

ASSESSING PROMEGA DNA IQ™ SYSTEM AND INVITROGEN CHARGESWITCH® KIT TECHNOLOGY FOR BONE AND TISSUE EXTRACTIONS

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The Armed Forces DNA Identification Laboratory (AFDIL) supports the Armed Forces Medical Examiner System by performing DNA identification of deceased military personnel from Operation Iraqi Freedom. Autopsy specimens, typically tissue and bone samples, are submitted to our laboratory for DNA testing. Nuclear DNA profiles are generated and compared with reference DNA profiles from known bloodstain cards maintained by the Armed Forces Repository of Specimen Samples for the Identification of Remains. Scientific identification via fingerprint and/or dental records may not be possible depending on the condition of the remains. In these cases, DNA is the primary method of scientific identification. DNA testing is also used to re-associate fragmented remains. When DNA testing is the sole method of identification and/or re-association, it is essential to expedite the laboratory processes in order to identify the individual and return the remains home in hopes to provide a sense of closure to the family members. Currently, AFDIL scientists use organic extraction methods to isolate DNA from challenging tissue and bone specimens. Due to the increase in work load with the ongoing war efforts and the rapid turn-around time required for a primary DNA identification, AFDIL is in the process of assessing the Promega DNA IQ™ System and the Invitrogen ChargeSwitch® Kit technology in order to increase efficiency in extraction procedures. Benefits of these kits over organic extraction methods include avoidance of harsh chemicals, less sample transfers, and availability to use with automated liquid handling systems. Promega's DNA IQ™ System uses paramagnetic resin for DNA isolation. Cells are lysed and the DNA is allowed to bind to the resin. The samples are then placed on a magnetic stand to pull the resin away from the liquid, and a series of wash steps is performed to remove inhibitors. The purified DNA is released from the resin with the addition of Elution Buffer. Invitrogen ChargeSwitch® Kit utilizes a covalent chemical linkage that binds and releases DNA by changing the pH of a solution. After cell lysis, DNA binds to the ChargeSwitch® magnetic beads by lowering the pH to <6.5. Contaminants are removed through wash steps, and the DNA is eluted from the beads by raising the pH to 8.5. Sets of autopsy tissue and bone specimens will be extracted with both the Promega DNA IQ™ System and the Invitrogen ChargeSwitch® Kit protocols. Samples will be quantitated using the Applied Biosystems Quantifiler™ Human DNA Quantification Kit and assessed for yields and presence of inhibitors. One nanogram of extracted DNA will be amplified using both the Promega PowerPlex® 16 and the Applied Biosystems AmpFLSTR® Identifiler® PCR amplification kits. Sensitivity and reproducibility, as well as quality of the data generated from both kits as compared to organic extraction procedures will be evaluated. Results will be presented at the meeting. The opinions and assertions expressed herein are solely those of the authors and are not to be construed as official or as the views of the U.S. Department of Defense or the U.S. Department of the Army.