

EVALUATION OF PROMEGA'S BONE DEMINERALIZATION BUFFER FOR PROCESSING DEGRADED FEMUR SAMPLES

BG Gallardo, DLT Apaga, MCA De Ungria and GC Calacal

DNA Analysis Laboratory, Natural Sciences Research Institute, Miranda Hall, University of the Philippines, Diliman

DNA analysis of bones poses a significant challenge to every forensic laboratory involved in human identification. Varying success in the recovery of DNA suitable for aSTR typing is due to low levels of endogenous DNA, post mortem DNA damage and the presence of inhibitors that can be co-extracted with DNA. Hot and humid weather condition prevalent in tropical countries like the Philippines could promote rapid tissue decomposition and DNA degradation.

DNA extraction is the most critical step that could determine if downstream analysis will be successful, especially when dealing with bone samples that are limited and compromised. Hence, we compared the efficiency of a beta test version of Promega's bone demineralization buffer vs. their current bone incubation buffer combined with a manual DNA IQ™ protocol, and with our standard organic procedure. Moreover, we reduced the starting bone powder from 200 mg to 25 mg to determine effectiveness of the demineralization process following the manual DNA IQ™ workflow. Both pre-processing bone incubation buffers were likewise evaluated using an automated DNA IQ™ method with the Maxwell® 16 LEV System. Femur samples (n=16) were obtained from recently deceased and exhumed human remains ~1 month post-mortem. DNA yield and quality were assessed by real time PCR using PowerQuant® and amplified in a PowerPlex® Fusion multiplex reaction.

There is no significant difference in DNA quantity using the standard organic and demineralization/DNA IQ™ method. However, better allele recovery was observed in majority of the demineralized bone extracts analyzed. Furthermore, a notable improvement in the quantity and quality of recovered and amplified DNA was observed using the demineralization buffer as compared to the current commercially available bone incubation buffer of Promega in both manual and automated DNA extraction format.

Results also showed that the demineralization/DNA IQ™ system is capable of isolating amplifiable DNA from a 25 mg starting bone material, generating full to partial (>70%) DNA profiles even with suboptimal DNA template (<500 pg) used in amplification. Finally, the automated process resulted to a four-fold increase in DNA quantity with efficient removal of PCR inhibitors and comparable amplification efficiency. Generally, this simplifies the bone extraction process and increases laboratory throughput.