

AXO Science has established a Freedom EVO®-based high throughput blood genotyping system, helping to meet the needs of blood banks and transfusion centers around the world.



AXO Science, based in Lyon, France, specializes in the development of high throughput and multiplexed microarray diagnostic solutions for characterization of biological samples. With extended blood group genotyping now seen as the next step towards even safer blood transfusion, AXO Science has collaborated with the French National Blood Service (EFS) to establish a rapid, automated high throughput blood genotyping solution based on its patented HIFI Technology. Samuel Serraz, Director of Sales and Marketing, explained: "Blood banks in France needed a large-scale blood genotyping solution, but were struggling to find a system that was both high throughput and cost effective. We collaborated with EFS, using our technology to develop the HIFI Blood 96<sup>™</sup> assay, establishing a robust, automated blood genotyping process on Tecan's Freedom EVO workstations."

R&D Manager Benjamin Corgier took up the story: "HIFI Blood 96 is a multiplexed assay performed in a 96-well plate, and is fully automated on two Freedom EVO 100 platforms. DNA extraction is performed using a MACHEREY-NAGEL NucleoSpin® 96 Blood Kit — a silica membrane technology — on a Freedom EVO system equipped with a four-channel Air LiHa air displacement pipetting arm with adjustable tip spacing, a Robotic Manipulator (RoMa) Arm, a Te-VacS™ vacuum separation module, and an Eppendorf ThermoMixer®."

"Initially, 200  $\mu$ l of whole blood is transferred to the lysis plate, and 40  $\mu$ l of lysis buffer is added. After lysis, the samples are moved onto the filter plate, where the DNA is bound to the silica membrane, washed and finally eluted, producing around 80  $\mu$ l

of extracted DNA. The plate is sealed and transferred to a thermocycler for DNA amplification, in preparation for the analysis. High throughput post-processing and hybridization is then carried out on the second Freedom EVO platform, which is equipped with a MultiChannel Arm™ 96, a RoMa, a HydroSpeed™ washer, a plate reader, three Pinnacle thermal heaters – two fixed at 37 °C, the other used to perform cooling temperature gradients – plate holders and tip racks."

"High throughput processing is based on hybridization of the amplification product on AXO Science microarrays printed in 96-well plates. The PCR product plates are placed on the workdeck and the amplified DNA is transferred to plates containing the microarrays. A cooling temperature gradient, from 90 to 60 °C, is then used to obtain specific hybridization on the microarrays. We characterize single nucleotide polymorphisms (SNPs), and can detect a single base difference in the amplicons. From this point onwards, the protocol is based on different wash steps, performed on the HydroSpeed, followed by incubation with labeling enzymes. Finally, incubation with the substrate results in precipitates forming at the different spot positions – each array has 49 spots – making them appear darker. Optical density measurements are then used to determine the signal, analyzing the images using the integrated AXOware software. We process 14 SNPs in duplicate, plus controls and dark spots for positioning the grid during detection and, because everything is automated, continuity and traceability are ensured from beginning to end."

The open architecture of the Freedom EVO workstation means that both the pre- and

post-PCR processing can be performed using the same platform and software, reducing the training requirements for new operators. Samuel continued: "We chose the Freedom EVO because it is a low maintenance system that we were already familiar with. Everything is controlled by Freedom EVOware® software, which launches the protocols and works with the AXOware image analysis software to create output files that allow us to analyze the results and create reports. HIFI Blood 96 received the CE IVD mark in January 2014, and we recommend the use of the Freedom EVO platform to automate our solutions."

"The Freedom EVO 100 is ideal for our purposes, and has allowed us to develop an automated, large-scale blood group genotyping process, meeting the need for a rapid, high throughput protocol that is reliable and cost efficient. For customers at blood banks needing even higher throughput, there is the flexibility to choose one of the larger Freedom EVO platforms and, if demand is sufficient, we also have the capability to print onto 384-well plates, which would enhance throughput further still," concluded Samuel.

To find out more about Tecan's clinical solutions, visit

www.tecan.com/clinicaldiagnostics

To find out more about AXO Science, visit www.axoscience.com

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K1; K2

K3; K4

JK3; JK2

MNS1; MNS2

MNS3; MNS4

FyY; FynoFy

FyX (FynoX

Fy1; Fy2

V11; V12

Co1; Co2

Do1; Do2

Ao(a+1; Io(a+)

Hy\*; HhLu1; Lu2

Comprehensive
genotype results

for 96 samples

The HIFI Blood 96 assay offers automated processing of 96 samples in just 4 hours and 20 minutes

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