EVALUATION OF AMP-FLP MARKERS AND SUMMARY OF PCR-BASED FORENSIC CASEWORK

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Polymerase Chain Reaction (PCR) based testing in forensic casework has been validated in numerous courts with the use of HLA DQα testing for forensic specimens. HLA DQα has been used in thousands of cases throughout the world over the past several years, where the evidence yielded DNA of too limited quantity or too degraded for Restriction Fragment Length Polymorphism (RFLP) analysis. HLA DQα PCR based testing has enabled our laboratory to analyze hundreds of cases which previously would have proven unsuitable for RFLP DNA analysis. Items of evidence from these cases include specimens such as microscopic slides from sexual assault evidence, cigarette butts, fingernail scrapings, swabbings from gun barrels as well as a variety of evidentiary specimens where DNA recovered from the specimen was too highly degraded to produce results with RFLP analysis. HLA DQα, however, represented only a single genetic marker available for PCR based testing in forensic analysis. The purpose of this study was to characterize and validate several additional amplification fragment length polymorphism (AmpFLP) markers for potential use in forensic casework. The use of AmpFLPs with forensic specimens will extend the application of VNTR polymorphisms to specimens which were either too limited in quantity or too degraded for traditional RFLP analysis. Initially, we evaluated the following four AmpFLP markers for their potential use with forensic specimens: D1S80, YNZ22, Apolipoprotein-B, and Collagen 2A1. The results of evaluation with forensic specimens included the optimization of extraction, amplification, and gel resolution conditions for each genetic marker as well as standard methods for interpretation of results. Population distributions for allele frequencies were analyzed for each AmpFLP marker within major North American racial groups.

DNA extraction methodologies were compared for a variety of tissue specific extraction protocols including extractions from liquid blood, blood stains, muscle specimens, bone

specimens, and hair specimens as well as a variety of mixed stain sexual assault evidence. Extraction of DNA from these specimens was accomplished utilizing both conventional organic-based extraction protocols as well as a modified chelex extraction. Although chelex extractions have been useful for amplifying DNA specimens for the HLA DQα amplitype system, it has been our experience that DNAs isolated via chelex extraction methodologies have not been suitable for amplification with these additional PCR based AmpFLP markers.

Amplification with D1S80 was accomplished as previously described by Kasai, et.al., Journal of Forensic Sciences, vol. 35, pp. 1196-1200 (1990), in a 50 μl reaction volume Amplifications were carried out in a Perkin Elmer 480 Thermal Cycler with the following thermocycling parameters: Denaturation, 95°C for 1 minute; annealing, 67°C for 1 minute; and extension, 70°C for 4 minutes for a total of 27 cycles. Amplified products were resolved on ultra thin polyacrylamide gels (7½%T/2%C) utilizing piperazine diacrylamide as a cross linker. Electrophoresis of specimens was carried out by discontinuous gel electrophoresis in a Tris/Formate (60mM) buffer system at 550 volts, 20 mAmps, and 10 watts for 15cm at 18°C. Size markers included the 100bp (LTI) ladder and 10bp ladder (LTI) as well as an allelic marker constructed at Genetic Design comprised of each D1S80 allele beginning with the 14 repeat allele through the 39 repeat allele. Gels were stained with silver, allowed to dry, and photographed. Population survey show the 24 repeat unit allele to be the most frequently occurring in both North American Caucasians and North American Blacks, (N=424 Caucasians and N=400 for Blacks). Additional population studies were conducted for East Coast North American Hispanics (N=190), West Coast North American Hispanics (N=314), North American Indian (N=216), and North American Orientals (N=210).

The following three case summaries show several types of cases where results obtained from HLA DQα may not be conclusive due to the limited number of alleles detected, especially where mixed stains or cases involving multiple parties are involved. The subsequent use of D1S80 has substantially improved our ability to resolve such cases.

Case 1: Both suspects share the HLA DQα type 3, 4 which was detected in the male fraction of the evidentiary material. D1S80 analysis included the first suspect (homozygous 18) and excluded the second suspect (heterozygous 28, 31).

Case 2: Both the suspect and victim shared an HLA DQα type 4, 4. Using D1S80 analysis, the victim was a type 26, 28 and the suspect was a D1S80 type 23, 29 consistent with the evidence in the male fraction.

Case 3: This case involved a sexual assault of one victim with four named suspects. HLA DQα testing produced multiple types in the male fraction of the evidentiary specimen. D1S80 analysis showed the presence of four alleles in the male fraction of the evidentiary specimen. D1S80 alleles 18 and 29 were consistent with the types obtained from the victim. The additional two D1S80 alleles observed (22, 23) were different from the results of all four suspects.

Amplification and gel conditions were optimized for the remaining three AmpFLP markers (YNZ22, Apolipoprotein B, and Collagen 2A1) and population data was accumulated; however, no additional validation work with forensic specimens has been completed because of the introduction of more promising genetic markers. These new markers are classified as short tandem repeats (STRs). To date, two of these markers have been studied in our laboratories (SE-33 and CYP-19). Validation work in our laboratory demonstrated the critical nature of the annealing temperature for SE-33. Annealing at 64°C resulted in secondary high molecular weight banding and annealing at 66°C resulted in the loss of some PCR product. Annealing at 65°C produced optimal results. In addition, experiments regarding the concentration of target DNA demonstrated that optimal conditions were achieved with 5 to 8 nanograms of target DNA. Amplification products with SE-33 were analyzed utilizing precast discontinuous Tris/Sulfate gels (LTI) electrophoresed in traditional 1X TBE buffer at 500 volts, 22mAmps, and 35 watts for 25cm. Amplification products were stained with silver and photographed. The second STR marker preliminary validated in our laboratory is CYP-19. This marker utilizes the same amplification and gel conditions as SE-33 with the exception annealing temperatures are optimized at 64°C. Population data has been collected for these two STR markers and preliminary validation with forensic specimens indicate promising results for use in forensic casework.