A singleplex HLA typing approach for nanopore sequencing

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Introduction

GenDx has recently developed the NGS-Pronto® workflow, featuring multiplex amplification and long-read sequencing of 11 classical HLA loci. Complementary to NGS-Pronto®, users could benefit from a supplemental singleplex amplification product. This is useful not only for (confirmatory) single-locus typing for transplantation purposes, but also for the typing of (several) specific HLA loci, in other clinical settings than transplantation, such as disease association. Here, we describe NGSgo-ProntoFLX, a singleplex or duplex HLA typing assay that is compatible with Oxford Nanopore Technologies (ONT) and Illumina sequencing (Figure 1). NGSgo-ProntoFLX will feature specific mixes of all 11 classical HLA loci, allowing for their fast and flexible amplification.

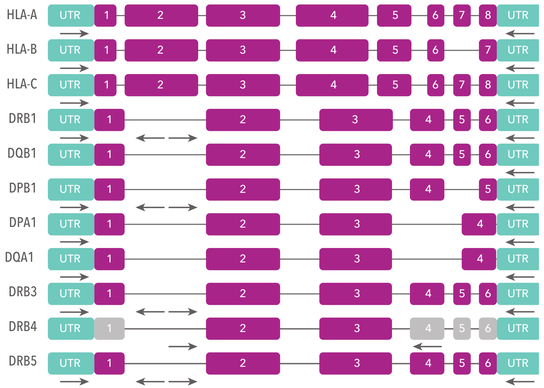

Figure 1. NGSgo-ProntoFLX coverage of all loci. NGSgo-ProntoFLX is designed for near full coverage of all 11 classical HLA genes.

Method

Whole-gene amplicons of 11 HLA loci were generated using 7 singleplex ready-to-use amplification assays, and two duplex assays (HLA-DRB1 + HLA-DRB3 and HLA-DQA1 + HLA-DQB1). Locus-specific amplicons were generated during a ± 120-minute PCR step and checked on a 1% w/v agarose gel. Specifically, performance of the HLA-A, -B singleplexes and HLA-DQA1/-DQB1 duplex was demonstrated using a high diversity HLA panel (GeT-RM HLA58, Coriell Institute). After amplification, NGS-ProntoPrep library preparation was used to obtain sequence-ready DNA libraries in ±90 minutes with ±60 minutes hands-on time. 10 hours of sequencing on an ONT GridION using MinION R10.4.1 flow cells and super accuracy (SUP) basecalling yielded at least 6000 reads per sample. Data was analyzed using NGSengine® 4.0 (GenDx).

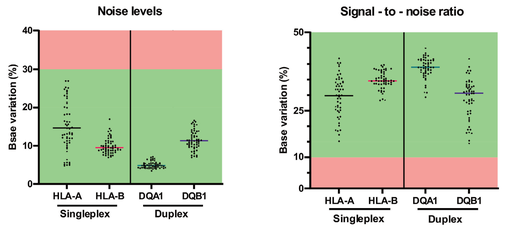

Figure 2. Noise levels and signal-to-noise percentages obtained when sequencing amplicons generated from the GeT-RM panel using NGSgo-ProntoFLX for HLA-A and HLA-B singleplex and DQA1/DQB1 duplex amplification.

	NGSgo-ProntoFLX		
Locus	HLA-A	HLA-B	DQA1/DQB1
# (concordant/typed)	116/116	116/116	232/232
Concordance (%)	100	100	100

Table 1. Typing concordance of NGSgo-ProntoFLX HLA-A, HLA-B singleplex and DQA1/DQB1 duplex amplifications of the 58 sample GeT-RM panel.

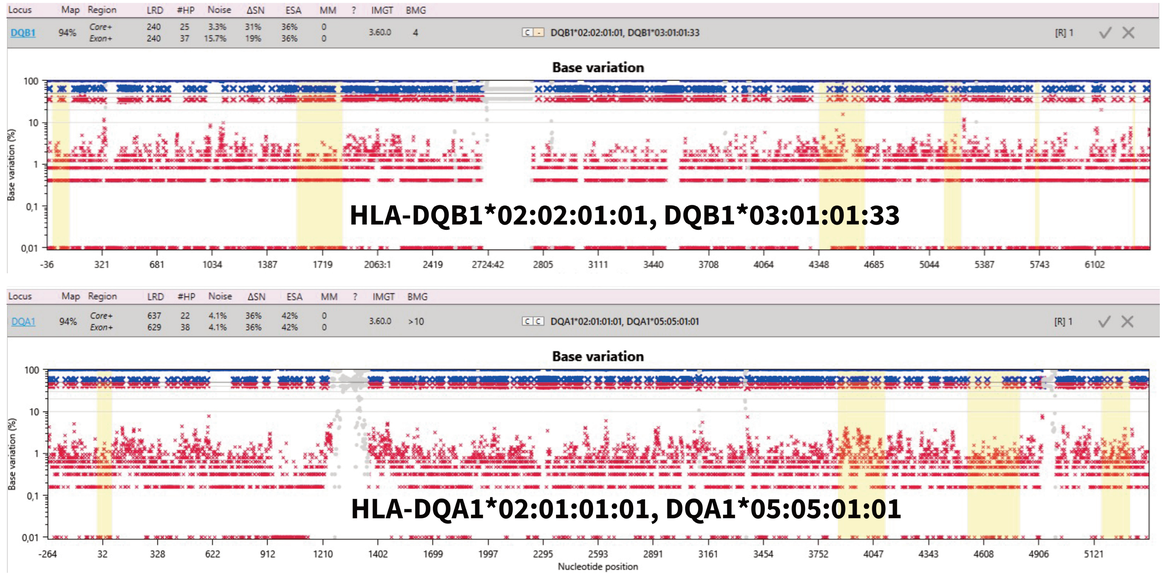

Figure 3. HLA-DQA1 and HLA-DQB1 base variation plots of a sample with a celiac association typical HLA-DQB1*02-DQA1*05 typing.

Results

NGSgo-ProntoFLX amplicons were confirmed on gel (data not shown). Assay performance for HLA-A, -B and HLA-DQA1/DQB1 yielded excellent quality metrics, as evidenced by low noise levels and high signal-to-noise percentages (Figure 2). The high data quality is corroborated by the base variation plots corresponding to a sample containing an allele combination relevant in celiac disease association (HLA-DQB1*02-DQA1*05) (Figure 3). Altogether, this contributed to a 100% typing concordance compared to the pre-typing, which was observed for all samples and all loci analyzed (Table 1).

Conclusion

Here we described the development of NGSgo-ProntoFLX. This amplification strategy enables fast and flexible typing of (combinations of) individual HLA loci. Initial validation studies on the HLA-A, HLA-B and HLA-DQA1/-DQB1 specific amplification assays have demonstrated their robust and specific amplification, yielding 100% typing concordance and excellent data quality metrics.

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