SHORT TANDEM REPEAT ANALYSIS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Genotyping based on short tandem repeat (STR) regions is extensively used in human identification, parentage testing, gene mapping studies, cancer diagnostics, and hereditary diseases determinations. Analysis of STR systems using slab gel electrophoresis requires lengthy and labor intensive procedures. Therefore, alternative methods such as capillary electrophoresis or high performance liquid chromatography (HPLC) have been implemented as techniques of DNA analysis. Ion-pair reversed-phase (EPRP) HPLC offers an attractive substitute to gel electrophoresis for STR analysis because of the reduced analysis time and elimination of hazardous waste disposal commonly associated with radioisotopic, enzyme-linked, or fluorescence methodologies. We evaluated the use of IPRP HPLC for the sizing and typing of STR alleles from the HUMTH01 locus on chromosome 11p15.5.

The IPRP HPLC conditions (column temperature, flow rate, and percent organic modifier per minute) were optimized for the separation of PCR products in the size range of 50-434 basepair. Using the optimized separation conditions, the alleles of the HUMTH01 system were sized in their native state (double stranded) with the use of internal markers. The typing results correlated 100% to accepted gel methods of typing performed at the Armed Forces DNA Identification Laboratory. In addition, to the TH01 analysis, amelogenin was multiplexed to allow for sex typing along with genotyping. The HPLC analysis time for the HUMTH01 locus was less than 14 minutes and the added capability of allele collection for further examination such as sequencing.