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SBT HLA-A High Resolution Typing Procedure

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Genomic DNA from individuals is prepared from 200µL of frozen whole blood (citrate/EDTA), PBLs, granulocytes, or EBV cell lines using the commercially available Qiagen (Valencia, CA) QIAamp Blood Kit according to the manufacturer's protocol.

Exons 2 and 3 flanked by introns 1, 2 and 3 act as a template for a primary (polymerase chain reaction (PCR). Locus-specific primers hybridize in introns 1 and 3 resulting in a primary amplicons of 914 base pairs in length. This primary locus-specific PCR product then serves as a template for four nested and heminested secondary PCR reactions.

Nucleotide primer mixes are used to generate separate exon 2 and 3 HLA-A amplicons of approximately 340 base pairs in length with an M13 universal primer site on one end and biotin on the other. The HLA-A exon 2 and 3 biotinylated PCR products are then bound to a streptavidin coated support upon which bi-directional DNA sequencing reactions are performed. Sequence data is analyzed using the HLA SequiTyper™ software, version 2.0 (Amersham Biosciences, Piscataway, NJ).

HLA-A ambiguous combinations are resolved using Reference Strand Conformational Analysis (RSCA) on the same hardware platform (ALFexpress; Amersham Biosciences) used for sequence based typing (SBT). This methodology has proven to be a fast, relatively inexpensive and efficient alternative to ambiguity resolution using sequence specific primer (SSP) technology since it does not require increasingly large inventories of different SSP primer combinations. RSCA data analysis is accomplished using the AlleleLinks software (Amersham Biosciences).