

DNA Analyst Training Laboratory Training Manual

Protocol 3.04

DNA Isolation: Isolation of DNA from Bone



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

Refer to the General Information on DNA Isolation section.

SAFETY CONSIDERATION

Refer to the General Information on DNA Isolation section.

PREPARATION

Refer to the General Information on DNA Isolation section.

INSTRUMENTATION

Refer to the General Information on DNA Isolation section.

MINIMUM STANDARDS AND CONTROLS

Refer to the General Information on DNA Isolation section.

PROCEDURE OR ANALYSIS

Bone which has been submerged or in the environment for a long period of time may not yield DNA which is amenable to nuclear DNA testing. Therefore, ask for entire bone samples (i.e., ribs or femurs). Request that the medical examiner does NOT saw on the bone.

Note: If soft tissue is present, extract 2-5 mm of it. If a DNA profile is not obtained, extract the bone.

1. Clean the bone and remove all of the flesh. Soak the bone in 10% bleach for 1-2 minutes. Rinse in sterile water and dry the bone thoroughly. Using sandpaper or a dremel tool, remove the outer layer of tissue. Avoid the sawed ends of the bone since these may be contaminated. If the sample is degraded, avoid the marrow as it will produce less high molecular weight DNA.
2. Obtain up to 15 grams of bone dust.
3. Place approximately 1 g of bone dust in a 15 ml conical bottom tube or 3 g in a 50 ml conical bottom tube.

4. Optional bone decalcification:
 - A. Fill the tube to the top with 500 mM EDTA pH 8.0. Vortex. Incubate the tube with rocking for 24 hr at 4EC. Spin the tube for 15 minutes at 2000 x g. Discard the supernatant. Repeat this process for a total of five times.
 - B. Fill the tube to the top with sterile ddi H₂O. Vortex. Spin 15 minutes at 2000 x g. Repeat this process for a total of three times.
5. Add a volume of Stain Extraction Buffer, proteinase K (20Fg/FI) and 390 mM DTT (in a ratio of 400/10/5) that is twice the volume of the bone dust. Incubate overnight at 56EC. Rock periodically until the pellet dissolves.
6. Extract the suspension three times with phenol/chloroform/isoamyl alcohol (PCI). Repeat until the aqueous phase becomes relatively clear (more than three extractions may be required). Follow with a chloroform/isoamyl extraction. Note: If the solution is not clear the filter may become plugged.
7. Remove the DNA from the aqueous phase using Centricon 100 filters. Rinse the filters twice with TE-4 before collecting DNA.

Note: Since decayed or buried bones may contain large amounts of non-human DNA, quantitation of total human DNA is required.

[Return to Laboratory Training Manual User Guide](#)