The robust NGSgo® workflow for high-resolution HLA typings also fully compatible with the Ion S5 platform

Aim

Current technologies for HLA Sequencing-Based Typing often show ambiguous results, most of which are caused by cis-trans allele combinations. Next Generation Sequencing (NGS) technology however generates sequences from single DNA molecules, enabling unique identification of paternal and maternal alleles. Therefore, NGS technology has great potential in the application of HLA typing for diagnostic purposes. The NGSgo® workflow that proved to be compatible with the Ion PGM platform was now validated on the Ion S5 platform. Here we present NGS data generated when processing pooled HLA loci on the Ion S5 platform using the fully automated Ion Chef system.

Methods

96 clinical samples were successfully amplified for 11 HLA loci with NGSgo®-AmpX (GenDx), enabling amplification of whole class I genes and the essential genomic regions for high-resolution typing of the class II genes. The amplicons generated were pooled per sample in an equimolar manner and processed in the NGSgo® library preparation that consists of three steps (Figure 1):

1. Fragmentation and end-repair, performed in a single step with the NGSgo®-LibrX (GenDx).
2. Ligation of a universal P adapter and 24 uniquely barcoded X adapters using the NGSgo®-IndX (GenDx). At this point the libraries were pooled in one tube in an equivolume manner.
3. A size selection / clean-up step with magnetic beads that removed all fragments <400bp.

The concentrations of resulting library pools were measured using the qPCR KAPA quantification reagents (Biosystems). An amount of final library of 60-80 pm was applied in the clonal amplification and enrichment steps with the fully automated Ion Chef system. The Ion S5 sequencing runs were each performed with 24 samples using 520 chips (4 runs in total). Data analysis was performed using NGSengine® software (GenDx).

Results

High quality data was obtained for all HLA loci tested, showing an average mappability of 88%, an average read length of 258 bp and average read depth of 393 (Figure 2). Full amplicon coverage and an even distribution of reads was achieved in all cases as presented by the alignment overview in NGSengine® (data example for HLA-A in Figure 3).

The level of resolution was examined. For HLA-A and DPA1 unambiguous allelic resolution typings were obtained for all samples tested. In most cases, unambiguous typings were obtained at the 2nd field resolution, except for HLA-B (n=10), HLA-C (n=22), HLA-DRB1 (n=14) and DRB5 (n=1). For HLA-B and –C, the presence of a read depth dip at the start of exon 2 caused inconsistencies due to imbalanced heterozygous positions. A read depth dip occurs when a region has a high GC content and/or GC repeats making it more difficult to be read by the sequencing polymerase. A new feature in NGSengine® allows editing of such heterozygous positions, generating unambiguous typing result when needed.

For HLA-DRB1 and HLA-DRB5, the allele ambiguities were caused by nucleotide differences outside the amplicon. Only for HLA-DRB1 typings (n=6) genotype ambiguities at the 1st field level were observed, caused by long distance heterozygous positions that could not be phased.

A typing concordance of 90-100% with the known pre-typing was achieved (Figure 4). No pre-typing was available for HLA-DQA1 (n=29), HLA-DPB1 (n=90) and HLA-DPA1 (n=87) samples, yet no drop-outs were observed considering the NGS data true typings. For HLA-A, a disconcordant typing result was observed proving to be a possible new allele.

Conclusion

The availability of several NGS workflows allows the end-user to choose the most preferred HLA typing NGS platform that fulfills the requirements of their laboratory for their specific applications. Here we have demonstrated that the NGSgo workflow developed for the Ion PGM is also compatible with the Ion S5 platform, making it a powerful method for high-throughput HLA typings by means of NGS.

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