NANOPORE-BASED HLA TYPING DEMONSTRATED HIGH ACCURACY AND ADVANTAGES IN RESOLVING CHALLENGING CASES

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Introduction

The advantages of using long-read sequencing technology in HLA typing have been increasingly recognized in recent years. This single-center, pilot study aims to evaluate the accuracy of one commercially available long-read sequencing-based HLA typing assay at different typing resolutions for potential applicability in both solid organ and hematopoietic cell transplant settings.

Materials and Methods

Forty-four patient and donor DNA samples previously typed with a clinically validated next-generation sequencing assay (MIA FORA NGS MFLEX HLA Typing Kit, Werfen, Spain) on the Illumina platform (Miniseq) were included in the study. Both common and rare HLA alleles were represented in the HLA genotypes of these samples. Based on the previous short-read sequencing analysis, some technically challenging samples with phasing ambiguity, coverage issues, repetitive elements, and HLA loss of heterozygosity (LOH) were also included. Eleven HLA loci were amplified using the NGS-Pronto kit (GenDx, Netherlands) followed by library preparation using Oxford Nanopore native barcoding kit. Sequencing was performed on Nanopore GridION for 12 hrs with the super accuracy setting, and data was analyzed using the NGSengine Turbo software. Concordance with prior typing results was evaluated at low and high resolutions, and to the third and fourth fields.

Results

	All HLA Loci (%)	Class I HLA Loci (%)	Class II HLA Loci (%)
Concordance at Low Resolution	100	100	100
Concordance at High Resolution	100	100	100
Concordance to the third field	99.7	100	99.6
Concordance to the fourth field	70.0	79.3	65.3

Figure 1: Phasing Ambiguity using MFlex HLA Typing Kit and NGS-Pronto Kit. (A, B) Werfen/Illumina library preparation using Werfen typing software had difficulty resolving the allele ambiguities due to the phasing limitation. (C, D) GenDx/Nanopore library preparation and software was able to generate the correct allele calls without phasing ambiguity.

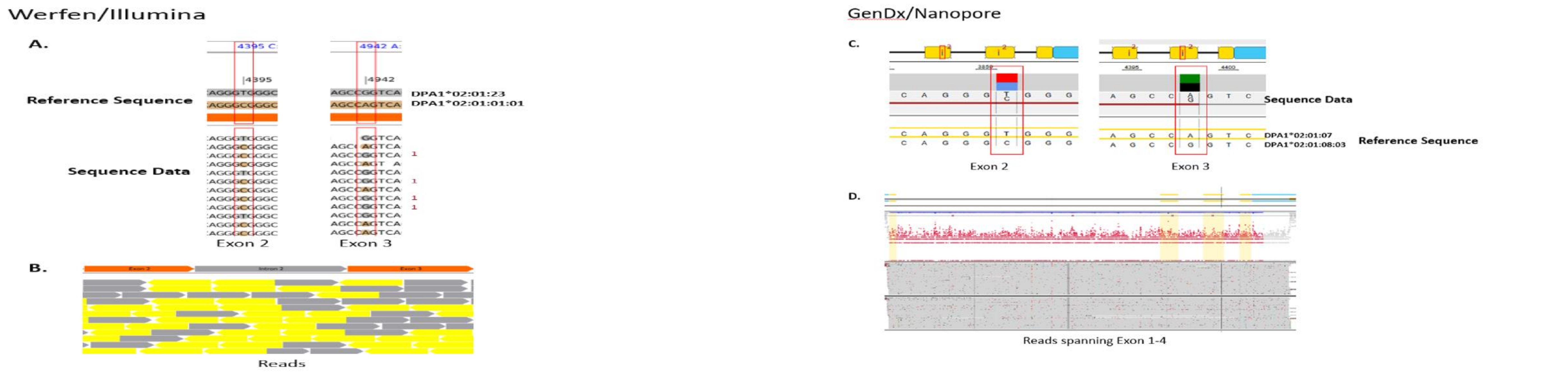


Figure 2: Repetitive Region of DRB1*15:01:01. (A) IMGT database showed there are 34 Ts in Intron 5. (B) Due to the limitations of sequencing homopolymer/STR regions, Werfen/Illumina generated variable-sized PCR fragments. (C) GenDx/Nanopore performed better at distinguishing base difference (G/T) between 15:01:01:01 and 15:01:01:06, providing sufficient coverage and reads in phase.

