ONE AMPLIFICATION – TWO ANALYSES: A COMBINED CE AND MPS WORKFLOW

Maiko Takahashi[1], Jonathan L. King[1], Xiangpei Zeng[1], Jennifer D. Churchill[1], Bruce Budowle[1,2]

[1]Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center
[2]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University

Currently, short tandem repeat (STR) typing using capillary electrophoresis (CE) is the most widely used technology in the DNA forensic laboratory. However, massively parallel sequencing (MPS) offers an alternative or an adjunct technology that can analyze STRs and provide standard allele results as well as increase discrimination power by detection of intra-allele variants.

With challenged samples, at times, allele drop-out can occur. In addition, more genetic information can be useful when results are limited. After PCR 1 µL of product is analyzed by CE. The remaining PCR product typically is unused or discarded. However, MPS-based analysis can accommodate a much larger volume of PCR products for library preparation and thus provides an opportunity for a follow up analysis without additional consumption of precious sample. An effective use by MPS of the remaining PCR products obtained from limited case work samples could provide a bridge workflow between CE and MPS and obtain additional genetic information.

A study was performed where standard CE typing was carried out and subsequently the remaining unused PCR product was subjected to MPS .Two amplification kits were used: GlobalFiler® kits (Life Technologies®) and PowerPlex® Fusion® System (Promega Corporation).

The samples were amplified according to the manufactures instructions. For CE analysis, 1 µL of PCR products was injected into ABI 3500xl® (Life Technologies®) genetic analyzer, and the data were interpreted with GeneMapper IDX v1.2® (Life Technologies®) software. For MPS analysis, various volumes of PCR products were used in library preparation and then sequenced using the Illumina® MiSeq (Illumina®) and the Ion Torrent™ Personal Genome Machine® (Thermo Fisher Scientific), then the allele calls were made using STRait Razor software.

The results demonstrate that a systematic STR typing system is feasible in which no additional consumption of evidence is required; complementary results are generated to better effect interpretation; and additional information can be obtained on a case-by-case basis. This study also demonstrates another practical approach to implementation of MPS.