

Report from the “HLA-SBT Training School – Basic Course”  
17 & 18 January 2006  
by Dr. Frantisek Mrazek

In the middle of January 2006 I attended the HLA–SBT Training School organized by Genome Diagnostics. The School consists of two separated courses, “Basic” and “Advanced”, which both took place at the University Medical Centre (UMC) Utrecht, The Netherlands.

The two days basic course is intended especially for individuals from laboratories which just start (or plan to start) with HLA high resolution typing by sequencing based typing (SBT). The first day of the course started with explaining the basic HLA items (biology, genetics, medical applications) and continued with a lecture reviewing the most frequently used techniques for the HLA typing (serology, SSP, SSOP). Both opening lectures provided good background to the main topic of the course: SBT in practice.

In the afternoon we continued with an introduction into the theory of HLA typing by SBT. Each step of the SBT procedure was discussed independently, including techniques for DNA isolation suitable for SBT, purification of the amplicon, sequencing reaction, purification of the sequenons, sequencing electrophoresis and data analysis. We were also familiarized with the strategy used for HLA-SBT of the Class I at the UMC Utrecht. The most important item of this strategy is the initial amplification of the whole HLA gene (i.e. all relevant exons together) of a particular HLA-locus. It enables, when required, extended sequencing of additional exons using the same amplicon. The first day of the basic course was finished with practical SBT work. Using the protocols provided by the organizers, each of the participants prepared and performed several amplification reactions for SBT. We also carried out purification of amplicons, prepared sequencing reaction and performed electrophoresis. In the meantime, we learned basic operation of the SBT data analysis software “SBTEngine®”.

The software “SBTEngine®” was used for data analyses at the second day of the course. It appeared to me as reliable, user friendly and highly versatile. I appreciated especially that the software is not dependent on the reagents used for sequencing (only the generated sequence data is important). The procedure of data analysis by SBTEngine® is simplified by the automatic alignment of the analysed sequence with the latest version of the IMGT/HLA library. The analysis is performed in three steps: 1) solving of possible differences with the IMGT/HLA allele library, 2) solving of possible differences between sequences (e.g. forward and reverse sequences), 3) checking for “crucial” positions. The analysis is very rapid and simple. All ambiguous alleles and allele combinations (if any) are listed, including the positions reflecting them. Other advantages of the software are the possibility of predefined operation and assignment of sequence files, and time-saving organization and assignment of the SBT results for each sample. We obtained a CD-ROM containing the trial version of SBTEngine® which allows us to practise SBT data analysis after the course.

Using SBTEngine®, we interpreted the data generated during the first day of the course. Furthermore, we practised analyses using sequencing data of varying quality from numerous samples. The crucial positions (positions potentially modifying results) and both allelic and genotype ambiguities were discussed in detail. Solving ambiguities was one of the most important items discussed during the advanced SBT course which took place next two days after the basic course.

In conclusion, I found the HLA-SBT Training School very useful for people implicated in HLA-DNA typing. All lectures, provided materials and protocols were of high quality. I particularly appreciated the possibility to meet and inquire the experts in the HLA-SBT field (e.g. Marcel Tilanus and Erik Rozemuller). The atmosphere of the courses was open to discussion and to exchange experiences between the participants from different countries. I would like to thank Genome Diagnostics for granting me the bursary to attend the HLA-SBT “Basic course”.

Participant: Dr. Frantisek Mrazek, Tissue Typing Laboratory, Dept of Immunology, Palacky University Hospital, Olomouc, Czech Republic.