AN INNOVATIVE METHOD FOR EXTRACTION OF DNA FROM CALCIFIED TISSUES AND BIOLOGICAL MATERIALS

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Isolation of DNA from forensic samples can be challenging because of variability in sample types, exposure to environmental insults, contamination by PCR inhibitors and limited quantities of starting material. The result of these complications is a bottleneck in the sample processing workflow. We developed the PrepFiler™ Forensic DNA Extraction Kit to enable high DNA recovery and removal of PCR inhibitors from various sample types. The method employs a proprietary multi-component surface chemistry that efficiently extracts highly purified genomic DNA from most forensic evidence samples. However, we have found that bone, tooth, and some other sample types present a challenge to the standard PrepFiler™ protocol, where DNA recovery is often less than expected. To improve DNA yield from these traditionally difficult sample types, we developed a new lysis method that is fully compatible with PrepFiler™ protocols.

We describe a lysis reagent that significantly improves the yield of DNA from calcified tissues like bone and tooth, and is designed for use with the PrepFiler™ Kit reagents. Simultaneous decalcification and lysis of the biological material embedded in calcified samples is essential for optimal DNA recovery. The Bone and Tooth Lysis Reagent (B&T) was developed to disrupt calcified tissue matrices and achieve effective extraction of nucleic acids from pulverized bone and tooth samples. Further we discovered that the B&T lysis reagent works well to mildly but efficiently extract DNA from other challenging substrates including tape, chewing gum, and cigarette butts. After lysing with the B&T reagent, the DNA is processed using established PrepFiler™ protocols.

We successfully extracted DNA from several powdered human bone samples, as well as different brands of chewed gum, tape lifts and smoked cigarettes using B&T lysis reagent and the PrepFiler™ Kit. The yields of DNA from the bone samples and multiple domestic and international brands of chewing gum and cigarettes tested were consistent and reproducible. This extraction method efficiently removed potentially contaminating PCR inhibitors from the samples tested, and C_T values for the internal PCR control (IPC) supplied with the Quantifiler® Human DNA Quantification Kit were consistent and in the normal range. Indeed, the DNA extracted from these samples provided conclusive profiles that were free of PCR artifacts when amplified using the AmpFℓSTR® Identifiler® PCR Amplification Kit.