NGSgo-ProntoFLX: Single- or Duplex HLA Amplifications for Confirmatory Typing or Disease Association

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Introduction

High-resolution HLA typing is essential for transplantation diagnostics and is increasingly employed in disease association studies. Our new HLA amplification product, NGSgo-ProntoFLX, featuring two singleplex assays (HLA-A and HLA-B) and one duplex assay (HLA-DQA1, -DQB1), is suitable for use in both contexts. Here we show that NGSgo-ProntoFLX can be used for low- and high-throughput typing (between 1 and 96 samples) and allows for seamless integration with our multiplex NGSgo-ProntoAmp workflow.

Methods

In this study, cell-line extracted DNA from the 58-sample GeT-RM high diversity HLA-panel (Coriell) were used. A low- (1-sample) and high-throughput (96-sample) amplification run were performed according to the NGSgo-ProntoFLX workflow (Figure 1). To evaluate whether NGSgo-ProntoFLX and NGSgo-ProntoAmp amplicons can be pooled in a single NGS-ProntoPrep workflow, NGSgo-ProntoFLX-derived HLA-A and HLA-DQA1, -DQB1 amplicons from a single sample were combined with NGSgo-ProntoAmp amplicons covering 11 HLA loci from eight samples. Sequencing of libraries was performed on a GridION and data was analyzed using NGSengine.

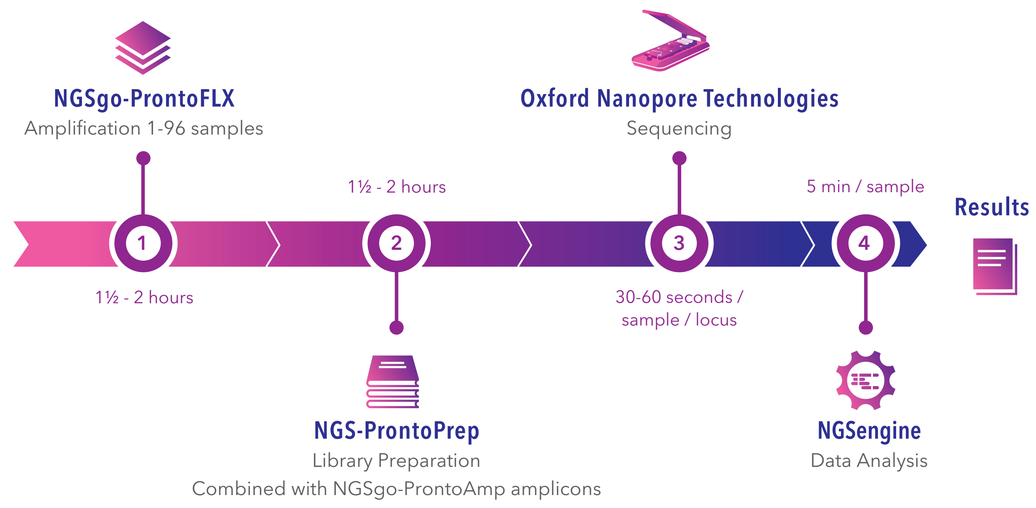

Figure 1. Schematic representation of the NGSgo-ProntoFLX workflow.

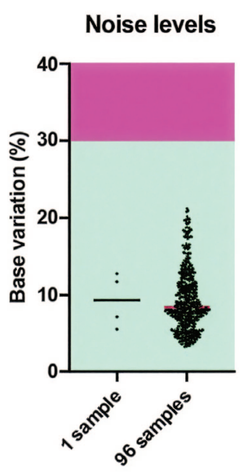

Figure 2. Low noise levels were obtained when sequencing either 1 or 96 samples in a single library using NGSgo-ProntoFLX amplifications for HLA-A and HLA-B singleplex and HLA-DQA1, DQB1 duplex.

Table 1. 3rd-field typing concordance of NGSgo-ProntoFLX HLA-A, HLA-B singleplex and DQA1, -DQB1 duplex amplifications of 1 and 96 samples in a single sequencing library.

