Multiplex Long Range PCR Enrichment Assay for HLA Typing and ABO Phenotyping

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Abstract

Purpose: Targeted amplification of HLA by PCR combined with Next Generation Sequencing (NGS) is widely implemented in transplant diagnostics to characterize up to 11 HLA loci in high (third field) resolution. Blood type (ABO) is also a critical piece of information that informs transplant decision-making and organ selection. Current methods for ABO phenotyping are largely performed using whole blood serological testing, but these tests can be ambiguous or inaccurate. Therefore, an accurate and efficient method to provide genetic resolution of ABO is needed.

Methods: An ABO-specific primer mix was designed to be combined with an 11 loci HLA NGS primer mix for co-amplification in the same PCR reaction. Twenty-four well characterized samples were amplified with the combined ABO and HLA primer mixes. The amplicons were then processed using the standard HLA NGS workflow and sequenced on an Illumina's NextSeq 1000.

Results: Analysis of sequencing reads containing ABO and HLA requires a modified algorithm in addition to catalog and reference files for ABO. HLA genotyping is unaffected by addition of ABO as the primer design is balanced to not impact the number of reads required for HLA. Results from 24 samples tested provided 100% HLA concordance. ABO results can be viewed as either phenotype or genotype (including ABO subtype), with the ability to toggle between both available in the software.

Conclusions: An ABO primer mix can be combined with 11 loci HLA primers for PCR amplification, NGS library preparation, and sequencing. The downstream analysis requires software that is calibrated for ABO, but there is no impact to HLA genotype results while there is significant added benefit to genotype level resolution of ABO in the same sequencing run. Furthermore, co-amplification of HLA and ABO reduces hands on time and cost.

Materials and methods

Sample Preparation

Twenty-four well characterized DNA samples were selected for this study that had the quality and quantity to meet the criteria (25 ng/µl) with the fluorometric quantification method with 0.1 mM of chelating reagent. DNA was extracted from cell pellets using phenol.

Test Method

11 HLA Loci Amplification was performed using the AllType FASTplex NGS Assay (One Lambda, West Hills, CA) with an internally developed ABO primer mix spiked into the amplification reaction. Amplicons were processed into sequencing libraries using the standard NGS protocol using the AllType FASTplex NGS Assay(One Lambda, West Hills, CA). Sequencing was performed using an Illumina NextSeq1000 P1 2x150 PE sequencing.

Data Analysis

TypeStream Visual 3.1 was used for data analysis.

Results

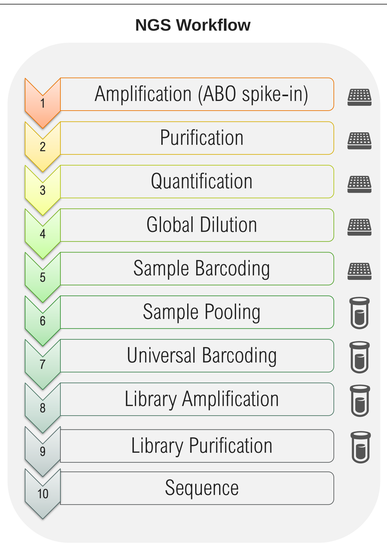

Figure 1. Modified NGS workflow showing the addition of the ABO primer mix added to the amplification step. The rest of the workflow is not changed.

Sequencing Metric	Result
Barcode Balance	18.59%
Locus Balance	17.43%
Average Exon 2 Allele Balance	> 70% all loci
Concordance	100%

Table 1. Sequencing Results for HLA assay results with ABO. Concordance and Coverage balance are not impacted by addition of ABO amplicon into the workflow.

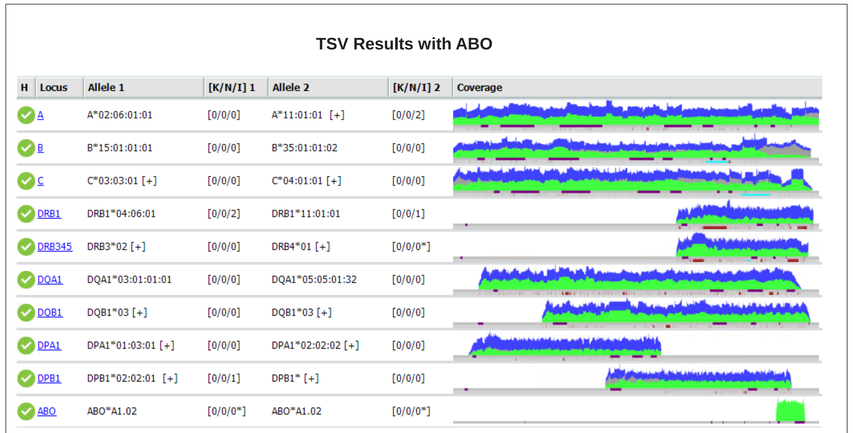

Figure 2. Representative genotyping results displayed within TSV software. HLA loci are presented as they would for standard results analysis with the additional coverage for ABO shown.

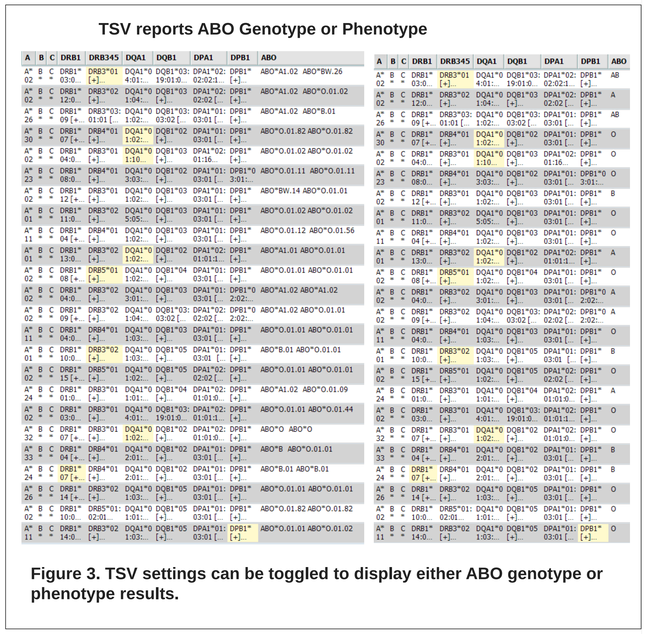

Figure 3. TSV settings can be toggled to display either ABO genotype or phenotype results.

Conclusions

- An ABO specific primer mix can be seamlessly introduced into the amplification reaction of an HLA NGS workflow to generate ABO and HLA typing results in parallel with readout in TypeStream Visual 3.1 software.
- Data quality remains exellent after addition of ABO primers with 100% concordance, 17.43% Locus CV, 18.59% Barcode CV, and average Exon 2 allele balance > 70% for all Loci.
- TypeStream Visual Software will show ABO genotype results alongside HLA results including coverage Histogram. The user can toggle between genotype and phenotype results for ABO.
- There is significant added benefit to the incorporation of high-resolution NGS ABO sequencing results that can be prepared and reported alongside HLA results as cost, hands on time, and potential ambiguities are removed from the analysis.

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