Development of an independent sequencing workflow for confirmation of homozygous HLA typing results.

A homozygous HLA typing result can have different causes. In most cases, the sample studied is truly homozygous, meaning both parents passed on an identical HLA allele. However, it is possible that a homozygous typing is introduced by the method used. In this case, only one allele is processed correctly and the second allele is missed. Such an analytical artifact can happen in both PCR based and capture based methods. With PCR this artifact is most commonly caused by a mismatch with the primer in the amplification reaction. Although most commercial HLA primers are designed to match the majority of HLA alleles, rare alleles can exist that will not match the primer.

Complementary PCR methods

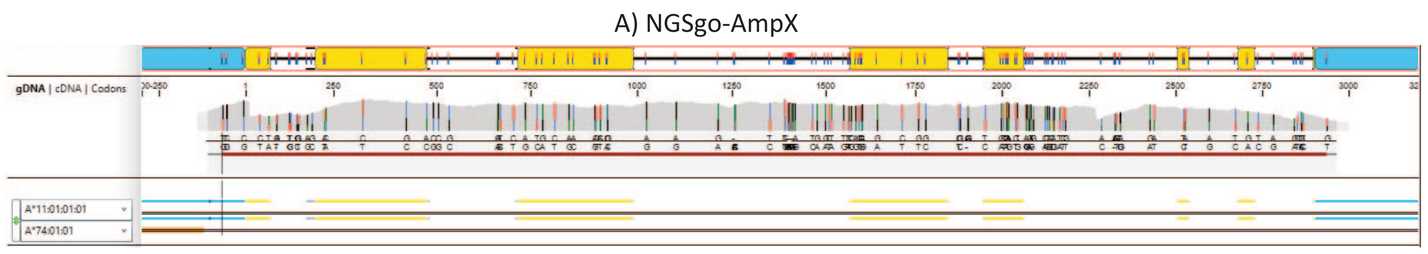
The best way to confirm zygosity is by using a second method. As GenDx offers multiple well tested amplification strategies for HLA typing, an alternative amplification method to confirm homozygous results could be defined (Table 1). For example, when using NGSgo-AmpX HLA-A, a second independent amplification method that can be used is AlleleSEQR HLA-A. The primers in these two methods do not overlap, meaning that a missed allele using method 1 will be detected using method 2.

Sanger to NGS

Originally, AlleleSEQR HLA amplfications were developed for Sanger-based HLA typing. Here we show results of the compatibility testing for AlleleSEQR HLA kits with NGS. Figure 1 shows NGS results of the HLA-A amplification using both the NGSgo and AlleleSEQR amplification strategy. The alignment and coverage plot shows that both amplicons yield high quality, phased data with concordant typing results. Moreover, the coverage plot shows that a different area of the gene is amplified by both methods. Indicating the feasibility to use AlleleSEQR HLA-A PCR to confirm homozygous results obtained with NGSgo-AmpX HLA-A. An example for HLA-B amplifications with 2 different methods is shown in figure 2 and gives equally good results.

locus	Method 1	Method 2
HLA-A	NGSgo AmpX MX6-1 SBTexcellerator	AlleleSEQR
HLA-B	NGSgo AmpX MX6-1 SBTexcellerator	AlleleSEQR
HLA-C	NGSgo AmpX MX6-1 SBTexcellerator	AlleleSEQR
HLA-DRB1	NGSgo AmpX MX6-1 SBTexcellerator AlleleSEQR	DRB1 whole gene
HLA-DQB1	NGSgo AmpX MX6-1 SBTexcellerator	AlleleSEQR
HLA-DP1	NGSgo AmpX MX6-1 SBTexcellerator	AlleleSEQR

Table 1. Independent amplification methods that can be used to confirm homozygous typing results.



