HLA-SuBiTo NGS*type*® & RBC-NGS*type*® CORE: Combining high-resolution HLA & ABO typing via third-generation sequencing in one test to allow for high-throughput typing

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Background:

Joseph *et al.*, 2023 reported that up to 65% more ABO*A2 deceased donors can be identified via sequencing in contrast to phenotyping. Up until recently, most commercially available methods focused on either HLA or blood group typing, requiring two separate experiments to obtain the full HLA and ABO typing.

With the introduction of third-generation sequencing methods by Oxford Nanopore Technologies, there is now the option to combine both assays into one experiment, allowing for a high-throughput approach that results in high-resolution HLA and blood group typing results.

Aims:

We present the validation of such a methodology that combines both amplifications in the same library preparation step, allowing for library preparation and sequencing of HLA and ABO typing to be run in one experimental approach.

Methods:

11-loci HLA typing was performed by HLA-SuBiTo NGStype®, ABO typing was performed by RBC-NGStype® CORE (both inno-train). Both kits use an NGS sequencing approach and can be combined with either Illumina or ONT machines.

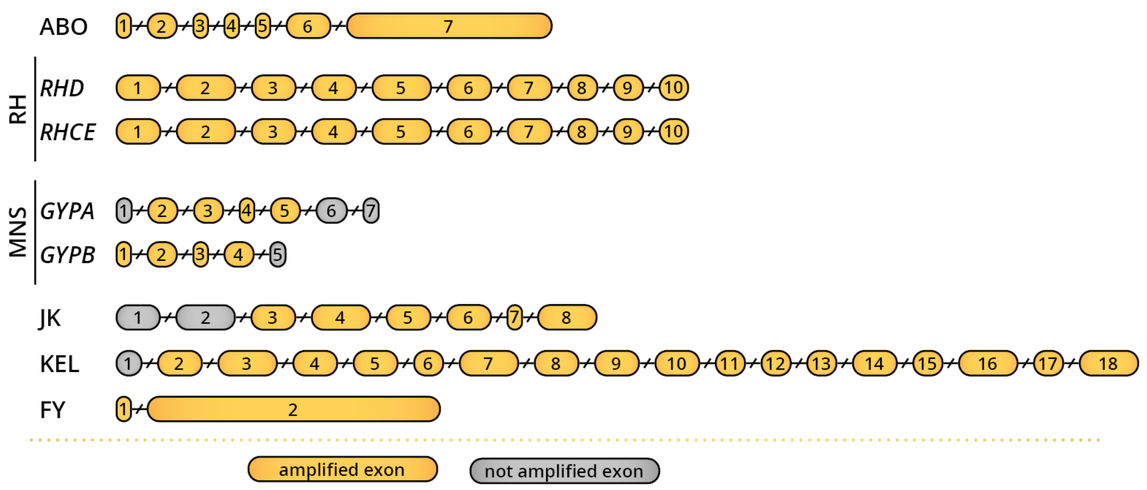


Fig. 1: Coverage of RBC-NGS*type*® CORE

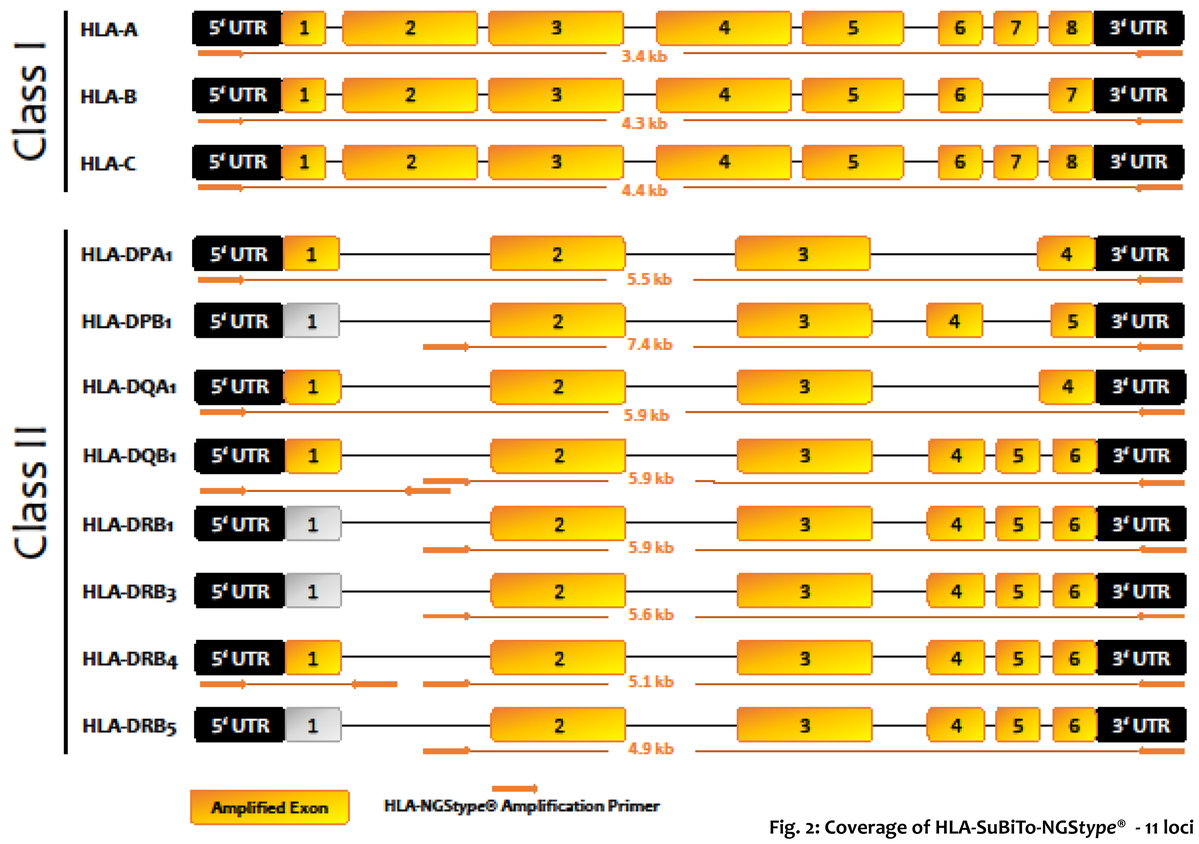


Fig. 2: Coverage of HLA-SuBiTo-NGS*type*® - 11 loci

Results:

The approach was tested on 20 samples. The results demonstrated a 100% concordance between the real-time PCR and the NGS approach.

