University of New Mexico

HLA and Molecular Diagnostics Laboratory

Principal Investigator

Thomas M. Williams, M.D.

Primary Staff dedicated to the NMDP Donor-Recipient Pair Project

Jin Wu, M.D.

SBT HLA-DPA1 High Resolution Typing Procedure

Last Revised February 2004

Genomic DNA is isolated from various sample types according to the appropriate manufacturer's protocol: transformed cells and blood spotted filter paper (PuregeneDNA Isolation Kit, Gentra Systems, Minneapolis, MN); frozen whole blood, granulocytes, and mononuclear cells (Qiagen QIAmp DNA Blood Mini Kit, Valencia, CA).

High resolution typing of HLA-DPA1 is accomplished using amplification and sequencing reagents developed and/or validated at the University of New Mexico. Products are amplified from genomic DNA for subsequent Sanger dideoxy thermal cycle sequencing with resolution of ladders on ABI 377 or 3100 instruments (Applied Biosystems, Foster City, CA). For HLA-DPA1, an internal sequencing primer and fluorescently labeled dideoxyterminators are used.

An HLA-DPA1 generic 1366 nucleotide PCR product including exons 2 through 4 is prepared from genomic DNA using primers E2.5 and 14.3 as previously described (Rozemuller EH, et. al. *Tissue Antigens* 1995: **45**: 57-62). Sequencing ladders for exon 2 are prepared from this template with the internal sequencing primer 12.3F annealing within intron 2 and dye terminator kit reagents (Perkin Elmer-ABI, Foster City, CA). Sequencing products are precipitated, denatured, and resolved on sequencing gels.

All sequences are processed using MatchTools and MT Navigator HLA typing software from Applied Biosystems. These programs allow for allele assignment and sequence editing, respectively.