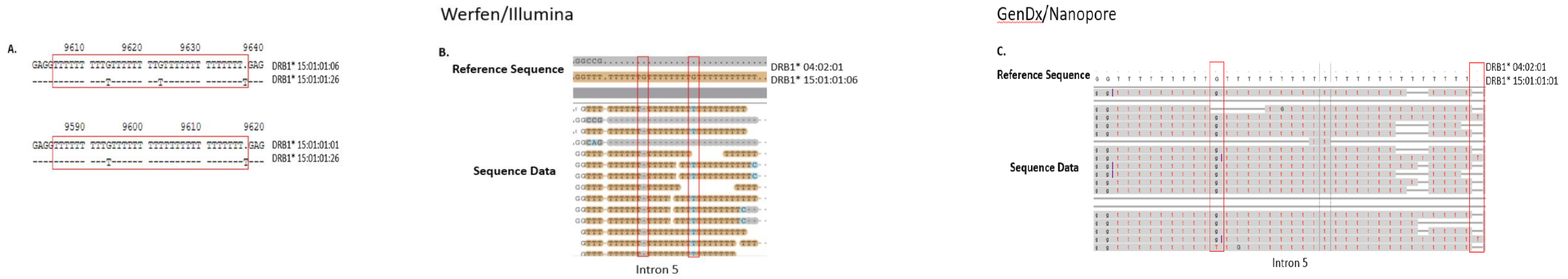
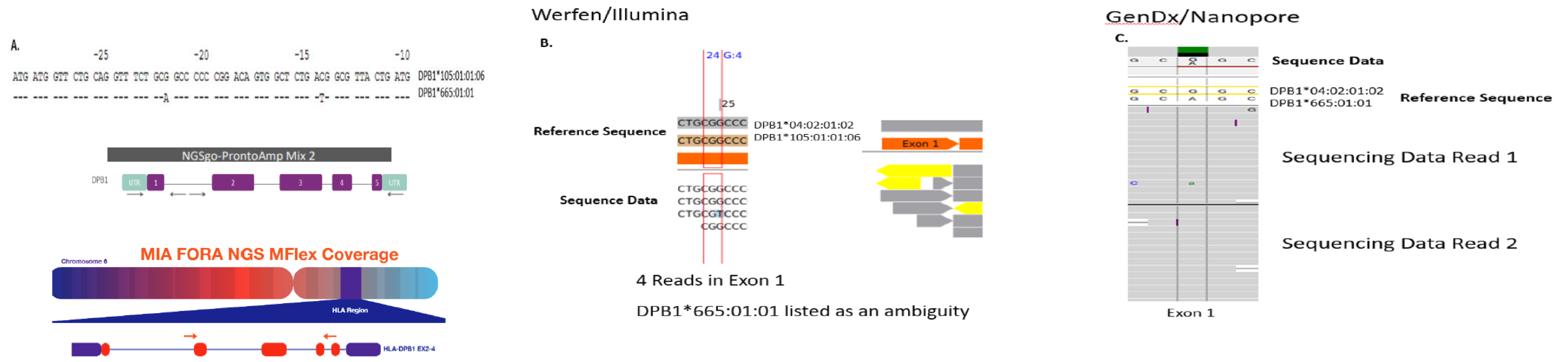


Figure 3: Lack of coverage in exon 1 of HLA-DPB1 and inability for phasing with Wenfen/Illumina assay. (A) Sequence differences between DPB1*105:01:01:06 and DPB1*665:01:01. Primer locations of MIA FORA NGS MFLEX HLA typing kit and NGS-Pronto kit are shown. (B) Lack of PCR primers targeting exon 1 and majority of intron 1 in MIA FORA NGS MFLEX HLA typing kit contributed to ambiguity in DPB1 allele calls. (C) GenDx/Nanopore was able to accurately type the DPB1 alleles with adequate coverage of exon 1 and intron 1.



Conclusions

This study demonstrated high accuracy of HLA typing results to the third field between the NGS-Pronto kit and MIA FORA NGS MFLEX HLA typing kit,. The long-read sequencing assay is particularly advantageous in resolving phasing ambiguity, coverage issues, repetitive regions and rare alleles. The data, though pending full clinical validation, supports the feasibility of utilizing Nanopore-based technology in generating accurate HLA typing results to meet the needs of both solid organ and stem cell transplantation.

Acknowledgement

We would like to thank GenDx for the library preparation reagents and the typing software.

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