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Health Sciences Center

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SBT HLA-DQA1 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.

Two rounds of polymerase chain reaction (PCR) amplification are performed. During the first, the genomic DNA is diluted and used as a template for nested biotinylated PCR reactions which amplify exon 2 locus-specific primers. A series of cycles run at varying temperatures and times on a thermocycler produces a PCR product. This product is then run on an agarose gel to check the length of the amplicon, which should be at least 270 base pairs. This process is then repeated with a second set of primers to generate an amplicon length of approximately 230 base pairs.

Using a Cy5 dye-labeled-21-mer M13 Universal primer and an Autoload Kit (Amersham Pharmacia Biotech, Piscataway, NY), direct sequencing of each of the nested PCR products is performed. Sequencing reactions are then loaded onto a 6% PAGE Plus gel (Ameresco, Solon, OH) and run on a Pharmacia ALFexpress (Amersham Biosciences) automated DNA sequencer. Data analysis is completed using the Pharmacia HLA SequiTyper software, version 2.0 (Amersham Biosciences).