Report on a High-throughput Blood Group Genotyping Prototype Using Matrix-assisted Laser Desorption / Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

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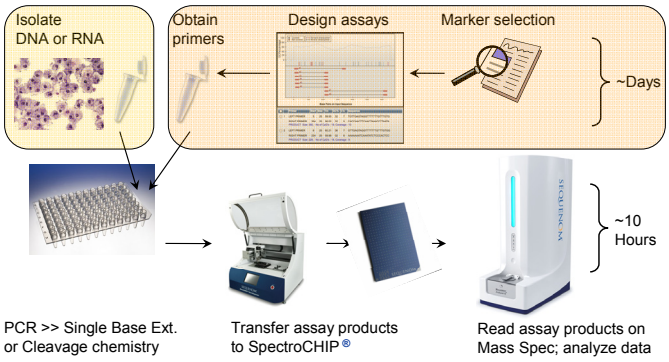
BLUTSPENDE ZÜRICH

Background

MALDI-TOF MS is an accurate, highly automatable and fast technology with the capacity of genotyping more than 150.000 single nucleotide polymorphisms (SNPs) per day (Figure 1). Therefore, genotyping of serologically Dneg, genetically *RHD* positive individuals, detailing blood donors' antigenic profiles and screening for blood donors with rare antigenic constellations, summarized as "high-through-put-blood group genotyping (ht-bg-GT), may easily be carried out using this technique.

Figure 1: Typical MassARRAY® Assay Workflow

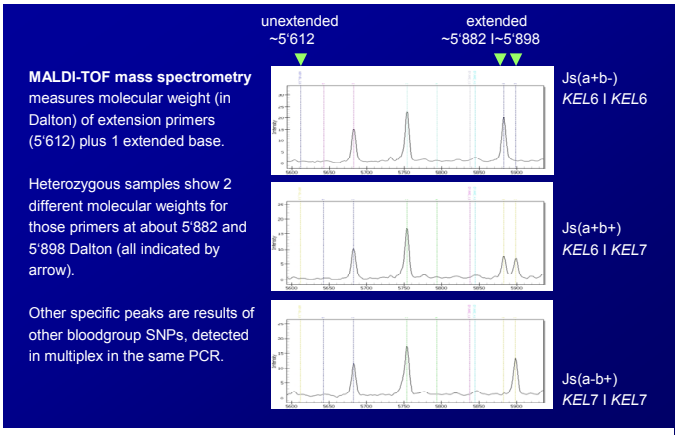
Using the 384 format: up to 150'000 genotypes per day, or e.g. 1'000 DNAs with 150 SNPs !



Methods

A set of SNPs, defining phenotypically relevant polymorphisms of *RHD*, *KEL*, *JK*, *FY* and *MNS* was defined and tested on a selectively compiled and genetically pretested donor DNA-panel. SNPs chosen were *RHD*-specific nucleotides for all 10 exons, and specific nucleotides for categories, partials, weaks, *RHD*dels and unexpressed *RHD*s (n=29), and RHC, c, E, e and W (n=5). For *KEL*, *JK*, *FY* and *MNS*, again, the major alleles and SNPs defining weakly or unexpressed alleles were considered (n=19). A total of 100 DNAs including rare alleles such as weak *RHD* type 15, *RHD*psi(null), *KEL2*(IVS3+1G>A)null, or very rare genotypes such as *KEL2*, or *FY**X homozygotes, were genotyped following the standard Sequenom MassARRAY iPLEX® Pro genotyping protocol (Figure 2).

