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SBT HLA-DQB1 High Resolution Typing Procedure

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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-DQB1 is accomplished using the commercially available AlleleSEQR (Forensic Analytical, Hayward, CA) reagents and protocol. A pre-formulated PCR pre-mix contains buffer, dNTPs, MgCl₂, oligonucleotide primers, and water. Per the American Society for Histocompatibility and Immunogenetics (ASHI) guidelines, AmpliTaq Gold (Applied Biosystems, Foster City, CA) is provided as a separate reagent to be added to the pre-mix at the time of amplification reaction preparation so as to eliminate the possibility of non-specific amplification during storage. ExoSAP-IT (USB, Cleveland, OH) is used to remove unincorporated PCR components, primers and dNTPs. This reagent is a mixture of exonuclease I, which digests single stranded oligonucleotides (i.e.: unincorporated PCR primers), and shrimp alkaline phosphatase which digests unincorporated dNTPs. The sequencing mix for HLA-DQB1 is based on the Sanger dideoxy chain terminator DNA sequencing method. The pre-formulated mixes are based on sequencing chemistry from Amersham Biosciences (Piscataway, NJ). The DYEnamic ET Terminators contain fluorescent dye labeled ddNTPs and DNA polymerase. When combined with oligonucleotide sequencing primers that recognize sequence motifs within the PCR amplicon, the sequencing reactions generate fluorescent sequence ladders. Sequencing primers are chosen to generate a sequence for exon 2 in both directions for accuracy and simplified editing. Sequence ladders are purified and concentrated by ethanol precipitation, heat denatured in formamide, and electrophoretically separated and analyzed on an ABI 377 or 3100 automated DNA sequencer from Applied Biosystems.

The exon 2 sequence of DQB1, from codon 9 to the end of exon 2, is generated. After preparation of genomic DNA, exon 2 of the DQB1 gene is amplified using a pre-formulated PCR mix containing custom PCR primers located at the 5' end of exon 2 and the 5' end of intron 2. Following PCR amplification according to the manufacturer's

instructions, the amplicon is processed with ExoSAP-IT. The enzyme-treated PCR is then added to each of 2 different pre-formulated sequencing reaction mixtures: exon 2 forward and reverse.

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.

While a multilayered approach is used for resolving ambiguous HLA-DQB1 heterozygous combinations, the primary approach involves use of a series of both group and allele specific SSP amplification primers as previously described (Bunce, M and Welsh, K. Laboratory Manual, A. Hahn, ed. Volume 2, V.C.1.1-19, 4th Edition, 2000).