

## **Immunogenetics and Transplantation Laboratory**

### **University of California, San Francisco**

#### **Principal Investigator**

Lee Ann Baxter-Lowe, Ph.D. d(ABHI)

#### **Primary Staff dedicated to the NMDP Donor-Recipient Pair Project**

Victor Corpuz

#### **SBT HLA-DPA1 High Resolution Typing Procedure**

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Genomic DNA is extracted from a 200 µl sample (i.e., frozen whole blood, PBLs, granulocytes or EBV transformed cell lines using a Qiagen QIAamp Blood Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) methodology employing locus-specific intron primers for the HLA-DPA1 locus are used to generate templates for sequencing reactions. The size and quantity of the amplicons are determined using agarose gel electrophoresis.

Unincorporated primers and nucleotides are removed from amplicons using a High Pure PCR Product Purification Kit (Roche, Indianapolis, IN) or a PCR 96 Cleanup Kit (Millipore, Bedford, MA). The purified HLA-DP amplicons serve as templates for an exon 2 cycle sequencing reaction using Big-Dye sequencing chemistry (Applied Biosystems, Foster City, CA). Sequences are determined for the sense and anti-sense strands using nested primers or M13 priming sites created by the PCR primers. Unincorporated nucleotides are removed using Sephadex spin columns. Primer extension products from the cycle sequencing reactions are separated on an ABI Prism 3100 capillary sequencer (Applied Biosystems). If templates are heterozygous, the sense and anti-sense strands are sequenced.

HLA data are analyzed using ABI Sequence Navigator and ABI MatchTools software and a preliminary HLA type is assigned. If only one allele is observed, a low resolution sequence specific primer (SSP) assay is used to confirm homozygosity. Sequencing ambiguities are resolved using one or more of the following approaches: (1) sequencing of templates generated by allele-specific amplification, (2) typing using SSP methods, and (3) sequencing of templates generated by cloning the locus-specific amplicons.

#### References:

Baxter-Lowe LA. (2000) Class II HLA Sequence Based Typing. *ASHI Laboratory Manual, 4th Edition*, H Noreen (ed), American Society of Histocompatibility and Immunogenetics, V.C.8.1

Tilanus MGJ, ed. *IHWG Technical Manual: Genomic Analysis of the Human MHC: DNA-Based Typing for HLA Alleles and Linked Polymorphism* 2000: Publication of the 13<sup>th</sup> International Histocompatibility Working Group. Seattle, USA: Cancer Research Center.