The NGS results often extended the Real-time PCR results, helping to clarify typing results. An example can be found in table 1. The table includes all common and well-documented alleles. If only common alleles are chosen, an even better resolution might be possible.

DNA	Method	HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DRB3		HLA-DRB4		HLA-DRB5		HLA-DPA1		HLA-DPB1		HLA-DQA1		HLA-DQB1		ABO	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Inno-train S5	RT-PCR	*02	*32	*40	*50	*02	*06	*07	*09	-	-	*01	*01	-	-	*01:03:01:01	*01:03:01:01	*02:01	*04:01:01:01	*02:01:01:01	*03:02:01:01	*02:02:01:01	*03:03:02:01	*A1.01	*O.01
Inno-train S5	NGS	*02:05:01:01	*32:01:01:01	*40:02:01	*50:01:01	*02:02:02	*06:02:01	*07:01:01:01	*09:01:02	-	-	*01:03:01:01	*01:03:02	-	-	*01:03:01:01	*01:03:01:01	*02:01:02	*04:01:01:01	*02:01:01	*03:02:01	*02:02:01	*03:03:02	*A1.01	*O.01.01

Table 1: Example of a typing result via RT-PCR and NGS

Summary / Conclusions:

The integration of 11-locus HLA typing and ABO genotyping within a single library preparation enables robust, high-throughput analysis of large cohorts. The high-resolution NGS results provide the accuracy required for confident donor-recipient matching in transplantation settings. In addition, the short turnaround time of approximately 3.5 hours per sample highlights the potential applicability of this approach for deceased donor typing.
Even further, the new RBC-NGS*type*® ABOnly long-range ABO NGS kit can even be run in the same initial PCR and pooled in the same library preparation workflow.