

VALIDATION AND STABILITY STUDY OF EXOSAP-IT® POST-PCR PURIFIED CASEWORK MTDNA SAMPLES AT THE ARMED FORCES DNA IDENTIFICATION LABORATORY

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Low copy number mitochondrial DNA analysis is used for degraded or difficult remains when the quality of DNA available is insufficient for nuclear analysis. Efficient post-PCR product purification is necessary to obtain high quality sequencing results and is currently accomplished at AFDIL using Centricon® 100 centrifugal filter columns. However, due to the increased sensitivity of ABI-3130®, it has been observed that the Centricon® 100 columns have been leaving excess amplification primers in the retentate, which leads to the presence of underlying opposite (forward or reverse) strand sequence in sequenced samples. In order to increase the efficiency of purification, reduce the amount of time, labor and sample manipulation involved, an alternative strategy of enzymatic purification using ExoSAP-IT® was investigated.

This study utilized AFDIL's high quality positive control DNA (DAL) as well as highly degraded non-probative casework amplicons to assess the quality of sequence product and stability of ExoSAP-IT® purified samples stored at 4°C for up to 6 months. The positive control DNA was amplified in duplicate at 1, 10 and 100pg and purified using either 1.5µl of ExoSAP-IT® for 15minutes or Centricon® 100 filter units. The sensitivity sequencing results demonstrated the need to either extend the ExoSAP-IT® incubation time or increase the amount of ExoSAP-IT® used to facilitate the complete digestion of unused primer. For this study, the incubation time was extended to 30-minutes to reduce cost and prevent glycerol inhibition. The extended incubation removed all excess primer from the sensitivity and casework samples, which differed from their concordant Centricon® 100 samples that exhibited underlying opposite strand sequence. Furthermore, the ExoSAP-IT® purified samples demonstrated no loss of sequence quality after 6 months of storage at 4°C.

Additional experiments involved identifying 16 previously Centricon® 100 purified casework amplicons where the appearance of the minor sequence component peaks on the electropherogram were greater than one quarter of the major sequence peaks, which resulted in the data not being used. These products were resequenced and analyzed on the ABI-3130®. The results were consistent with the original casework sequencing: 5 out of 32 products had no background while the remaining 27 still displayed underlying sequences. After ExoSAP-IT® purification, all 27 sequencing products resulted in high quality data absent of underlying sequence. The adaptation of ExoSAP-IT® for use with casework grade samples shows considerable benefit in streamlining the purification process by reducing the effort and time spent by the analyst doing sample manipulation, as well as reducing laboratory costs.