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A multi-center case study to evaluate HLA typing resolution using novel SBTessenz® assays suitable for donor registries

Introduction

Many countries have set-up donor registry HLA-typing programs to facilitate unrelated hematopoietic stem cell transplantation. The NMDP requires that each newly recruited donor is typed at the intermediate resolution level for HLA-A, -B, -C and -DRB1. For bone marrow transplantation, HLA-DQB1 is also required. We developed novel SBTessenz® assays that enable unique identification of HLA-A, -B, -C, -DQB1 and -DRB1 gene regions that code for the antigen recognition site using sequencing-based typing (SBT) (Figure 1). The SBTessenz® assay strategy accommodates intermediate resolution in the first round of sequencing through application of a specified set of group-specific sequencing primers (GSSPs) in addition to the Core sequencing primer set (Figure 1).

Aim

In this multi-center study the performance of the SBTessenz® assays was evaluated by BFR Gene Diagnostics, UMASS Memorial Health Care and Leiden University Medical Centre, using different gDNA isolates, including blood, buccal swabs, and cell-line material of divers DNA qualities.

Results

Strong HLA locus-specific amplicons of the expected sizes were obtained for the different sample panels tested: LUMC (n=192), BFR (n=96), and UMASS (buccal swaps: n=42) using the SBTessenz® assays (Figure 2). Sequences with high quality scores were generated for all amplicons (Figure 3). Typing results obtained (total of n=330 samples) using the HLA-A, -B, -C, -DQB1, and –DRB1 SBTessenz® assays were concordant with the pre-typing results provided by the centers.

The resolution levels obtained for the Chinese and LUMC sample panel were analyzed for the SBTessenz® assay strategy (Core plus GSSPs) versus a generic approach (Core only) (Figure 4). The percentage of genotype ambiguities drops significantly for all loci when applying the SBTessenz® strategy (Figure 4).

When the Common and Well-documented (CWD) filter is applied, thereby ignoring CWD -/- ambiguous combinations, the level of genotype ambiguities reaches: HLA-A [19%], HLA-B [50%], HLA-C [26%], HLA-DRB1 [17%], and HLA-DQB1 [5%] (Caucasian); HLA-A [19%], HLA-B [13%], HLA-C [7%], HLA-DRB1 [13%], and HLA-DQB1 [4%] (Chinese). Further analysis, ignoring CWD -/- and CWD +/- ambiguous combinations, show that the majority of samples have a single common genotype (Figure 5).

Figure 1. Locations of EAP participants

Exons 2 and 3 are amplified for HLA-A, -B, and –C, exon 2 for HLA-DQB1 and –DRB1 (red arrows). Sequencing reactions are performed using the core sequencing primers (blue arrows) and the SBTessenz® specified GSSPs (green arrows).

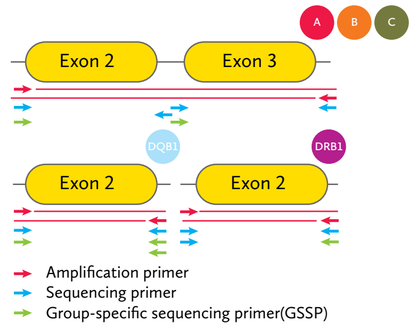


Figure 2. Robust HLA locus-specific amplification on multi-center sample panels

Agarose gel-picture depicting a representation of amplicons obtained from sample panel LUMC (n=192), BFR (n=96), and UMASS (n=42) using SBTessenz® HLA-A, -B, -C, DQB1, and –DRB1 typing assays.

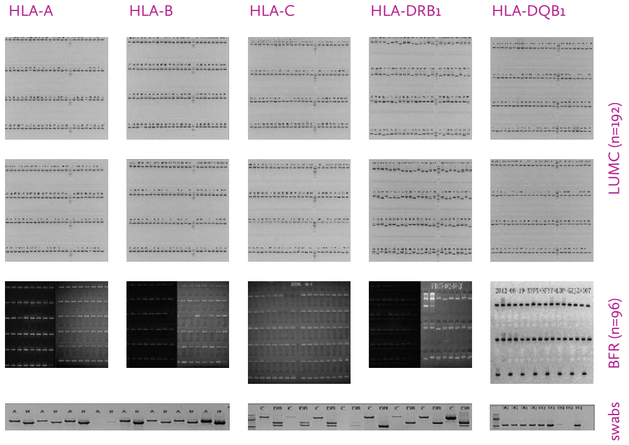


Figure 3. HLA-DRB1 sequence data showing high quality sequencing scores on buccal swab isolates.

