High-Throughput Genotyping of HLA-DRB3, -DRB4, -DRB5, -DQA1, and -DPA1 by Next Generation Sequencing

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Motivation

The polymorphic HLA-class II gene families HLA-DR, -DP, and -DQ on chromosome 6 are in strong linkage disequilibrium and inherited together as a haplotype. Nowadays, matching for HLA-A, -B, -C, -DRB1, and -DQB1 loci (10/10 match) is the gold standard for unrelated hematopoietic stem cell transplantation (HSCT). However, there is increasing evidence that all class II loci affect HSCT outcome. Currently, consideration of HLA-DRB3/4/5, -DQA1, and -DPA1 for donor selection is hampered by the very low numbers of donors with available genotyping information in the registries. To overcome this limitation, we extended our genotyping profile for newly registered donors by including HLA-DRB3/4/5, -DQA1, and -DPA1. This reflects the recommendation of the current NMDP matching guidelines to use these genotypes for selection among multiple similar donors. Here, we focus on the implementation of these loci in our high-throughput NGS-workflow.

Workflow

Our genotyping workflow so far covered seven HLA-Loci (A, B, C, E, DRB1, DQB1 and DPB1), the blood groups ABO and rhesus, the CCR5Δ32 mutation, the complete KIR gene family and MICA/B. We extended this profile to HLA-DRB3/4/5, -DQA1, and -DPA1 by one additional multiplex PCR reaction. A set of 30 primers targeting exons 2 and 3 was designed to amplify all described allelic variants (Figure 1).

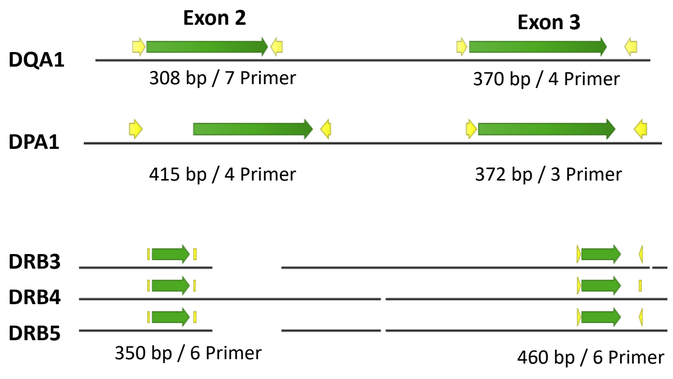

Figure 1. Primer set for the amplification of HLA-DRB3, -DRB4, -DRB5, -DQA1 and -DPA1. Length of the amplification products without adapter sequences and number of primers used. [yellow: primer; green: exon; black lines: genomic sequence, shown as alignment (gaps = non-homologous regions)].

PCR is performed in 384 well plate format in one multiplex PCR. After pooling with amplicons from other loci, barcoding and cleaning, the samples are subjected to Illumina next-generation sequencing, and then analyzed with our in-house genotyping software ‘neXtype’ (Figure 2). Validation of the extended workflow was carried out using a set of 95 reference samples with known genotypes for HLA-DRB3/4/5, -DQA1, and -DPA1. All results were concordant, confirming the fitness of the primer set and software algorithms for application in the high-throughput NGS-workflow.
So far, we analyzed more than 1.1 million samples with this workflow.

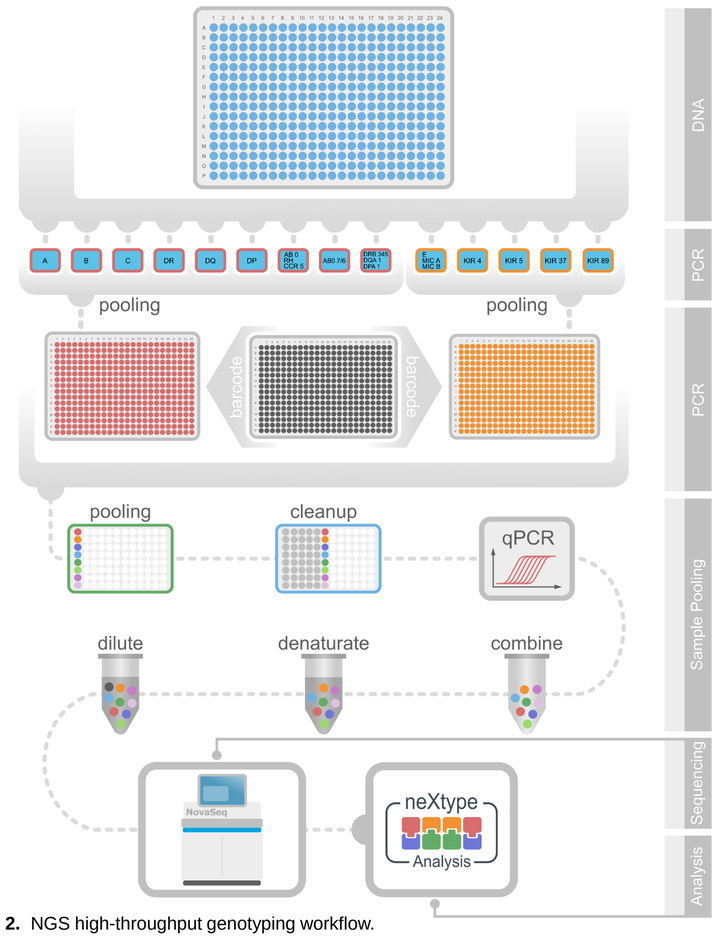

Figure 2. NGS high-throughput genotyping workflow.

