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. 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-DQA molecule, encoding the DQA1*03 variant and DQB1*03:02 alleles. 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. We developed a HLA genetic screening test NGSgo-HLALinkX that identifies HLA-B, DQA, and DQB1 typings by NGS using a multiplexed amplification strategy in a single tube.

**Methods**

A multiplexed amplification assay was established that encompasses HLA-B, HLA-DQA1 and HLA-DQB1 locus specific amplification of exons 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 (1195 samples) to verify robustness, (82 different HLA-B alleles, 83 HLA-DQA1, and 93 different DQB1 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). The assay was compatible with the general workflow (Fig. 3) and the NGSgo-HLALinkX multiplexed PCR on validation panel (Fig. 4). The assay was compatible with the general workflow (Fig. 3) and the NGSgo-HLALinkX multiplexed PCR on validation panel (Fig. 4).

**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 the presence of DQB1*03 alleles. By lowering the DQB1 allele threshold to 15% in the presence of DQB1*03 alleles. We could exclude all B*27 N-alleles. All samples carrying B*27 (n=6) were confirmed at the 1st field resolution, or higher (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 DQ2.75), 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.

**References**

2) Ludwig M et al Immunogenetics 2012, Volume 64, Issue 6, pp. 455-460

**Figure 1.** NGSgo-HLALinkX amplification strategy.

**Figure 2.** NGSgo-HLALinkX 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