Motivation

Next generation sequencing (NGS) has the potential to deliver fully resolved human leukocyte antigen (HLA) genotyping with very low error rates. However, many NGS assays are still based on manual sample handling, processing and tracking, and are therefore hardly scalable. In addition, manual sample handling intrinsically carries the risk of human errors. Thus, we recently developed a high-resolution HLA typing workflow based on long-range PCR and next generation shotgun sequencing, in which all critical aspects are fully automated.

Assay features

✓ optimized for buccal swab, whole blood, and DNA samples
✓ in-house developed primers targeting all exons of HLA-A, -B, -C, and -DQB1 and exons 2 to 5 of HLA-DRB1 and -DPB1
✓ extended profile via amplicon-based sequencing: ABO, RhD, CCR5-Δ32
✓ automation solutions for all critical aspects of the assay using Analytik Jena, Beckman Coulter, and Tecan robotic platforms
✓ in-house developed robotic-tracking interface for continuous and complete tracking of all sample processing steps
✓ automated data processing and verification
✓ quality assurance tools for workflow monitoring
✓ fully scalable for up to 94 samples per run + option of multiple runs per day
✓ up to 4 field unambiguous HLA typing using GenDx NGSengine® software with manual verification of the obtained results
✓ results are reported electronically in customer specific formats and transferred to the customer via secure cloud storage
✓ 5 workdays turnaround time from sample receipt to genotyping report
✓ workflow development and optimization in 2018, followed by validation of the complete workflow including genotyping, data processing, and tracking based on 147 samples à 6 loci → 100 % typing accuracy

Summary & Outlook

Our newly developed long-range HLA genotyping workflow has proved to be robust and accurate in daily operation as well as time- and cost-efficient. In total, 1574 samples were genotyped in Q1 2019 with a repetition rate of 2.2%. And there is more to come: the assay is planned to be extended by the loci HLA-DRB3, -DRB4, -DRB5, -DQA1, and -DPAl.