MITOCHONDRIAL DNA ANALYSIS OF CRIME SCENE SAMPLES USING PROTOTYPES OF THE 'LINEAR ARRAY MITOCHONDRIAL DNA HVI/HVII REGION-SEQUENCE TYPING KIT'

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Roche Applied Science, Inc. (Indianapolis, IN) is preparing to release the 'LINEAR ARRAY Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit'. This new system for mitochondrial DNA typing of forensic samples uses the proven technology of reverse dot blotting (used previously in the AmpliType® PM+DQA1 nuclear DNA testing kit) with the exception that the probes are arranged in a linear fashion versus being arranged as a 'dot'. The system is a simple, rapid, and inexpensive way of performing mitochondrial DNA analysis when compared to the standard sequencing analysis. The 'LINEAR ARRAY' kit provides an effective mechanism for screening samples to minimize the number of samples that must be sequenced. The discrimination power of the system is significant, but it is not as powerful as sequencing analysis.

In addition to the developmental validation that a manufacturer must perform prior to releasing a product to the forensic community, forensic laboratories must perform internal validation studies prior to bringing a DNA typing system on-line. The study presented here was undertaken to investigate how the typing system works with crime scene samples. The samples chosen for analysis were bloodstains collected at actual crime scenes in San Bernardino County, CA sometime prior to 1993. The samples are considered 'secondary reference samples', meaning that their source can be logically inferred (e.g. blood collected from a pool adjacent to a body with a gunshot wound to the head). These samples had been exposed to an array of environmental conditions (e.g. snow, heat, rain) and were deposited on a wide variety of substrates (e.g. carpet, clothing, asphalt, dirt). Also analyzed were the actual reference (origin positively known) blood samples.

Existing DNA extracts from these samples produced from either the organic (phenol/chloroform) method or the Chelex method were used. These samples were not extracted with mitochondrial DNA analysis in mind. Also, various analysts performed these extractions in either 1993 or 2000 and some samples were extracted multiple times.

Using prototypes of the 'LINEAR ARRAY Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit', all but three of the crime scene samples were successfully amplified and typed following the appropriate protocols. For one sample, no additional extract remained for further testing. It was determined that the two remaining non-amplifying samples likely contained substances inhibitory to the PCR. Varying parameters such as amount of input DNA, using alternate primer sets, and the use of bovine serum albumin were employed in an effort to overcome the inhibition. Successful amplification was achieved for both samples simply by decreasing the volume of the input DNA into the PCR.

No contamination was observed when comparing crime scene samples with the corresponding reference samples. Cross-hybridization likely occurred with one prototype of the LINEAR ARRAYS for some samples. When the same PCR product from these samples was typed on a newer version of the LINEAR ARRAYS, the cross-hybridization disappeared. Studies such as this one have been helpful in probe design and in determining optimal DNA input for the final version of the typing kit.