

American Red Cross Blood Services

New England Region

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SBT HLA-DQB1 High Resolution Typing Procedure

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Sequence based typing (SBT) is the primary typing methodology used for high resolution typing of the HLA-DQB1 locus.

Transformed cells are thawed and washed once with PBS. Genomic DNA is prepared from 200 μ L of frozen blood or washed cell suspensions using Qiagen M96 Bio-Robot or QIAamp 96 Blood Kit from Qiagen (Valencia, CA).

Exon 2 of the HLA-DQB1 gene is amplified using primers listed on the International Histocompatibility Working Group (IHWG) Web site by Dr. Tilanus and subsequently sequenced using BigDyeTM Terminator chemistry from Applied Biosystems (Foster City, CA). TECAN Genesis workstation (Maennedorf, Switzerland) is used for PCR set up to reduce labor and avoid human error. Sequencing is carried out using ABI 3100 Genetic Analyzers (Applied Biosystems). Direct SSOP kits (Orchid Diagnostics, Stamford, CT) are used as a complimentary and confirmatory method for DQB1 typing.

Ambiguous typing is resolved by a subsequent group-specific amplification step followed by hybridization with proper probes selected by the IHWG Class II working group. Currently, Luminex LabType SSO kits (One Lambda, Canoga Park, CA) are also validated for use.