An HLA genotype-informed dPCR assay for monitoring donor-derived cfDNA

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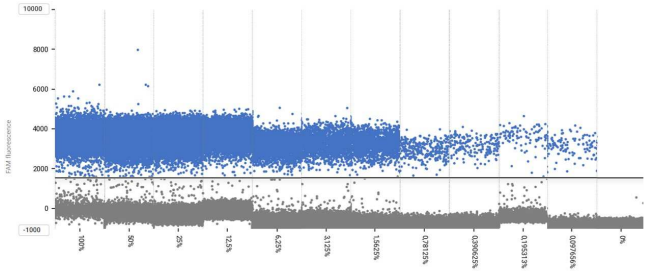
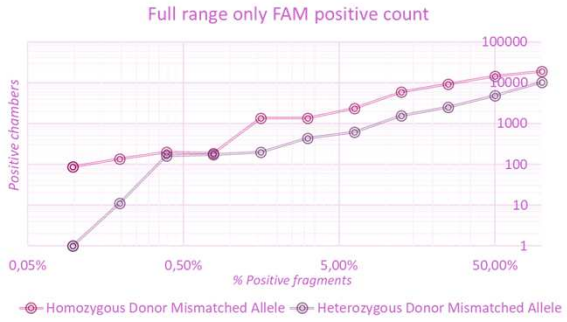
Aim:
Donor-derived cell-free DNA (cfDNA) is being evaluated as a biomarker for monitoring graft injury in solid organ transplantation. Monitoring cfDNA levels in peripheral blood could be used to confirm or substitute biopsies. The assay presented here, SOtrack™, uses markers based on HLA typing in combination with dPCR. we test the performance of HLA-DRB1 and HLA-DQB1 allele group-specific SOtrack markers using the Quantstudio Absolute Q dPCR system on artificial chimeric cfDNA samples.

Selection assay → Pre-amp multiplex → Digital PCR → Data analysis





Results:
The current protocol shows a correct trending of the FAM signal (R2>0.99 for heterozygous samples from 0.2% to 100% and R2>0.98 for homozygous from 0.2% up to 50% (Figure 3a&b, Figure 4), but requires further loading optimization for the homozygous FAM and for the HEX channel in general.

Conclusion:
Sensitive and specific results can be achieved using SOtrack. The use of HLA markers allows for a rapid test with a turnaround under 3.5 hrs without the need for a pre-typing step.