An improved and reliable SBT strategy for HLA-DQB1 typing

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Introduction
Sequencing-based typing (SBT) is the gold standard for high-resolution tissue typing, which is required for optimal HLA matching between donor and recipient in stem-cell transplantation settings. Almost all currently used SBT strategies for HLA-DQB1 typing employ amplification and/or sequencing primers located within exon 2 and 3 resulting in a partial coverage of exon 2 and 3. For example, DQB1*030101 cannot be differentiated from DQB1*0319 due to the lack of sequencing information at the 3’ end of exon 3.

Here we present an improved SBTecaccelerator HLA-DQB1 strategy that accelerates unambiguous DQB1 typing and provides high-resolution HLA-DQB1 typing results for routine practice (see Fig. 1).

SBTecaccelerator HLA-DQB1 features:
1. Exons 2 and 3 amplified in one single PCR reaction.
2. Group-specific sequencing primers (GSSPs) for genotype ambiguity resolution.
3. Complete exon 2 sequence information provided (NEW).
4. Complete exon 3 sequence information for enhanced allele ambiguity resolution (NEW).

Results 1)
Exons 2 and 3 amplified in one single PCR reaction
The improved SBTecaccelerator HLA-DQB1 kit enables the amplification of the entire exon 2 and exon 3 in a single, non-multiplex PCR reaction. Amplifying exon 2 and exon 3 in a single PCR product assures that exon 2 and 3 are present in equimolar amounts. This strategy ensures the amplification of both alleles present in the whole amplified region, i.e. exon 2 and 3.

Complete exon 2 and 3 sequence information provided
The amplification and sequencing primers of the improved SBTecaccelerator HLA-DQB1 kit are located at positions that enable sequencing of the complete exon 2 and exon 3, thereby significantly increasing the accuracy of DQB1 typing (see Fig. 2).

Results 2)
Complete exon 3 sequence information for enhanced allele ambiguity resolution
HLA-DQB1 typing based on exon 2 and exon 3 ensures the reliable identification of alleles differing only in exon 3 (e.g. DQB1*030101 and DQB1*0319 that differ at position 650 of exon 3) (Fig. 3).

Figure 1. Overview of the improved SBTecaccelerator HLA-DQB1 kit.
SBTecaccelerator HLA-DQB1 kits, SBTengine software, QIAquick PCR purification Kit and DyeEx Kits are for Research Use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Figure 2. Overview of sequences obtained using different commercial HLA-DQB1 SBT strategies.
The exon 2 and 3 sequences obtained using a commercially available HLA-DQB1 typing kit (A), the previous version (B) and the improved SBTecaccelerator HLA-DQB1 kit (C). The regions that provide supplementary sequence information. The red indicator bars show the sequence parts that are not sequenced. Please note the increased sequence lengths in (C) as compared to (A) and (B).

Figure 3. Sequencing data of HLA-DQB1 exon 3.
Sequence data generated at the 3'-end of exon 3 using the new improved SBTecaccelerator HLA-DQB1 kit. The heterozygous sample carrying DQB1*030101, 050301 demonstrates that allele DQB1*030101 can clearly be distinguished from DQB1*0319 by analyzing position 650 (see black arrow).

Results 3)
The new SBTecaccelerator HLA-DQB1 kit provides unambiguous typing results
A set of 7 group-specific sequencing primers (GSSPs) was developed, resolving 97% of all theoretical resolvable genotype ambiguities. In daily practice this strategy will lead to an unambiguous allele assignment for more than 98% of the samples (see Fig. 4).

Conclusions
The improved SBTecaccelerator HLA-DQB1 kit accelerates unambiguous DQB1 typing results and provides high-resolution HLA-DQB1 typing (see Fig. 1).

The novel strategy has several important and unique features:
1. Exons 2 and 3 are amplified in a single robust PCR reaction, thereby increasing accuracy and minimizing the number of amplifications.
2. Exons 2 and 3 can be sequenced in forward and reverse direction.
3. Complete sequence information of both exons is provided (see Fig. 2 and 3).
4. Complete exon 3 sequence information facilitates the resolution of allele ambiguities, for instance HLA-DQB1*030101 and HLA-DQB1*0319.
5. A set of 7 GSSPs are provided to resolve 97% of all theoretical resolvable genotype ambiguities.

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