Bone Extraction Protocol to be Used With the DNA IQ™ System

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Description

This protocol was developed externally in collaboration with Promega to preprocess pulverized bone samples in preparation for DNA purification using the DNA IQ™ System (Cat.# DC6700 and DC6701) (1). The resulting purified DNA can be used as a template in amplification reactions for both genomic and mitochondrial sequences. This protocol was tested externally on mass grave remains under various soil conditions, but due to the nature of this sample type (i.e., the samples may have been exposed to environmental factors for long periods of time and the amount of biological material may be limiting), DNA capable of being amplified may not be obtained from all samples.

Bone must be preprocessed to efficiently extract DNA from the calcium matrix. The success of the extraction process depends on the degree of grinding, which can be accomplished by physical grinding or with a drill operated at low speed to reduce heat buildup. The extraction process works most efficiently with finely ground bone in which cells interspersed in the bone matrix are more accessible for lysis.

The success of purifying nuclear or mitochondrial DNA from bone also depends on DNA integrity. Soil conditions and humidity have a profound effect on DNA quality. The DNA IQ™ Resin removes very small DNA fragments, which can act as PCR inhibitors, leading to improved amplification results. However, if the DNA is degraded to very small fragments no DNA will be obtained. With the DNA IQ™ System, contaminating microbial DNA will be isolated in addition to the human DNA so discarding or scrubbing the outer layer of bone is advisable, especially when examining mitochondrial DNA.

Promega offers the DNA IQ™ System, Proteinase K (V3021), DTT (V3151), DNA IQ™ Lysis Buffer (Cat.# A8261), as well as custom manufacturing of the Bone Incubation Buffer used in this protocol.

Reagents Available From Promega

- Proteinase K (Cat.# V3021)
- Incubation Buffer, Custom* (for reconstituting lyophilized Proteinase K)
- Bone Incubation Buffer*
- DNA IQ™ System (Cat.# DC6700 or DC6701, for DNA purification)
- DNA IQ™ Lysis Buffer (Cat.# A8261)
- DTT (Cat.# V3151)
- PolyATract® System 1000 Magnetic Separation Stand (Cat.# Z5410) or MagneSphere® Magnetic Technology Stand (Cat. #Z5332 or Cat.# Z5342)

*Available by custom order. Please contact Promega for additional information.

Storage Conditions: Store Proteinase K and DTT at –20°C. The Bone Incubation Buffer and Incubation Buffer can be stored at –20°C to 25°C. The SDS in the Bone Incubation Buffer may form a precipitate if stored below room temperature. Warm the Bone Incubation Buffer at 37°C to dissolve the precipitate prior to use. Store all other components at 22–25°C.

Protocol for DNA Extraction From Bone

Materials to Be Supplied by the User

- 56°C water bath
- 65°C heat block
- 95–100% ethanol
- isopropyl alcohol
- 15ml tubes
- microcentrifuge tubes, 1.5ml
- aerosol-resistant micropipette tips
- Proteinase K (Cat.# V3021)
- Incubation Buffer, Custom* (for reconstituting lyophilized Proteinase K)
- Bone Incubation Buffer*
- DNA IQ™ System (Cat.# DC6700 or DC6701)
- DNA IQ™ Lysis Buffer (Cat.# A8261)
- DTT (Cat.# V3151)
- PolyATract® System 1000 Magnetic Separation Stand (Cat.# Z5410) or MagneSphere® Magnetic Technology Stand (Cat. #Z5332 or Cat.# Z5342)

*Available by custom order. Please contact Promega for additional information.

A. Preparation of Reagents

Preparation of Stock Proteinase K Solution

1. Add 5.5ml of Incubation Buffer per 100mg Proteinase K, and gently swirl to dissolve. The final concentration of Proteinase K will be 18mg/ml.
2. Dispense the Stock Proteinase K Solution into smaller aliquots that reflect usage, and store at –20°C for up to 1 year. Each sample requires 168µl of Stock Proteinase K Solution. The Proteinase K can be frozen and thawed up to 5 times with no significant loss in activity. Prior to use, Proteinase K should be thawed and stored on ice.

Preparation of Proteinase K Digestion Solution

1. This solution should be made up fresh and used immediately. Each sample requires up to 3ml of Proteinase K Digestion Solution. Prepare the Proteinase K Digestion Solution by combining the Bone Incubation Buffer and Stock Proteinase K Solution in the proportions indicated below.

