Optimization, Validation, and Automation of DNA Extraction from Bones and Teeth using the DNA IQ™ System and the BioMek® 2000 Automation Workstation

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Bone and tooth samples represent a small percentage of cases in forensic science, yet those types of samples can be encountered in large numbers as the result of mass disasters. The attack on the World Trade Center focused research into streamlining the processing of thousands of skeletonized remains. The project reported here explored the implementation of the DNA IQ™ system on bone and tooth samples for use in a robotic extraction system.

Methods of pre-cleaning, obtaining pulverized sample, and DNA purification were evaluated. The first of these steps typically has employed sanding, but more recently the use of bleach cleaning or proteinase K digestion has been described. The three main considerations of this step was the ease of use, effectiveness in removing exogenous DNA, and the effect on typing results. This research evaluated the before and after results of three chemical pretreatments—ethanol, bleach followed by ethanol, and a proteinase K digest—in terms of the ability to remove DNA, ease of use, and ensuing results from the bone/tooth sample.

The second of these steps exploited methods already in place for liberating the encapsulated DNA from its vessel, but integrated those methods developed for use with organic DNA purification with the DNA IQ™ System of DNA purification. Tooth samples were pulverized using a hammer and the bone drilling approach utilized that was developed by The Bode Technology Group in response to the World Trade Center bombings.

The third step of automating bone and tooth extractions required that not only the proteinase K containing buffers used be compatible with the DNA IQ™ System, but also that the robotic method could successfully accommodate the large digestion volumes employed. The current laboratory protocol digested teeth and bones in a proteinase K digest using Stain Extraction buffer overnight (10mM Tris pH 8, 10mM EDTA, 10mM NaCl, 2% SDS). This buffer was known to be incompatible with the DNA IQ™ system since the Guanidium Thiocyanate in the DNA IQ™ Lysis buffer would precipitate out of solution. Thus, a buffer compatible with the DNA IQ™ System needed to be created. The buffer concocted—called *buffer A* (50mM Tris pH 8, 50mM EDTA, 100mM NaCl, 2% Sarkosyl) —produced similar yields and STR typable results comparable to the stain extraction buffer. Results were evaluated by comparing the DNA IQ™ extraction to a standard phenol/chloroform extraction with Microcon purification. The DNA IQ™ protocol for bone samples does offer a Bone extraction buffer (10mM Tris pH 8.0, 50mM EDTA, 100mM NaCl, 0.5% SDS) in conjunction with an extraction method, but we opted to use an alternate buffer and method that was readily available and easily integrated into current operations. The main differences between our method and the DNA IQ™ bone protocol was the composition of digest buffer, amount of digest buffer used, and length of digest. The method was otherwise the same, but implemented on the robotic system.

Teeth and bone samples were obtained from the Medical College of Virginia dental clinic and the Office of the Chief Medical Examiner for the state of Virginia. Thus, samples that mimicked casework and true casework samples were utilized. A newly developed large volume method (LV method) accommodates samples which require a large (500 µL or greater) Proteinase K digestion buffer for optimal performance, either because the sample is diffuse or problematic for use the the DNA IQ™ Lysis buffer alone. This large digestion volume is necessary for bone and tooth samples, therefore the automated extraction procedure utilized the LV robotic method.

The preliminary results indicated that the buffer systems produce comparable yields and that the DNA IQ™ System performs as well as the organic extraction, if not better. The advantage of the DNA IQ™ System is the ability to rinse away potential PCR inhibitors and the luxury of robotic extraction for typical casework or the high-throughput associated with mass disasters. Preliminary data suggest the use of the bleach pre-cleaning process is easiest to perform and produces equivalent, if not superior removal of exogenous DNA to that of the Proteinase K digest or Ethanol wash.