GENDX

personalizing diagnostics

Performance of a Multiplex PCR Amplification for NGS-Based Typing of Six HLA Genes

Introduction

Accurate NGS-based HLA typing heavily depends on a reliable PCR amplification of HLA genes. Most protocols apply a method in which each HLA gene is amplified separately, after which amplicons are pooled and processed in a library preparation procedure. We have developed a reliable multiplex PCR for HLA-A, B, C, DRB1, DQB1 and DPB1, bringing down the number of amplifications from six to only one reaction per sample. In development of a multiplex amplification strategy, no compromises were made on robustness of the amplification and genotyping result.

Materials & Methods

HLA gene-specific amplification primers, based on NGSgo-AmpX primers (GenDx), were combined in one PCR using the GenDx-LongMix mastermix. Amplicons were processed in the NGSgo® workflow (GenDx) for library preparation and sequenced on a MiSeq (Illumina) with application of paired-end sequencing (2x151 bp). Data was analyzed in HLA typing software NGSengine® (GenDx).

Results

Our multiplex PCR protocol was developed to obtain a robust amplification with 6 HLA genes in one PCR (Figure 1). For time efficiency, the PCR protocol had been reduced from 5 to 2.5 hours, making it feasible to do the entire NGS workflow in one day (Figure 2). Data analysis in NGSengine® demonstrated that the quality parameters were well within acceptance range, showing a generally high mappability (Figure 3a: map. >90%) and low noise level (Figure 3c: noise <6.3% and delta Signal/Noise >23%), with balanced allele levels (Figure 3d: allele ratio of ~47% for heterozygous samples). The assay was optimized such that the read depth of each gene was similar (Figure 3b) to make cost-efficient use of the output of the flow cell.

Figure 1. Robust amplification with NGSgo® multiplexed HLA amplification primers, demonstrated by gel electrophoresis of the amplicons.

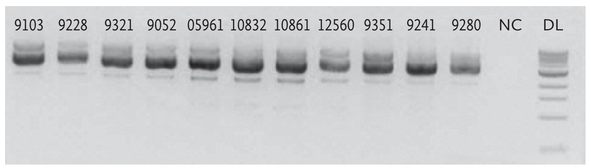
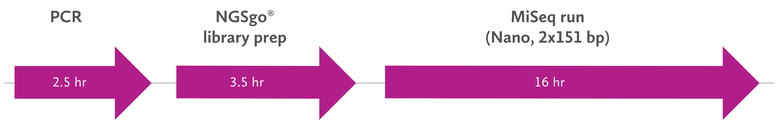


Figure 2. One-day workflow for 12 samples with the multiplexed NGSgo® HLA PCR.

