B14 Retrofitting Massively Parallel Sequencing (MPS) for HLA-DQA1 and Polymarker (PM) in Forensic Casework

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Learning Overview: After attending this presentation, attendees will be able to understand the potential use of MPS in forensic casework where no original biological evidence remains, but HLA-DQA1 and PM forensic analysis results are present.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an alternative method to approach cold cases and cases in which biological evidence, originally processed with the AmpliType® PM+DQA1 PCR Amplification and Typing Kit, is no longer available.

The objective of this research is to retrofit MPS to establish the genetic profile of an alleged suspect in a cold case, where the sole remaining evidence for comparison are results from the AmpliType® PM+DQA1 Kit. The AmpliType® PM+DQA1 Kit simultaneously amplified six loci: HLA Class II Histocompatibility Antigen gene coding for the DQ Alpha 1 Chain (HLsA-DQA1), Low-Density Lipoprotein Receptor (LDLR), Glycophorin A (GYPA), Hemoglobin G Gammaglobin (HBGG), Group-specific Component vitamin D Binding Protein (GC), and D7S8.[1] The methodology was based on a reverse dot blot assay, which utilized an allele-specific oligonucleotide probe, immobilized on a nylon membrane, to hybridize with the complementary DNA sequence.[1] Following hybridization, a colorimetric assay indicated the presence of the corresponding allele.[1] The AmpliType® PM+DQA1 PCR Kit was one of the first Polymerase Chain Reaction (PCR) -based DNA typing kits. It emerged in the early 1990s as a popular technique that provided improved sensitivity compared to Restriction Fragment Length Polymorphism (RFLP) assays, although it had a lower power of discrimination. The assay was soon replaced by Short Tandem Repeat (STR) -based assays in the mid to late 1990s. This presentation aims to communicate the development of an MPS assay to type the six original loci and depict the results of preliminary testing on reference samples.

Primer and probe sequences were obtained from the literature.[2,3] Primers were synthesized for compatibility with the Ion AmpliSeq™ targeted sequencing technology in collaboration with Thermo Fisher Scientific™. Additional primers were designed for redundancy. Initially, samples of known genotypes were amplified in a singleplex reaction using conventional PCR with AmpliTaq Gold® DNA Polymerase. Successful amplification was verified utilizing agarose gel electrophoresis. PCR products were subsequently pooled and processed with the Ion AmpliSeq™ manual library preparation protocol. The barcoded samples were later templated and sequenced on the Ion Chef™ and Ion S5™ platform using the Ion S5™ Precision ID Chef & Sequencing Kit. In parallel, primers were pooled and samples were processed with the automated library preparation Precision ID DL8 kit and sequenced as described above. The sequenced samples were analyzed using Integrated Genome Viewer (IGV) software through localization of probe sequences for each marker to determine the genotype of each sample and compared to known references for verification. The most challenging region to type was GYPA due to the similarities between GYPA, Glycophorin B (GYPB), and Glycophorin E (GYPE). GYPA, GYPB, and GYPE are all sialoglycoproteins found on the human erythrocyte membrane, and they help determine the MNS blood groups. There is 95% homology among them.[4]

Preliminary results indicate that this MPS-based assay is able to effectively type the loci targeted in the original AmpliType® PM+DQA1 PCR Amplification and Typing Kit. Results from the manual library preparation are consistent with the results obtained utilizing an automated approach. As expected, sequencing the HLA-DQA1 region is more discriminating than the original assay as it allows for detection of microvariants indiscernible to the probe hybridization-based methodology.

Reference(s):

1. Lincoln, Patrick J. and Jim Thomson. 1998. *Forensic DNA Profiling Protocols*.
2. Fildes, Nicola Jane and Rebecca Lynne Reynolds. 1994. *Methods and reagents for Glycophorin A typing.* 5643724, issued 1994.
3. Saiki, Randall K. and Shanavaz L. Nasarabadi. 1992. *Methods and reagents for gamma-globin typing*. 5273883, issued 1992.
4. Palacajornsuk, P. 2006. Review: Molecular Basis of MNS Blood Group Variants. *Immunohematology*.

HLA-DQA1 Polymarker, Massively Parallel Sequencing, Cold Case

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