IMPLEMENTATION OF A 96-WELL HIGH-THROUGHPUT METHOD FOR MITOCHONDRIAL DNA DATABASING

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The analysis of the mitochondrial DNA (mtDNA) of thousands of bloodstains is necessary for the establishment of a family reference database to support the mission of the identification of missing soldiers. Hypervariable regions 1 and 2 (HV1 and HV2) of the mtDNA provide substantial information to allow the discrimination of maternal lineages. In order to facilitate the efficient sequencing of those regions, a 96-well method of processing bloodstains was implemented.

Currently, the method is implemented at the stage of amplified product purification. The amplified product is purified by using a combination of a Solid Phase Oligo/Protein Elimination (SOPE) resin and a 96-well gel filtration block supplied in the Edge Quickstep™ 96 Well PCR Purification Kit. The SOPE resin binds excess primers, single-stranded DNA, enzymes, and other proteins. The gel filtration block, which contains 96 gel matrix columns, removes salts, buffers, dNTPs, and other small molecules.[1] The recovered purified product is then cycle sequenced in a 96-well microtitre plate. Multichannel pipettes are used throughout the procedure to ensure the rapid and accurate transfer of products. Following cycle sequencing, the product is purified using the 96-well gel filtration block. Samples are then loaded onto 64-lane acrylamide gels, using an 8-channel loader.

Refinements of the basic method have substantially improved productivity and the quality of results. The amount of DNA template used during both amplification and cycle sequencing was increased from 2 µl to 4 µl. During amplified product purification, the SOPE resin must be resuspended frequently to prevent precipitation. When spinning product through the gel filtration block, unequal amounts of product may be recovered throughout the block. The block's orientation in the centrifuge carriage can be reversed and the block spun a second time to obtain consistent amounts of product. At the cycle sequencing stage of processing, caps should securely fit the tubes to prevent evaporation of the sample. Caps that fit easily on the tubes decrease the amount of time that the enzyme is in contact with reagents prior to being placed in the thermal cycler. When loading gels, the amount of loading buffer in which the DNA is resuspended is lowered to increase the concentration of DNA being loaded. The multichannel gel loader is rinsed with distilled water between each load. No evidence of cross contamination has been observed. Buffer should not be used to rinse the loader due to the potential of residue build-up.

In the future, the 96-well system will be implemented during the amplification phase of processing. Also, the use of 96-lane sequencing gels will be re-evaluated. Previous attempts with 96-lane gels were unsuccessful. The 96-well method of DNA processing is an intermediate step leading to eventual robotic automation.

[1]Edge Quickstep™ 96 Well PCR Purification Kit Recommended Protocol. Edge BioSystems, Gaithersburg, MD