Figure 2: Spectrogram of three different Jsa/Jsb (*KEL6* I *KEL7*) genotypes



Results

For 10 out of 43 *RH* specificities tested, no positive control DNAs were included in the tested DNA-panel. However, PCR amplification for those alleles was still carried out in the various multiplex PCRs to emulate realistic amplification conditions. Of the remaining 33 specificities, 5 (e.g. weak *RHD* type 3), did not give any results, whereas all other (n=28) gave correct results. Additionally, testing for *RHD* exon specific SNPs also gave correct *RHD* gene copy number measurements. (Figure 3)

In *KEL*, *JK*, *FY*, and *MNS* genotyping, 4 out of 19 specificities were not included as positive control DNAs. However, 13 of a total of 15 SNPs were correctly typed. All *KEL*, *JK* and *FY* specificities could be multiplexed in one single multiplex PCR. Overall, 85% of all specificities with control DNAs available (28 plus 13 of 48) were already operating perfectly in the first test of the described prototype.

Figure 3: Measurement of gene copy numbers, e.g. zygosity of *RHD*

Genes of *RHD* and *RHCE* are shown with their 10 exons in scale, introns with 1/10 of their actual length, respectively. The 16 *RHD*-specific positions detected on *RHD* and *RHCE* are indicated in green and orange. Signal intensity of *RHD* is measured versus *RHCE* and ist proportional relative to *RHD* gene copy content. Plot on the lower left corner shows a theoretical picture of 113 DNAs with no *RHD* gene (no *RHD* signal), *RHD*/d heterozygosity (0.5 relative *RHD* signal) and *RHD/RHD* homozygotes (1.0 relative *RHD* signal). Plots above are actually obtained data of 113 DNAs at 3 different positions.

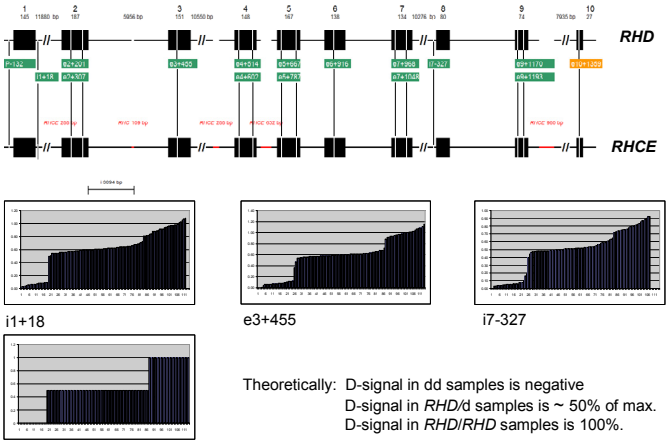
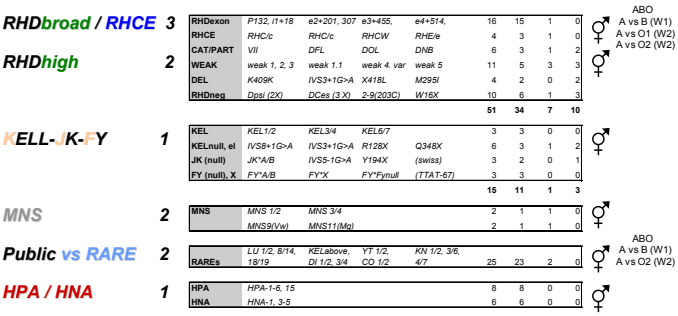


Figure 4: Specificities included in the various modules and current status

Modules for blood group genotyping include RHDbroad/RHCE, RHDhigh, KELL-JK-FY, MNS, Public vs RARE, and HPA/HNA and need 3, 2, 1, 2, 2, and 1 multiplex reaction each, respectively. Examples for various specificities in the various modules are given. Every module includes genetic sex testing and module RHDbroad/RHCE and Public vs RARE include raw ABO genotyping. This will allow a crosslink to phenotypically obtained donor data and avoid sample mixup for a cohort of e.g. 24 DNAs in a row.



Conclusions

The observed success rate of the first prototype using MALDI-TOF MS for ht-bg-gt, is highly impressive. The described method is independent of fixed formats like DNA-chips, and users are therefore free to choose and configure modules for their needs. E.g., the presented *KEL, JK* and *FY* could represent such a module (Figure 4). An improved and extended second prototype, including additional specificities for rare blood group specificities such as e.g. Lu^a, Yt^b, Co^b and Kn^b is under construction.