Optimizing gene coverage for HLA class II gene sequencing

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For unambiguous typing results, sequencing of whole HLA genes is required. For the Class I genes, whole gene strategies are already available from GenDx. However, for Class II genes, achieving this coverage is more challenging, mainly due to the large intron 1 region.

Figure 1a

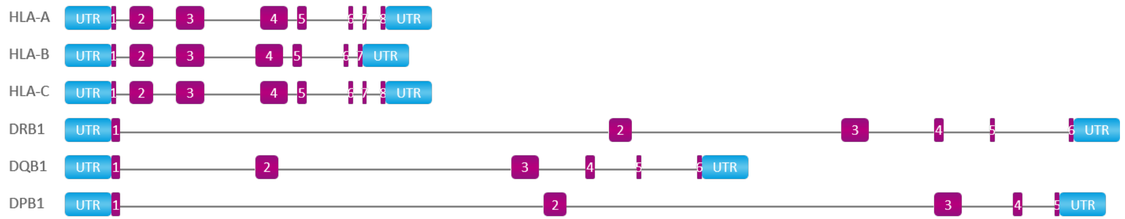
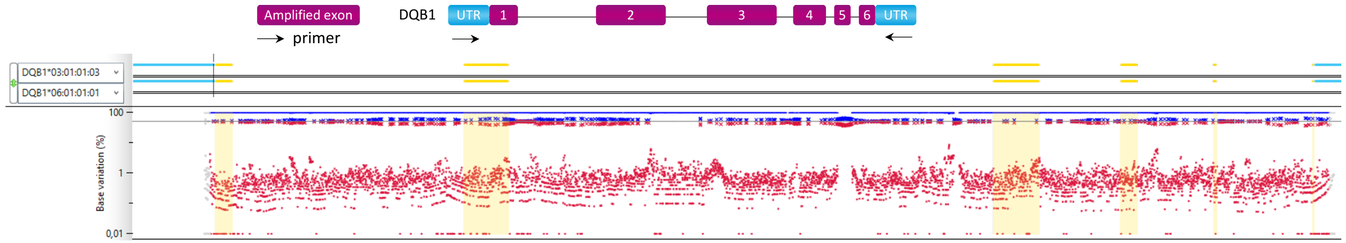


Figure 1b



In **Figure 1a** HLA Class II genes are longer than class I genes. Genes are drawn to the same scale. Exons are numbered and are coloured purple. Robust amplification of the whole DQB1 gene using primers in the 5'and 3'UTR was still possible. **Figure 1b** shows the amplification of a sample with a DQB1*03:01:01:03, *06:01:01:01 genotype. The amplification shows little noise (max 3% in the exon regions) and is well balanced. As shown in figure 1a, DPB1 and DRB1 are the largest HLA genes. For DPB1 and DRB1 both a two amplicon and one amplicon strategy was investigated (slide 2 and 3).

Figure 2a Sequencing results of a DPB1 Whole Gene two amplicon strategy. A small part of intron 1 is excluded from the amplification. The amplicons are sequenced on an Illumina MiSeq. This strategy gave robust and concordant typing results but could not be completely phased **Figure 2b** shows a one amplicon strategy. DPB1 is amplified from UTR to UTR and sequenced on a PacBio Sequel II. This strategy was successful when using gDNA samples that showed a high integrity. Low quality gDNA samples proved troublesome at times and further investigation into the one amplicon strategy is ongoing. The bold red line shows that all heterozygous positions are phased. This indicates that allele specific sequences from 5' UTR to 3' UTR could be generated with the one amplicon strategy for DPB1.

