High-throughput and cost-effective NGS strategy for intermediate-resolution HLA typing

Introduction

The National Marrow Donor Program (NMDP) requires that each newly recruited donor is typed at intermediate resolution level for HLA-A, -B, -C, -DRB1 and optionally -DQB1. Therefore we developed an NGSessenz assay specifically designed for intermediate resolution HLA typing (class I exon 2 & 3, and class II exon 2) that is suitable for high-throughput. PCR conditions were optimized such to reduce the costs of reagents and increase the sample capacity per run. Here we present NGS data obtained when processing 3 x 96 samples within one MiSeq run using the NGSessenz workflow conditions.

Materials and Methods

Sixty-four blood DNA samples were provided by Sanquin (The Netherlands) and 32 oral DNA samples were provided by Stefan Morsch Stiftung (Germany). Of the oral DNA samples, 16 were collected by buccal swabs and 16 were collected by ORAcollect (Genotek Inc). These 96 samples were processed in triplicate, resulting in a total of 288 samples. Loci were amplified in a 10 µl volume using Taq polymerase (Qiagen). After amplification, loci were pooled at an equimolar ratio for each sample prior to sequencing. The NGSessenz strategy is optimized to achieve a balanced mappability across the HLA loci, with the maximum complexity being in the non-coding regions.

Results

The NGSessenz amplification method, specifically designed to amplify the peptide-binding domain of HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 (Figure 1), was applied to 96 DNA samples in triplicate (288 samples in total). Samples were derived from various sources: blood, buccal swabs, and ORAcollect (a combination of buccal mucosa and saliva). Amplicons were obtained for all 5 loci and were strongest in blood and ORAcollect samples (Figure 2). The percentages of successful amplifications are shown in Table 1.

All amplicons obtained were suitable for processing into the NGSessenz workflow for Illumina MiSeq (Figure 3). After library preparation and sequencing, data was analyzed by GenDx NGSengine® software.

Reproducibility of the NGSessenz assay was presented by a balanced distribution of the insert sizes between the triplicates (Figure 4). The median library insert size was >370 bases for all DNA sample inputs and was 430 bases on average (Figure 5). Read depths showed variation between the DNA sources used yet was balanced between the 5 loci. On average the read depth was >400 reads for each locus, sufficient to generate reliable typings (Figure 5). Typings were compared with the available 2nd field level pre-types (Figure 5); 100% typing concordance was demonstrated for HLA-A, -B, -DRB1, and -DQB1 with pre-typings and between triplicates. Only for 0.5% of blood samples and 2.6% of buccal swab samples, typings were not concordant for HLA-C due to allelic imbalance of C*04 and C*07 alleles.

The NGSessenz amplification method presented here sequences a smaller fragment of the HLA gene compared to the whole-gene approach. This allows for a higher number of samples to be sequenced in a single run, as demonstrated here by running 288 samples (5 loci) on a micro flow cell. Combining a larger amount of samples into one single run facilitates high-throughput and leads to a reduction in costs.

Conclusion

The NGSessenz strategy is optimized to obtain a robust and reproducible HLA typing based on class I exon 2 & 3, and class II exon 2. Amplification can be performed in a 10 µl volume using Taq polymerase, and at least 288 samples (5 loci) can be sequenced in a reproducible manner in a single MiSeq run. Taken together, this new NGSessenz strategy is suitable for high-throughput and cost-effective HLA registry typing by means of NGS.