

Children's Hospital Oakland Research Institute HLA Laboratory

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SSOP HLA-DPA1 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.

To type the HLA-DPA1 locus, non-biotinylated primer pairs are used to amplify exon 2. HLA-DPA1 PCR products are then denatured and immobilized onto a nylon membrane using vacuum followed by U.V. cross-linking (dot blot format). The dots are then hybridized to a series of (currently) 19 biotinylated SSO probes specific to exon 2 of HLA-DPA1. The membranes are then stringently washed to remove unbound probe, and then developed using a non-radioactive, colorimetric detection system. The developed membranes are photographed for permanent storage and analysis. To determine the genotypes, the membrane pictures are analyzed using a proprietary computer pattern matching program developed in-house. If no definitive genotype can be deduced from the analysis of the general amplification, group-specific amplifications are performed to amplify the alleles separately for re-analysis with the SSO probes.

An immobilized probe typing system for HLA-DPA1 is currently under development in collaboration with Dr. Henry Erlich's laboratory at Roche Molecular Systems (Alameda, CA). 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 will sufficiently determine genotype.

Our HLA-DPA1 typing system is continuously upgraded as new alleles are discovered.