Figure 2a

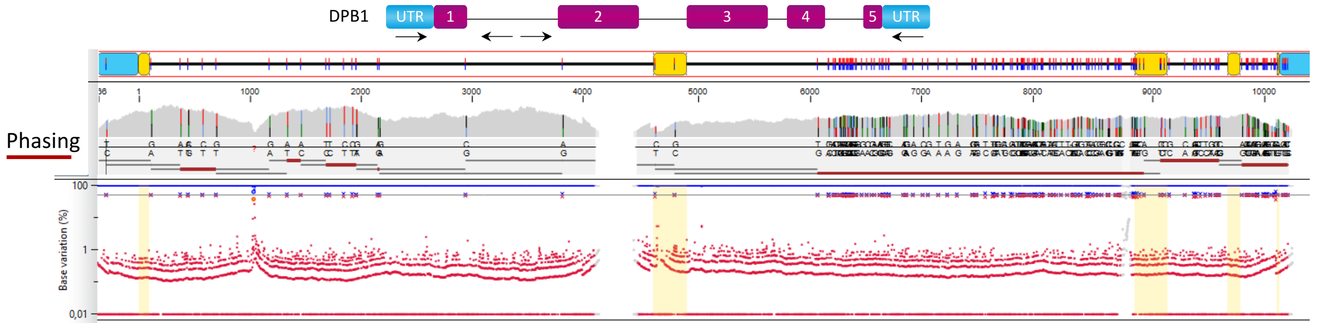


Figure 2b

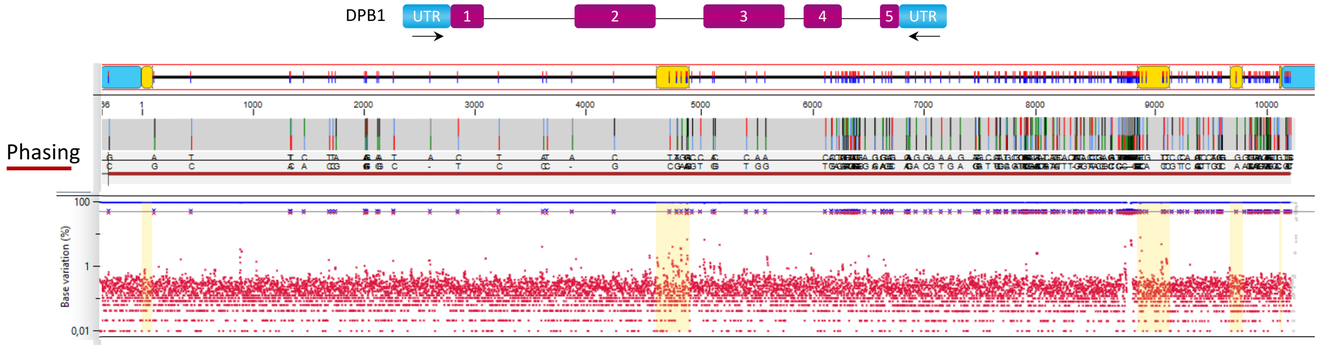


Figure 3a Sequencing results of a DRB1 Whole Gene two amplicon strategy. A small part of intron 1 is excluded in the amplification. The amplicons were sequenced on an Illumina MiSeq. This strategy gave robust and concordant typing results

Figure 3b shows the one amplicon strategy for DRB1. DRB1 is amplified from UTR to UTR and sequenced on a PacBio Sequel II. This strategy was successful when using gDNA samples that showed a high integrity. DRB1 could be phased completely.

Figure 3a

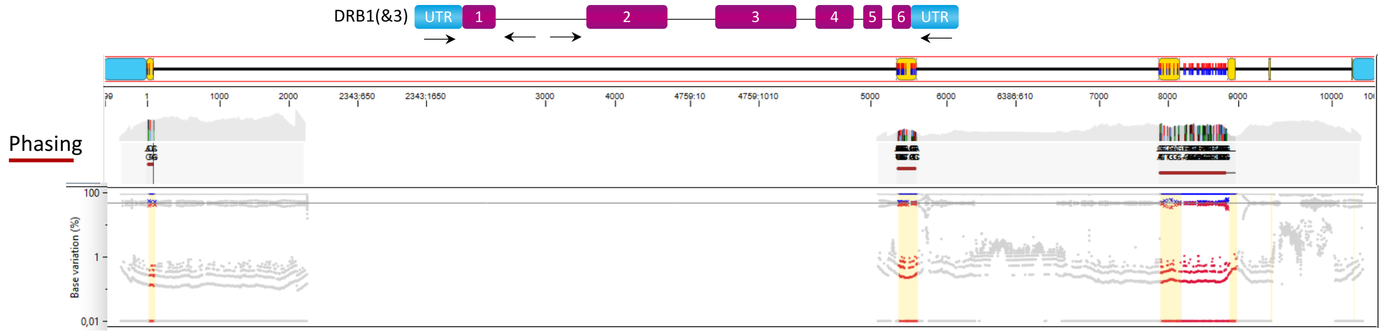


Figure 3b

Conclusions

HLA-DQB1 can be covered by a single amplicon PCR strategy, yielding robust and high quality sequence data. HLA-DRB1 and HLA-DPB1 can be amplified as one amplicon as well. However, a two amplicon strategy, that separates exon 1 from the rest of the gene, shows superior robustness.