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HLA disease association assignment by multiplexed NGS assay

Aim

Strong association of HLA molecules with autoimmune and inflammatory diseases have been identified, strengthening the value of HLA genetic screening for diagnostic purposes. There is a strong association between Ankylosing Spondylitis (AS) and HLA-B27. In ~90% of European patients, B27 alleles strongly predispose for AS[1]. In ~90% of patients with Celiac Disease (CD), the HLA-DQ2.5 phenotype is expressed, encoding HLA-DQA1*05:01 and DQB1*02:01 alleles, with the remaining ~10% mostly expressing the HLA-DQ8 molecule, encoding the DQA1*03 variant and DQB1*03:02 alleles [2,3]. Also in the field of pharmacogenetics there is a growing interest in the role of HLA. HLA-B*57:01 screening to prevent Abacavir hypersensitivity syndrome is now a routine clinical use in the developed world[4]. We developed a HLA genetic screening test NGSgo-HLALinkX that identifies HLA-B, DQA1, and DQB1 typings by NGS using a multiplexed amplification strategy in a single tube.

Methods

A multiplexed amplification assay was established that encompass HLA-B, HLA-DQA1 and HLA-DQB1 locus-specific amplification of exon 2 and 3 in a single tube (Fig. 1). The assay was compatible with the multiplex PCR kit (Qiagen) and required a PCR enhancer (GenDx). Analytical performance studies were assessed including screening of a large gDNA validation panel (n=95 samples) to verify robustness, (82 different HLA-B alleles, 83 HLA-DQB1, and 95 different DQA1 alleles). Amplicons were pooled and processed into the same NGSgo library preparation workflow for Illumina as established for HLA typing by NGSgo-AmpX (GenDx) (Fig. 2). Data was analyzed by NGSengine software (GenDx).

Results

For all samples tested (n=95) strong amplicons of the expected sizes were generated in a multiplexed PCR (Fig. 3). The no template control was clean (position H2, Fig.3). NGS data showed high locus mappability (>92%), average insert sizes >300 (Fig. 4) and full coverage of the amplicon with even distribution of reads, especially for HLA-B, and HLA-DQA1, having average read depths > 1500 (data not shown). The multiplexed assay showed to be capable of detecting both alleles when present in a balanced manner.
Preferential amplification was only detected in the presence of DQB1*03 alleles. By lowering the DQB1 allele threshold to 15% in NGSengine, still reliable typings were obtained.
The NGS typing results were compared with the available pre-types at the level of amplicon input, meaning that ambiguities outside Ex2 and Ex3 were excluded from analysis. Full typing concordance was obtained for HLA-DQB1, HLA-DQA1 and HLA-B. All samples carrying B*27 (n=6) were confirmed at the 1st field resolution, or higher (Fig. 5). We could exclude all B*27 N-alleles. All samples carrying DQ2.5, DQ8, and DQ2.2 phenotypes, and B*51 and B*52 could be assigned at the required resolution level. For DQ7.5, DQB1*06:02, and B*57:01, P-group assignments were obtained (Fig. 5). We could not exclude the low frequent N-alleles B*57:79N and B*51:11N (Fig. 5).

Conclusions

We developed a robust NGS-based HLA genetic screening test NGSgo®-HLAlinkX that identifies HLA-B, DQA1, and DQB1 typings associated with Ankylosing Spondylitis (B*27), Celiac Disease (DQ2.5, DQ2.2, DQ8 and DQ7.5), narcolepsy (HLA-DQB*06:02), Behcet's disease (HLA-B*51 and HLA-B*52), and Abacavir hypersensitivity (B*57:01). The multiplex strategy and compatibility with our general NGSgo HLA typing strategy, reduces laboratory workload and standardizes the workflow.

Figure 1.
NGSgo®-HLAlinkX amplification strategy.

