

## **University of New Mexico**

### **HLA and Molecular Diagnostics Laboratory**

#### **Principal Investigator**

Thomas M. Williams, M.D.

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

Jin Wu, M.D.

#### **SBT HLA-DQA1 High Resolution Typing Procedure**

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Genomic DNA is isolated from various sample types according to the appropriate manufacturer's protocol: transformed cells and blood spotted filter paper (PuregeneDNA Isolation Kit, Gentra Systems, Minneapolis, MN); frozen whole blood, granulocytes, and mononuclear cells (Qiagen QIAmp DNA Blood Mini Kit, Valencia, CA).

High resolution typing of HLA-DQA1 is accomplished using amplification and sequencing reagents developed and/or validated at the University of New Mexico. Products are amplified from genomic DNA for subsequent Sanger dideoxy thermal cycle sequencing with resolution of ladders on ABI 377 or 3100 instruments (Applied Biosystems, Foster City, CA). For HLA-DQA1, the PCR primers as sequencing primers and fluorescently labeled dideoxyterminators are used.

HLA-DQA1 exon 2 PCR products are prepared with primers M5QA (exon 2, codons 11-18) and M3QA (exon 2, codons 80-87). In some cases, M3QA is paired with MQA01 (intron 1) to generate the HLA-DQA1\*01 group specific products. Reactions are performed in a 50µL volume with 0.25 µM primers, 250 ng genomic DNA, 1.25 mM MgCl<sub>2</sub>, 0.2 mM dNTP, and 1.25 U Taq Gold polymerase with 35 cycles of the following: 96° C for 60 seconds / 60° C for 30 seconds / 72° C for 60 seconds. The generic PCR product size is 265 nucleotides; the HLA-DQA1\*01 specific product is 400 nucleotides. The PCR Product is purified using "Qiagen Columns" to achieve a final concentration of approximately 5ng/ul. Sequencing reactions are performed with Big Dye Terminator sequencing kits (Perkin Elmer ABI, Foster City, CA) using M5QA, MQA01, or M3QA primers. Sequencing products are precipitated in ethanol, denatured and loaded on a sequencing gel for analysis.

All sequences are processed using MatchTools and MT Navigator HLA typing software from Applied Biosystems. These programs allow for allele assignment and sequence editing, respectively.

While a multilayered approach is used for resolving ambiguous HLA-DQA1 heterozygous combinations, the primary approach involves use of a series of both group

and allele specific SSP amplification primers as previously described (Bunce, M and Welsh, K. Laboratory Manual, A. Hahn, ed. Volume 2, V.C.1.1-19, 4th Edition, 2000).