Cryogenic Grinding of Teeth and Bones for DNA Extraction

Reference:

Sweet DJ, Hildebrand DP. *Recovery of DNA from human teeth by cryogenic grinding.* J Forensic Sci 1998;43(6):1199-1202.

Supplies:

- SPEX™ Freezer Mill Model 6750 and polycarbonate tube assembly
- Liquid Nitrogen 2L
- Sterile weigh boat and sterile 15 mL conical tube

Technique:

1. *Remove soil or debris:* Place tooth in 50 mL conical tube; add 25 mL filtered, distilled H_20; replace cap; sonicate for 1 min; remove water to waste; repeat until gross soil or debris is no longer visible.

2. *Decontaminate:* Wipe tooth thoroughly with a sterile Kimwipe™ moistened with 10% bleach; woipe with sterile Kimwipe™ moistened with 95% EtOH; air dry under UV light exposing each side for 10 min.

3. *Documentation:* Place tooth in sterile weigh boat and record weight; photograph tooth from all directions; expose radiograph using traditional intraoral radiography angles and exposure factors.

4. *Cryogenic Preparation:* Insert sterile anvil into one end of sterile polycarbonate tube; insert tooth (root down) into tube; insert sterile impactor; seal tube by inserting second sterile anvil; load assembled vial into freezer mill.

5. Fill freezer mill with liquid nitrogen; allow instrument to cool 5 min; top-up with more LN2; start grinding protocol: a) 2 minutes at 6 impacts/sec, b) wait 30 sec (cooling time), c) repeat once (total= 2 cycles). Remove vial from freezer mill.

6. Remove one anvil with tool provided; pour powder into same sterile weigh boat; scrape remaining powder from tube; transfer powder from weight boat to sterile 15 mL conical tube using sterile spatula. *Caution*: static may be present.

7. Label and photograph conical tube containing powdered sample. Calculate and record weight of powder.

8. Add 3 mL extraction buffer and 100 □L proteinase K (20 mg/mL) to conical tube; mix gently but thoroughly without producing froth; incubate overnight at 56˚C with shaking.

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February 2003