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SBT HLA-A 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 intronic primers specific for the HLA-A locus (Cereb et. al.) is used to generate locus-specific 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-A amplicons serve as templates for four cycle sequencing reactions to generate bi-directional sequence for exons 2 and 3. Locus-specific sequencing primers which are located in introns are used with 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.

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 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 sequence-specific priming (SSP) methods (PelFreez, Milwaukee WI or GenoVision, West Chester, PA), and (3) sequencing of templates generated by cloning the locus-specific amplicons.

References:

Baxter-Lowe LA. Class II HLA Sequence Based Typing. In: Noreen, H, ed. *ASHI Laboratory Manual, 4th Edition American Society of Histocompatibility and Immunogenetics 2000*, V.C.8.1

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