	NGSgo - ProntoFLX		
Locus	HLA -A	HLA -B	DQA1/DQB1
1 - sample library (concordant/typed)	2/2	2/2	2/2
96 - sample library (concordant/typed)	182/182	182 /182	364 /364
Concordance (%)	100	100	100

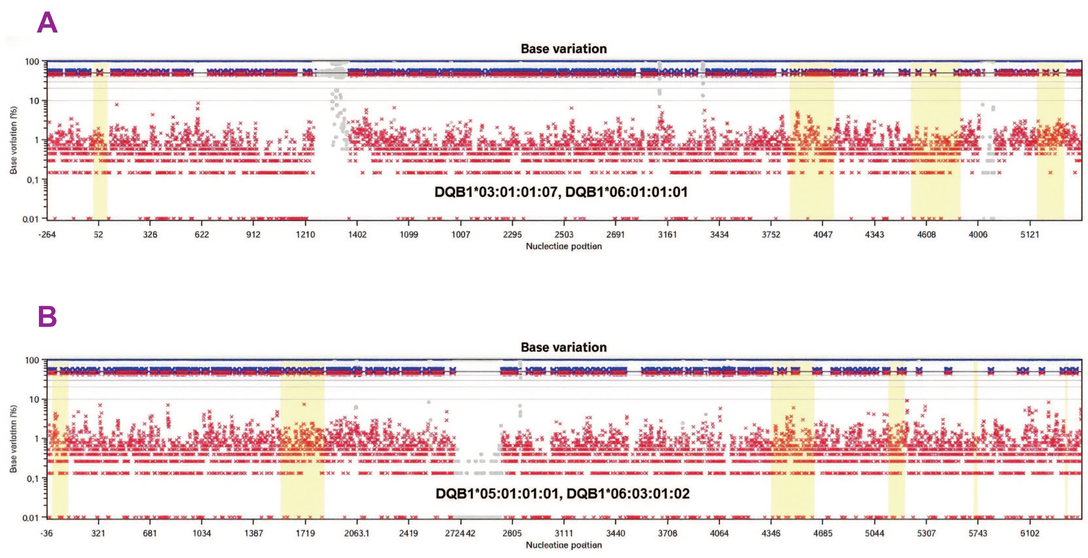

Figure 3. High sequencing data quality as observed in two representative HLA-DQB1 base variation plots of one NGSgo-ProntoFLX (A) and one NGSgo-ProntoAmp (B) sample in the combined workflow.

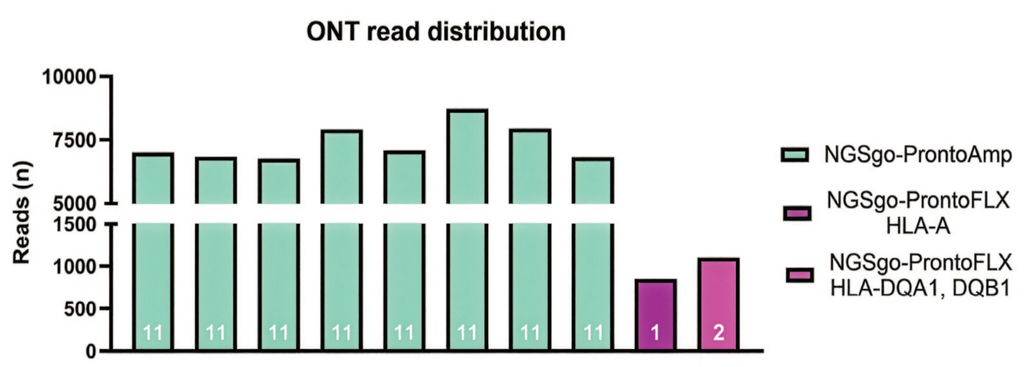

Figure 4. Equimolar pooling of 8 samples amplified with NGSgo-ProntoAmp (11 loci) and 1 sample amplified with NGSgo-ProntoFLX (1 or 2 loci) ensured reads were generated proportionally to the number of loci in each sample in combined NGSgo-ProntoPrep workflow.

Results

- Low and high-throughput NGSgo-ProntoFLX runs yield excellent data quality, evidenced by low noise levels (Figure 2) and full 3rd-field typing concordance with the pre-type of all loci (Table 1).
- NGSgo-ProntoFLX and -ProntoAmp amplicons can be combined in a single NGS-ProntoPrep workflow, as demonstrated by the full 3rd-field typing concordance and high-quality data (Figure 3).
- 30 seconds of sequencing time per locus were achieved by equimolar pooling of NGSgo-ProntoFLX and NGSgo-ProntoAmp amplicons, ensuring reads were generated proportionally to the number of loci per sample (Figure 4) when combined in a single workflow.

Conclusion

Here we demonstrate that NGSgo-ProntoFLX is a flexible and versatile assay, yielding reliable high-resolution HLA genotyping of between 1 to 96 samples and allowing smooth integration with NGSgo-ProntoAmp workflows (Figure 1). These features make NGSgo-ProntoFLX suitable for use in transplantation diagnostics and disease association studies.

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