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SBT HLA-A High Resolution Typing Procedure

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Following the extraction of genomic DNA using the QiaAmp kit (Qiagen, Valencia, CA), sequencing of HLA-A locus proceeds by polymerase chain reaction (PCR) and cycle sequencing techniques to amplify and sequence the most informative segments of the gene.

The first step consists of a single amplification reaction using allele specific 5' and 3' amplification primers. These amplification products include the informative exons 2 and 3, as well as the intervening intron 2, and are used as a template for sequencing.

Four separate sequencing reactions, one for each base, are required resulting in four series of different sized fragments. When these fragments are combined, each differs in size by one base. These fragments are then separated by electrophoresis on the MicroGene Clipper automated sequencer and interpreted by GeneObjects software (both from Visible Genetics Corporation, Toronto, Canada) in order to generate an accurate sequence of the test sample. Four sequencing primers for HLA-A are used for production of DNA sequence information of exons 2 and 3. In a majority of cases, these primers are sufficient to unambiguously determine the alleles present in a test sample.

A final typing result is generated by comparing the sample sequence to all combinations of known A gene alleles using the GeneLibrarian software (Visible Genetics Corporation).