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SBT HLA-A 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-A 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 (ie: unincorporated PCR primers), and shrimp alkaline phosphatase which digests unincorporated dNTPs. The sequencing mix for HLA-A 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 each exon 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 automated ABI 377 or 3100 DNA sequencer from Applied Biosystems.

Complete sequence information for HLA-A exons 2, 3 and 4 is generated. After isolation of genomic DNA, a region of the HLA-A gene is amplified using a pre-formulated PCR mix containing custom PCR primers located at the 5' end of exon 1 and the 5' end of intron 4. Following PCR amplification according to the manufacturer's instructions, the

amplicon is processed with the ExoSAP-IT reagent. Next the enzyme-treated PCR is added to each of 6 different pre-formulated sequencing reaction mixtures: exon 2 forward and reverse, exon 3 forward and reverse and exon 4 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.

A multilayered approach is used for resolving ambiguous HLA-A heterozygous combinations. One approach is the 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). Also used are a series of group specific amplification primers for the HLA-A*02 alleles developed by the Forensic Analytical group which will eventually become available commercially. Finally, the single allele amplification method developed by the University of New Mexico laboratory is also employed. This method relies on a dilution of genomic DNA prior to the PCR so that hemizygous PCR products can be prepared.