

American Red Cross Blood Services

New England Region

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SBT HLA-A 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-A locus. Sequence specific oligonucleotide probe (SSOP) methodology is also used for confirmation of homozygous results and for resolving ambiguous typing.

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

Exons 2 and 3 of the HLA-A gene are amplified and subsequently sequenced using Amplification Module and BigDye™ 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 three ABI 3100 Genetic Analyzers (Applied Biosystems). NMDP approved group-specific amplification primers used in SSOP methodology are also used as alternative amplification primers for the HLA-A SBT method after being tagged with M13.

Homozygous samples are confirmed using either Direct (Orchid Diagnostics, Stamford, CT) or Reverse (Dynal Biotech, Lafayette Hill, PA) SSOP kits. Samples with ambiguous typing are resolved using SSOP and/or sequence specific primer (SSP) methodology.