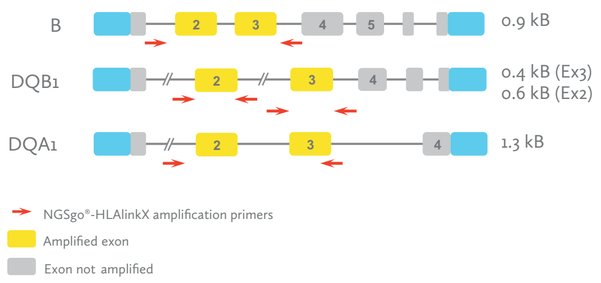

Figure 2.
NGSgo® workflow

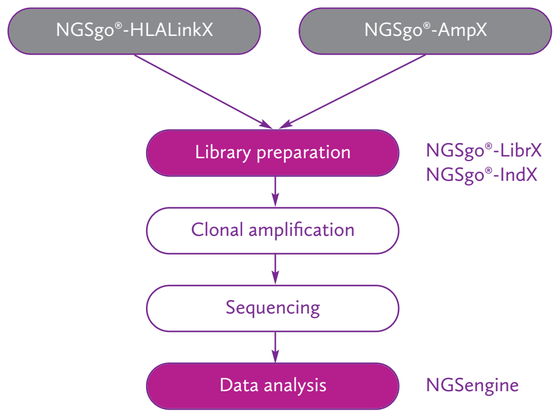

Figure 3.
NGSgo®-HLAlinkX multiplexed PCR on validation panel.

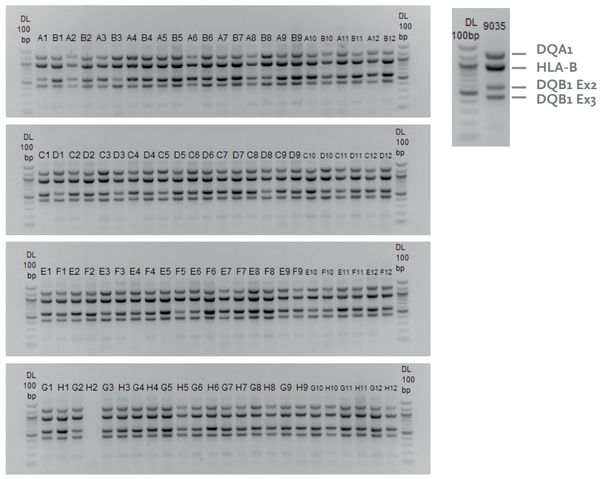

Figure 4.
NGSgo®-HLAlinkX multiplexed PCR on validation panel.

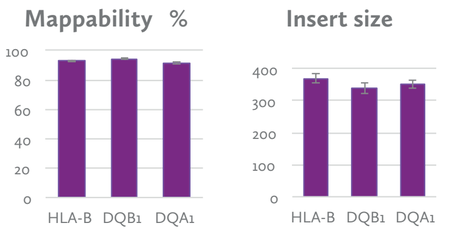

Figure 5. NGS typings obtained using NGSgo-HLALinkX

	Consensus typing	Validation panel		Ambiguities		Concordance pre-type
		NGS typings obtained	# of samples	1st field	2nd field	
Disease	B*27	B*27:03 B*27:04 B*27:05	1 1 4	no	B*27:04P B*27:05P	yes
Celiac disease	DQ2.5	DQA1*05:01, DQB1*02:01	8	no		yes
	DQ8	DQA1*03, DQB1*03:02	16		DQA1*03:01P	
	DQ2.2	DQA1*02:01, DQB1*02:02	12			
	DQ7.5	DQA1*05:05, DQB1*03:01	13		DQA1*05:05P DQB1*03:01P	
Abacavir hypersensitivity	HLA-B*57:01	B*57:01	3	no	B*57:01P B*57:79N (Ex4, 876delG)	yes
Narcolepsy	DQB1*06:02	DQB1*06:02	7	no	DQB1*06:02P	yes
Behcet's disease	HLA-B*51	B*51:01 B*51:02	5 1	no	B*51:01P B*51:11N (Ex4, 627insC)	yes
	HLA-B*52	B*52:01	5	no	B*52:01P	yes

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References.
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2) Ludvig M et al Immunogenetics 2012, Volume 64, Issue 6, pp 455–460
3) Karell K, et al 2003 Hum Immunol 64: 469–477
4) Martin MA et al. Pharmacotherapy. 2013 Jul; 33(7): 765-75.

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