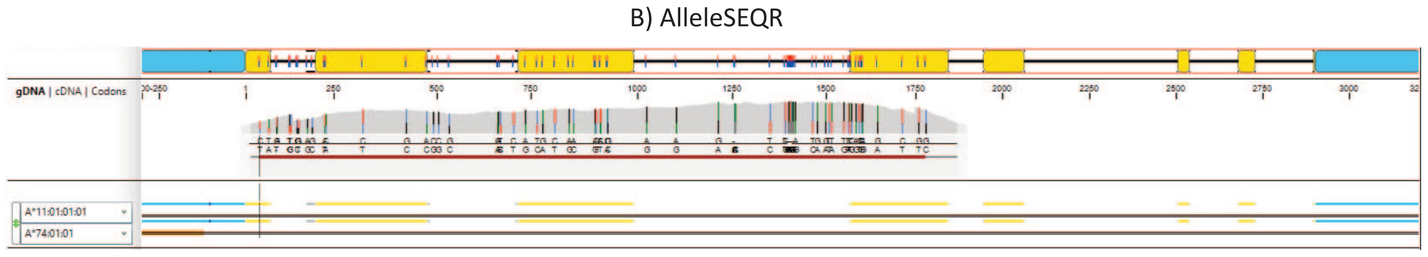
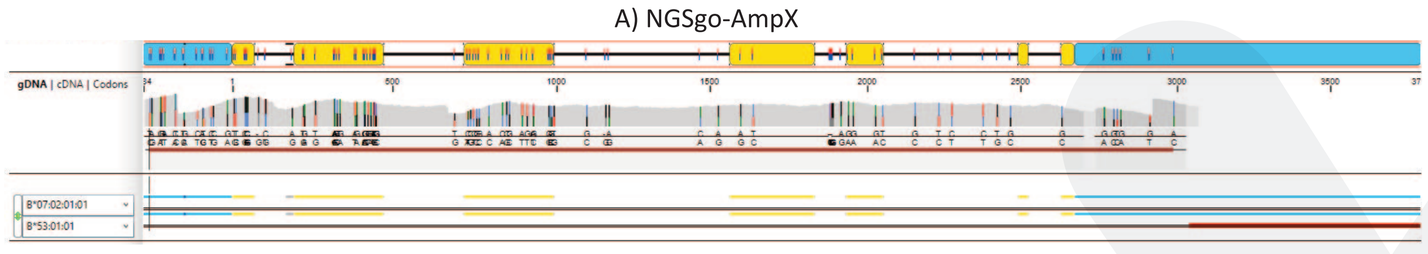


Figure 1. The same sample was amplified for HLA-A with (A) NGSgo-AmpX and (B) AlleleSEQR and analysed in NGSengine. Exons are in yellow, UTR in blue.



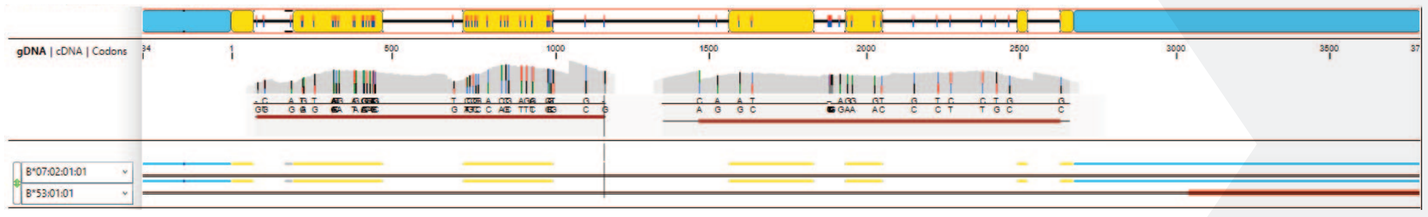


Figure 2. The same sample was amplified for HLA-B with (A) NGSgo-AmpX and (B) AlleleSEQR and analysed in NGSengine. Exons are in yellow, UTR in blue.

Conclusion

Although determined in a small cohort, the Independent methods are available for confirmatory typing for HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 and HLA-DPB1. Moreover, we show that the amplicons generated with the AlleleSEQR method can also be reliably sequenced with NGS and analyzed using NGS based HLA typing software.

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