Example of HLA-DRB1 sequence data obtained from exon 2 forward and reverse sequencing primers, as analyzed by the SBTengine® software.

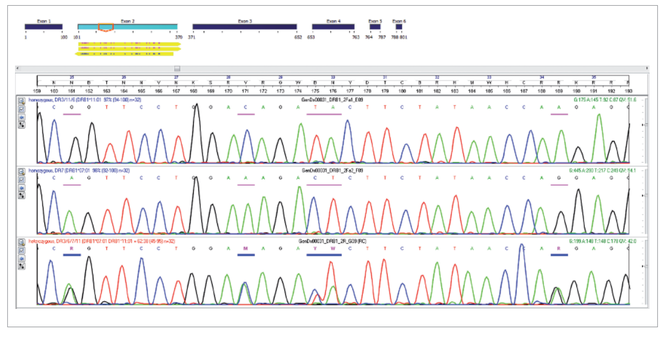
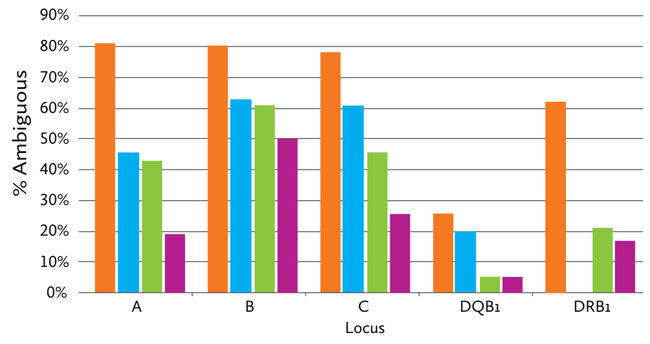


Figure 4. Enhanced genotype resolution when applying the SBTessenz® strategy

Genotype resolution was evaluated at the second field level for the Chinese and Caucasian sample panels. Comparison was made between the SBTessenz strategy (Core plus GSSPs) versus a generic approach (Core only). "Generic CWD / SBTessenz CWD" represents the resolution levels when CWD (-/-) ambiguous allele combinations are ignored. (CWD: source Cano et al. 2007).

Caucasian case study



Chinese case study

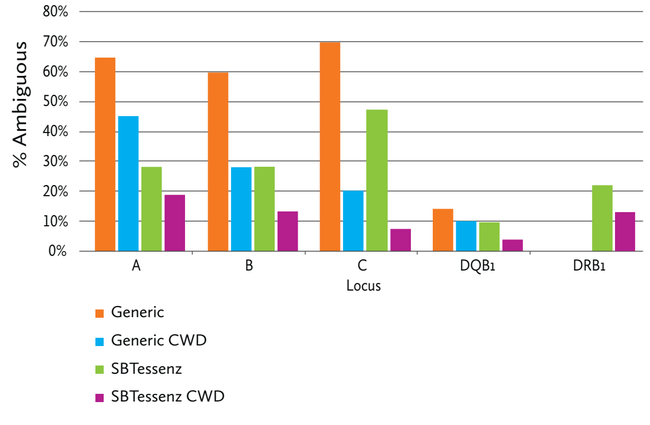


Figure 5. Genotype resolution when applying SBTessenz

Genotype resolution is shown for the number of samples that have a common genotype when CWD (-/-) and CWD (+/-) combinations are ignored.

Sample panel	Locus	Single common genotype CWD +/+
Caucasian	HLA-A	98%
	HLA-B	86%
	HLA-C	99%
	HLA-DQB1	97%
	HLA-DRB1	97%
Chinese	HLA-A	99%
	HLA-B	99%
	HLA-C	99%
	HLA-DQB1	98%
	HLA-DRB1	97%

Key Features SBTessenz®

- Unique identification of the antigen recognition domains for HLA-A, -B, -C, -DQB1 and –DRB1
- One-step DNA sequencing strategy with enhanced ambiguity resolution
- Robust performance on different gDNA materials including buccal swabs
- Plate set-up for high throughput of large sample panels
- Reduced technical effort for improved accuracy
- Low-cost typings suitable for donor registries

Conclusions

The multi-center study shows that the SBTessenz® assays identifies the antigen recognition site of HLA-A, -B, -C, -DQB1, and -DRB1 in an accurate and robust manner and provides enhanced genotype resolution in one DNA-sequencing step. The SBTessenz® assays are good complementary assays to the full gene SBT approach as it enables reliable HLA typings on buccal swab isolates and gDNA of lower quality.

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