Highlights

One challenge of multiplex PCR is finding the optimal balance between all loci and amplicons. We have succeeded in determining a recipe that produces stable amplicons with the required number of reads per exon.

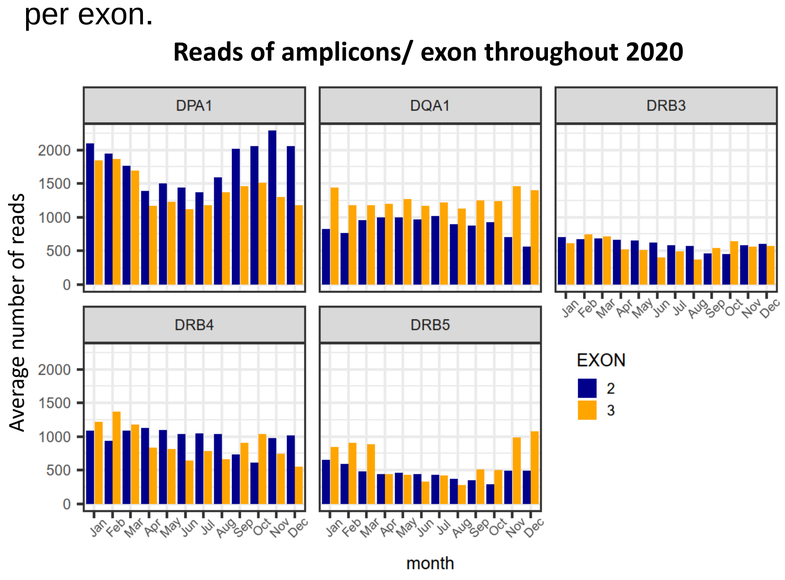

Figure 3. Average read coverage for each amplicon throughout the year 2020.

However, the ratio of amplicons to exons is a varying quality parameter that accompanies our workflow throughout the year (Figure 3).

Our analysis software ‘neXtype’ matches the reads to alleles in the IMGT database. It also flags potentially novel alleles with mismatches to the described allele sequences. In 2020, we identified 1815 samples with sequence mismatches in exon 2 which encodes for the antigen recognition site (Figure 4). This includes both, unique and recurrent novel alleles. We are planning to submit the more frequent variants to the HLA/IMGT database upon characterization of the complete genes with our DR2S workflow.

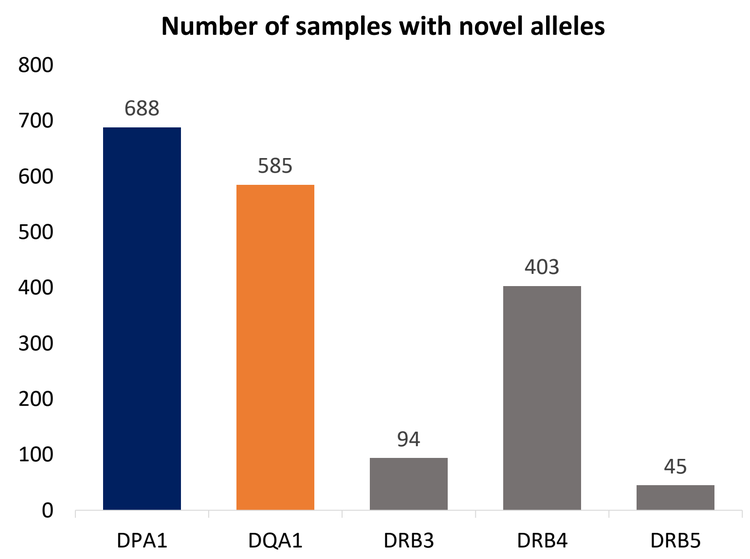

Figure 4. Number of samples with novel alleles identified throughout the year 2020 among 1.1 mio genotyped samples. Only mismatches within exon 2 were considered.

Conclusion

We started routine operation in October 2019 and genotyped more than 1.1 million potential stem cell donors with the extended profile so far, thereby facilitating the implementation of the current NMDP matching guidelines into clinical practice.

For information about the algorithmic aspect and observed allele frequencies please visit poster P245.

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