

USE OF MAGNETIC BEADS IN THE AUTOMATION OF SEQUENCE EXTENSION PRODUCT PURIFICATION

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The Armed Forces DNA Identification Laboratory (AFDIL) performs high throughput sequencing of mitochondrial DNA from family reference samples, for comparison to case samples from missing military service personnel from the Vietnam War, Korean War, and World War II. The method currently in use for removal of unincorporated dye terminators after cycle sequencing involves 96 well purification blocks (Edge Biosystems) that require multiple manual centrifugation and plate transfer steps. AFDIL is presently converting the sequencing system to a robotically automated platform, and we have therefore evaluated methods for post-cycle sequencing clean up that do not require steps such as centrifugation which require manual intervention and/or transfer off the robotic platform. We report here evaluation and incorporation of a protocol for purification that uses magnetic DNA-affinity beads. CleanSeq[®] kits (Agencourt Bioscience Corporation, Beverly, MA) contain solid-phase paramagnetic beads that selectively bind to sequencing extension products. Unincorporated dyes, nucleotides, salts, and other contaminants are subsequently washed away by 85% EtOH rinses while the beads are pulled to the sides of each well while placed in a magnetic field. Eluted product can be directly loaded on sequencing instruments.

The conditions of the CleanSeq[®] purification were first optimized utilizing two 85% EtOH washes instead of one to remove additional G-Blob stretches found at the beginning of the sequence data reported on the ABI PRISM[®] 3100 Automated Sequencer. The elution buffer was optimized to a final concentration of 0.2mM EDTA to eliminate the overloaded capillary as seen with samples eluted in dH₂O. Sequence data provided excellent peak discrimination into the 400bp range. The magnetic bead protocol was then programmed into the Tecan Genesis 200 Workstation and optimized there. The final four-hour procedure allows for seven 96 well cycle sequencing plates to be completely purified with no manual intervention.