IMPROVED ANALYSIS OF BONE SAMPLES BY PERFORMING SAMPLE TYPE-SPECIFIC SENSITIVITY STUDIES

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Validation studies are routinely performed by operational laboratories using extracted DNA from buccal or blood samples. The emphasis of these studies has been placed on the characterization of the selected markers and routine use of commercially available chemistry kits, due to requirements and recommendations from regulatory bodies. Challenged samples, such as DNA extracted from skeletal remains, may be included in the testing of non-probative sample types; however, these sample types have not previously been explored as a source of DNA for sensitivity studies. In order to determine the effect of sample type selection on analytical parameters, such as limit of detection (LOD) and recommended input quantity of DNA, a sensitivity study was performed on DNA extracted from bone samples.

The right femur of an unembalmed human cadaver was obtained from the Willed Body Program at UNT Health Science Center. The cut bone was cleaned and pulverized using a 6770 SPEX Freezer/Mill (Sample Prep, Metuchen, NJ) according to validated procedures. Extraction was performed using a modification of the method described by Dukes et al., which replaces 20 mg/mL of Proteinase K with 50 mg/mL of Collagenase Type II for sample digestion, shown to improve STR profile quality by Barrett. DNA purification was performed using the large volume protocol on an EZ1® Advanced XL instrument (Qiagen, Hilden, Germany). DNA was quantified using the Investigator® Quantiplex HYres Kit (Qiagen). The initial sample had an estimated quantity of 10.9 ng/μL of human DNA, which was serially diluted to create a sensitivity series. The sensitivity series (in ng/μL) was: 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0. This series was amplified in triplicate using the Investigator® 24plex QS Kit (Qiagen) and fragment analysis was conducted on an Applied Biosystems 3500xL Genetic Analyzer (Thermo Fisher Scientific, Carlsbad, CA). Data analysis was conducted using GeneMapper® ID-X v.1.4 (Thermo Fisher Scientific) and Excel 2013 (Microsoft Corp., Redmond, WA).

The linear response range for allele peak heights was from 0.25 to 5.0 ng/μL, and the optimal input template amount was 1.0 ng in a 25 μL reaction. Using the instrumental noise peaks ≥1 RFU for these samples, the LOD was calculated for each dye channel: 50 RFU for 6-FAM™ (blue), 136 RFU for BTG (green), 123 RFU for BTY (yellow), 98 RFU for BTR2 (red), and 186 RFU for BTP (purple). Using bone-specific template amounts and analytical parameters, improved profiles were obtained versus those using parameters derived from blood and buccal samples.

References:

1. Dukes MJ, Williams Al, Massey CM, Wojtkiewicz PW. Technical note: Bone DNA extraction and purification using silica-coated paramagnetic beads. American Journal of Physical Anthropology. 2012; 148(3): 473-482.
2. Barrett, L.C., " Effect of Collagenase Type 2 and Proteinase K Digestion on DNA Yield from Bone Samples Purified on the EZ1 Advanced XL" Fort Worth, TX: University of North Texas Health Science Center; (2015).