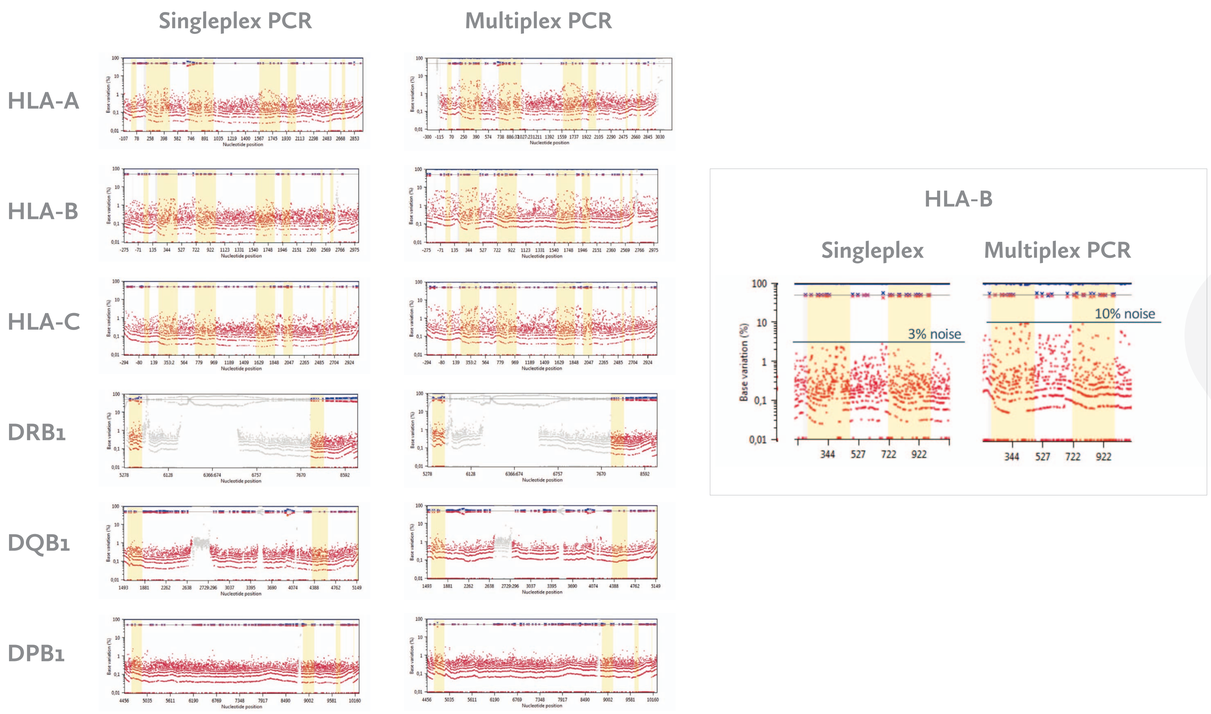


Comparison of the multiplex PCR data with the NGSgo®-AmpX singleplex PCR revealed slightly elevated noise levels in Class I genes (Figure 4). This was due to formation of hybrid reads, a PCR-artefact inherent to the multiplex PCR. Other multiplex strategies showed noise levels up to 20% (data not shown) which hampers accurate HLA typing. The new optimized multiplex PCR protocol described here, has brought the noise levels down to well below <12%, crucial for reliable HLA typing.

Figure 3. Representative example of typing results in NGSengine® of sample IHWG9351 with multiplexed NGSgo® HLA amplification primers.

Locus	Map	Region	LRD	#HP	Noise	ΔSN	ESA	MM	?	IMGT	BMG		a
P IHWG9351	a		b		c	c	d						85679/93330 (91%)
HLA-A	99%	*Exon+:*	694	57	3.9%	39%	49%	0		3.31.0	1		A*24:02:01:01, A*26:01:01:01
HLA-B	99%	*Exon+:*	491	45	6.3%	37%	48%	0		3.31.0	3		B*40:02:01:01, B*54:01:01
HLA-C	99%	*Exon+:*	885	24	3.9%	38%	47%	0		3.31.0	2		C*01:02:01:01, C*03:04:01:02
DRB1	97%	*Exon+:*	414	32	1.6%	23%	40%	0		3.31.0	3 !		DRB1*04:05:01:01, DRB1*11:06:01
DQB1	99%	*Exon+:*	478	27	2.6%	41%	47%	0		3.31.0	1		DQB1*03:01:01:14, DQB1*04:01:01:01
DPB1	99%	*Exon+:*	488	0	1.4%	-	-	0		3.31.0	>10	H	DPB1*05:01:01:01, DPB1*05:01:01:01

Figure 4. Noise level comparison of singleplex PCR versus multiplex PCR. The multiplex PCR results in slightly elevated noise levels in HLA Class I genes but not in Class II genes. Right panel shows a close-up of HLA-B noise levels.



Conclusions

GenDx has developed a multiplex PCR for HLA-A, B, C, DRB1, DQB1 and DPB1 that is fully compatible with the NGSgo® HLA typing workflow for Illumina. PCR artefacts have been minimized with this new protocol that generates high-quality data with low noise, and supports reliable NGS-based HLA typing.

Van de Pasch LAL, Van Heck M, Van Ham K, Penning MT, Rozemuller EH
GenDx, Utrecht, The Netherlands

GenDx.com