

American Red Cross Blood Services

Penn-Jersey Region

Principal Investigator

Susan H. Hsu, Ph.D.

Primary NMDP Donor-Recipient Pair Project Contact

Wei Dong, M.D.

Carly Carozza, B.S.

SBT HLA-A High Resolution Typing Procedure

Last Revised February 2004

For high resolution typing of HLA-A, AlleleSEQR kits from Forensic Analytical/MG (South San Francisco, CA) are used. Included in the A 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 and reverse sequencing mixes for exons 2, 3 and 4.

The HLA-A PCR primers amplify a 2 Kb fragment spanning from exon 1 to intron 4. Sequencing of exons 2 and 3 identifies the majority of HLA-A polymorphisms. Information obtained in sequencing exon 4 assists both in the identification of rare null alleles as well as in the resolution of certain alleles defined by exon 4 polymorphisms.

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-A locus is accomplished by a series of 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-A.

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 software (Applied Biosystems) 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.

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) are used to resolve HLA-A ambiguities following initial sequencing. 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.