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Theme: “Meeting the challenges of globalization and minaturisation.”

POSTER PRESENTATIONS: Additional abstract

P311. MHC Block Matching on the MegaBACE Technology Platform : A improved tool for hematopoietic stem cell transplant donor selection.

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In view of the current biotechnological developments and better alternatives to ultra-thin gel electrophoresis, we utilized high quality archived DNA samples from twelve families consisting of 36 individuals, to develop this new methodology. The principle of this test is based on genomic amplification and fragment analysis on a new capillary platform. Fragments of interest comprising beta and delta blocks of the MHC genome were amplified using Taq DNA Polymerase, 10X PCR buffer, MgCl₂, dNTPs and FAM-labeled forward and reverse primer of each block. In the initial stages, products were evaluated on a 2% agarose gel prior to loading on the Megabase 500 platform. Positive amplicons were then purified with Qiagen QIAquick purification kit before proceeding to precipitation with 95% and 70% ethanol. For capillary electrophoresis, loading solution containing 0.1% Tween 20, MB ET550 size standard and sdH₂O was added to samples. Samples were denatured for 1 minute at 95°C and loaded into the MegaBACE using the Genotyping Set 2 parameters at 10kv for 75 min. Data were processed with Genetic Profiler software. Patient and potential donor profiles were merged and best matched pair selected for full HLA typing. Profiles from archived generated using this technology were similar yet provided better resolution compare with previous Block Matching on ultra thin gels. Best matched pairs from newly extracted DNA from fresh blood were fully matched at the HLA level. From DNA samples, results results of up to 14 individuals (with triplicates for QA) can be obtained in 1 working day. The MegaBace 500 method eliminates difficulties in preparing glass plates, ultra thin gels and is very cost effective screen, prior to full HLA typing for best matched pairs.