Accurate HLA typing by NGS using the IonTorrent PGM with a platform specific developed and tested workflow

Aim

Next-generation sequencing (NGS) technology has great potential for the future application of HLA typing in routine diagnostic purposes. NGS sequencing allows sequencing of single DNA molecule sequences. This means that the paternal and maternal alleles can be uniquely identified. Currently, various NGS platforms are available on the market with each platform being based on a different technology and each technology in need of their own requirements to make them compatible for HLA typing. At GenDx we have developed a full NGS workflow from target generation to data analysis for platforms such as Illumina MiSeq, Ion Torrent PGM and PacBio RS Sequencer. Here we would like to show you our latest developments for the Ion Torrent PGM platform.

Materials & methods

We developed a NGS workflow for the Ion Torrent PGM platform that enables high-resolution HLA typing by NGS. In general the workflow can be described in five steps;

1. Target generation. The HLA locus-specific DNA sequences were amplified using the NGSgo®-AmpX kit (GenDx) that specifically amplifies the different HLA genes. After amplification, the locus-specific amplicons were pooled.

2. Library preparation. The fragmentation and end repair of the amplicons were performed in a single reaction, creating blunt-end DNA fragments that were directly ligated to the barcode-labelled X adapter and the ISP compatible P adapter. After ligation, bead size selection was performed using AMPure XP Beads to remove small fragments and adapter dimers and to select for fragments of an average size of 400bp. Separate libraries were pooled in equimolar concentrations and the final library concentration was determined using KAPA (Biosystems).

3. Clonal amplification. Using the ion One Touch 2 system and Ion PGM Template OT2 400 kit, DNA fragments were bound to Ion Sphere Particles (ISPs) with the P adapter. Subsequently each DNA fragment was amplified using adapter specific primers and properly amplified ISPs were selected.

4. Sequencing. The sample was prepared using the Ion PGM Sequencing 400 kit and a 316 v2 chip with a capacity of 3 million reads was loaded manually. After the PGM sequencer was initialized and the chip was applied, the sequencing run was started.

5. Data analysis. The generated data was imported in NGSengine, a software package that is developed by GenDx for analysis of NGS data consisting of HLA sequences. The software is NGS platform independent and can separate sequences of each locus from the other loci to form an unambiguous typing result.

Results

We have taken 18 samples of a standardized genomic DNA reference panel and for each sample determined the typing result for 5 loci; HLA-A, -B, -C, -DRB1 and -DQB1. Amplification of all loci was successful as seen in Figure 1. Weaker DRB1 amplicons could successfully be typed. The complete workflow including library preparation and sequencing (Figure 2) was achieved in 3 days and included 6 hours of hands-on time. The NGS data was analyzed using the NGS platform independent NGSengine® software, developed by GenDx for HLA sequence analysis (Figure 3). An average read length of 279 bp and an average read depth of 1284 was achieved resulting in coverage throughout the gene for each locus/sample (Fig. 3). A correct typing result was achieved in 95% of all cases: 100% for HLA-A, 94% for HLA-B, 94% for HLA-C, 94% for HLA-DRB1 and 94% for HLA-DQB1 (Figure 4).

Conclusions

The availability of different NGS workflows allows the end-user to choose the most preferred HLA typing NGS platform that fulfills the needs and requirements of their laboratory for their specific applications. Here we demonstrate the NGS workflow developed by GenDx for the IonTorrent PGM platform to be a powerful method, generating reliable, accurate and high-throughput HLA typing by means of NGS.


GenDx, Yalelaan 48, 3584 CM Utrecht, the Netherlands

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