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SBT HLA-DRB1/3/4/5 High Resolution Typing Procedure

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Genomic DNA is extracted from a 200 µl sample (i.e., frozen whole blood, PBLs, granulocytes or EBV transformed cell lines using a Qiagen QIAamp Blood Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) methodology employing group-specific exon primers for the HLA-DRB1 locus or locus-specific primers for the HLA-DRB3, HLA-DRB4, and HLA-DRB5 loci is used to generate templates for sequencing reactions. The size and quantity of the amplicons are determined using agarose gel electrophoresis.

Unincorporated primers and nucleotides are removed from amplicons using a High Pure PCR Product Purification Kit (Roche, Indianapolis, IN) or a PCR 96 Cleanup Kit (Millipore, Bedford, MA). The purified HLA-DRB amplicons serve as templates for an exon 2 cycle sequencing reaction using Big-Dye sequencing chemistry (Applied Biosystems, Foster City, CA). Unincorporated nucleotides are removed using Sephadex spin columns. Primer extension products from the cycle sequencing reactions are separated on an ABI Prism 3100 capillary sequencer. If templates are heterozygous, the sense and anti-sense strands are sequenced.

HLA data are analyzed using ABI Sequence Navigator and ABI MatchTools software and a preliminary HLA type is assigned. If only one allele is observed, a low resolution sequence specific primer (SSP) assay is used to confirm homozygosity. Sequencing ambiguities are resolved using one or more of the following approaches: (1) sequencing of templates generated by allele-specific amplification, (2) typing using SSP methods (PelFreez, Milwaukee, WI or GenoVision, West Chester, PA), and (3) sequencing of templates generated by cloning the locus-specific amplicons.

References:

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