

## **Children's Hospital Oakland Research Institute HLA Laboratory**

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### **SSOP HLA-HLA-DPB1 High Resolution Typing Procedure**

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QIAGEN (Valencia, CA) genomic DNA extraction kits are used to obtain high quality DNA from blood, cells, tissue, and blood spots.

An immobilized sequence-specific oligonucleotide probe (SSOP) line blot typing system (Nicklas et.al. submitted) is used to type the HLA-DPB1 locus. In this method, unlabeled oligonucleotide probes are immobilized onto a backed nylon membrane. The PCR product is labeled during the amplification process by the incorporation of biotinylated primers and hybridized to the immobilized probe array. A distinct advantage of the immobilized probe system is that, providing there is no allele ambiguity problem with the sample, only one hybridization reaction is sufficient to determine the genotype. Locus and exon-specific primers are used in the first tier analysis; group-specific primers are used in subsequent tiers of analysis.

To begin the allelic HLA-DPB1 typing procedure, a biotinylated primer pair is used to amplify exon 2. HLA-DPB1 PCR products are then denatured and hybridized to the immobilized SSO strip membranes using an automated hybridization/stringent wash and colorimetric detection instrument, the SLT Profiblot (Tecan US, Research Triangle Park, NC) or AutoRELI™ (DynaL Biotech, Brown Deer, WI). The immobilized probe strip used for HLA-DPB1 genotyping currently includes 47 SSO probes from exon 2. The developed probe patterns on the strips are then photographed for permanent storage and analysis. To determine the genotypes, strips or photographs are analyzed using a proprietary computer matching programs developed in-house. If no definitive genotype can be deduced from the strip, group-specific amplifications are performed to amplify the alleles separately for re-analysis on the strips. We currently use 6 different group-specific primers to separate alleles at HLA-DPB1.

Our HLA-DPB1 typing system is continuously upgraded as new alleles are discovered.