

American Red Cross Blood Services

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SBT HLA-B High Resolution Typing Procedure

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For high resolution typing of HLA-B, AlleleSEQR kits from Forensic Analytical/MG (South San Francisco, CA) are used. Included in the B locus-specific kit are the following reagents: PCR pre-mix, AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA), a DNA control, PCR purification reagents (ExoSAP-IT, USB, Cleveland, OH), sodium acetate/EDTA buffer, forward sequencing mixes for exons 2, 3 and 4 reverse sequencing mixes for exons 2, 3 and 4.

A complimentary approach of two sets of reagents is used to type HLA-B locus alleles. The first is a generic B kit which provides one set of primers (in a multiplex PCR) that amplify a region from exon 1 to exon 7. Sequencing of exons 2, 3 and 4 identifies the majority of HLA-B polymorphisms. The second set of reagents provides resolution of ambiguous heterozygous typing through the specificity of a number of group specific amplifications.

The primary PCR reaction is carried out using 10-50 ng/ μ L genomic DNA, extracted using the QIAamp DNA mini kit (Qiagen, Valencia, CA), and Taq Gold polymerase and is completed with the following series of thermal cycler steps: 1 soak (95° C for 10 minutes), 36 cycles (96° C for 20 seconds; 60° C for 30 seconds; 72° C for 3 minutes) and a final soak (4° C indefinitely).

Electrophoresis on a 2.5% gel is used to evaluate the PCR amplification product. ExoSAP-IT is then used to purify the PCR amplicons in a two step (37° C for 15 minutes followed by 80° C for 15 minutes) thermal cycler reaction.

Sequencing of the HLA-B locus is accomplished by a series 25 thermal cycler steps consisting of 96° C for 20 seconds followed by 60° C for two minutes. A total of 6 reactions (both a forward and a reverse reaction for exons 2, 3 and 4) are used to sequence HLA-B.

The ABI 3100 sequencer (Applied Biosystems) is used to collect the sequencing data. Currently Forensic Analytical AlleleSEQR class I kits use the ABI instrument software for data collection and sequence analysis. Specifically, the Matchtools (Applied

Biosystems) software processes the sequence file to create a preliminary report; MT Navigator inspects the electropherograms and edits the sequences; and Matchtools creates a final allele typing report.

A considerable number of HLA-B locus typings contain ambiguities. To resolve them, a second level of sequencing is performed using the following five group specific amplification kits (BG1-BG5):

- BG1 to resolve B07, 08, 14, 16 (38, 39), 21 (49, 50), 4001, 41, 45 48 and 67 groups
- BG2 to resolve B05, 13, 17 (57, 58), 18, 27, 35, 37, 4002, 44, 47, 78 and 81 groups
- BG3 to resolve B15 and 46 groups
- BG4 to resolve B05 (51, 52), 07, 08, 14, 15, 16 (38, 39), 17 (57, 58), 21, (49, 50), 35, 4001, 41, 45, 46, 48, 67, 73 78 and 81 groups
- BG5 to resolve B05 (51, 52), 13, 17 (57, 58), 18, 22 (54, 55, 56), 27, 35, 37, 4002, 44, 47, 59, 78 and 81 groups

High resolution sequence specific primer (SSP) reagents from Pel-Freez Clinical Systems, LLC (Brown Deer, WI), Olerup (distributed by GenoVision, West Chester, PA) and One Lambda (Canoga Park, CA) can also be used to discriminate HLA-B ambiguous combinations. Other technical problems such as PCR failures, weak or failed sequencing reactions, small peaks, dye blobs or "noisy" sequences are resolved via consulting the Forensic Analytical version 1.0 manual.