Bone Incubation Buffer	944µl
Stock Proteinase K Solution	56µl
Total Volume	1000µl

2. Mix gently, and store at room temperature prior to use.

Preparation of 1M DTT

1. Dissolve 5g of powdered DTT in nuclease-free water so that the final volume is 32.4ml.
2. Dispense into aliquots that reflect usage, and freeze at –20°C.

Preparation of Lysis Buffer

1. Determine the total amount of Lysis Buffer to be used (see Step 4 of Section B below).
2. Add 1µl of 1M DTT per 100µl of Lysis Buffer.

Preparation of 1X DNA IQ™ Wash Buffer

1. For the DNA IQ™ System (Cat.# DC6700: 400 samples) add 35ml of 95–100% ethanol and 35ml of isopropyl alcohol to the 2X Wash Buffer.

   For the DNA IQ™ System (Cat.# DC6701: 100 samples) add 15ml of 95–100% ethanol and 15ml of isopropyl alcohol to the 2X Wash Buffer.
2. Replace cap, and mix by inverting several times. Mark label to record the addition of alcohols. Solution can be stored at room temperature. Make sure bottle is closed tightly to prevent evaporation.

B. DNA Extraction from Bone

1. Pulverize up to 2g bone, observing appropriate precautions to avoid contamination and excess heat. The amount of bone used initially will depend on the quality of bone sample. Avoid the outer surface of bone, especially if mitochondrial DNA is being examined, to reduce contamination.
2. Place the bone in up to 3ml of freshly prepared Proteinase K Digestion Solution in a 15ml tube, and incubate at 56°C for 1 hour (see Notes 1–2).

   Notes:

   1. Coarsely ground bone may require longer incubation times.
   2. Add Proteinase K Digestion Solution to cover the entire sample.
3. Remove the remaining bone by centrifugation at 5000rpm for 5 minutes. Transfer solution to a new 15ml tube.
4. Add 2 volumes of DNA IQ™ Lysis Buffer to the solution.

5. Vortex the DNA IQ™ Resin bottle for 10 seconds at high speed or until resin is thoroughly resuspended. Add 15µl of resin to the sample. Keep the resin resuspended in the stock bottle while dispensing to obtain uniform results.
6. Vortex the sample/Lysis Buffer/resin mixture for 5 seconds at high speed.
7. Incubate at room temperature for 10 minutes, mixing one to three times by inverting the tube.
8. Vortex for 5 seconds at high speed, and place the tube in the magnetic stand. Carefully remove the solution. If resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place back in the stand.

   Note: If the PolyATtract® System Magnetic Separation Stand is not available for 15ml tubes, a MagneSphere® Technology Magnetic Separation Stand for 1.5ml tubes (Cat.# Z5332 or Z5342) can be used. To use one of the MagneSphere® Technology Magnetic Separation Stands, vortex solution as described in Step 8. Place up to 1.5ml sample/Lysis Buffer/resin mixture in a 1.5ml tube, and place the tube in the magnetic stand. After the resin has collected at the side of the tube, carefully remove and discard the solution without disturbing the resin. Add more sample/Lysis Buffer/resin mixture. Repeat until all resin is captured and all of the solution has been removed.
9. Add 100µl of Lysis Buffer, vortex for 2 seconds at high speed. Carefully transfer the mixture to a 1.5ml tube, making sure all resin is transferred.
10. Vortex for 2 seconds at high speed, place the tube in the magnetic stand, and carefully remove and discard the solution.
11. Add 100µl of prepared 1X Wash Buffer (see *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin* #TB296). Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.
12. Return tube to the magnetic stand. Dispose of all Wash Buffer.
13. Repeat Steps 11 and 12 two more times for a total of 3 washes, making sure all of the solution is removed after the last wash.
14. With the tubes in the magnetic stand and the lids open, air-dry the resin for 5 minutes.

    Note: Do not dry for more than 20 minutes, as this may inhibit removal of DNA from the resin.
15. Add 25–100µl of Elution Buffer, depending on how much biological material was used. Lower elution volume ensures a higher final concentration of DNA.
16. Close the lid, and vortex tube for 2 seconds at high speed. Place at 65°C for 5 minutes.
17. Remove tube from the heat source, and vortex for 2 seconds at high speed. Immediately place in the magnetic stand.
18. Transfer the solution to a container of choice.

    Note: The DNA solution can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

Reference

1. Primorac, D., personal communication.