products

Products and Catalog Number
Genomic DNA and RNA Purification

Phone: (858)-909-0079) **Bioclone Inc**. Fax: (858)-909-0057

One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Bacteria DNA Purification Kit, Cat. No. AE101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ101	One-Step Fungi & Yeast DNA Purification Kit, Cat. No. AL101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	One-Step Saliva Viral RNA DNA Purification Kit, Cat. No. AR101
One-Step Touch DNA Purification Kit, Cat. No. AS101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Dandruff Cells DNA Purification Kit, Cat. No. AAA101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
Bone & Teeth DNA Purification Kit, Cat. No. AB101	Cell Free DNA Purification Kit, Cat. No. AC101
Rootless Hair DNA Purification Kit, Cat. No. AD101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101	One-Step RNA Removal Kit, Cat. No. AU101
One-Step DNA & RNA Removal Kit, Cat. No. AV101	One-Step PCR Inhibitor Removal Kit, Cat. No. AX101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step DNA & RNA Cleanup Kit, Cat. No. AH101
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
One-Step DNA Fluorescent Labeling Cleanup Kit, Cat. No. AK101	Quick Oligo DNA Conjugation Kit, Cat. No. CA101
Quick DNA & RNA Conjugation Kit, Cat. No. CA103	Pure Midiprep Plasmid DNA Purification Kit, Cat. No. AY101

F. General Reference

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3. Jakubowska J, Maciejewska A, Pawłowski R. Comparison of three methods of DNA extraction from human bones with different degrees of degradation. Int J Legal Med. 2012 Jan;126(1):173-8.
4. Emery MV, Bolhofner K, Winingear S, Oldt R, Montes M, Kanthaswamy S, Buikstra JE, Fulginiti LC, Stone AC. Reconstructing full and partial STR profiles from severely burned human remains using comparative ancient and forensic DNA extraction techniques. Forensic Sci Int Genet. 2020 May;46:102272.
5. Prediction of autosomal STR typing success in ancient and Second World War bone samples. Zupanič Pajnič I, Zupanc T, Balažic J, Geršak ŽM, Stojković O, Skadrić I, Črešnar M. Forensic Sci Int Genet. 2017 Mar;27:17-26.