Genetic fingerprinting of crime scene samples

Forensic scientists at the Landeskriminalamt police office in Stuttgart, Germany, have commissioned a semi-automated sample processing system from Tecan to handle about 20,000 crime scene samples it receives annually for genetic fingerprinting. The specially designed system includes one MiniPrep™ and two Tecan Freedom EVO® liquid handling workstations. In addition, the system includes an integrated thermal cycler, real-time PCR and capillary electrophoresis systems from Applied Biosystems.

The Landeskriminalamt (LKA) is the central criminal police office for the federal state of Baden-Württemberg (BW), in South-West Germany, and its forensic services division is responsible for investigating most of the biological crime scene samples received from the deferring police departments in the state. Approximately 20,000 samples per year come from crimes of all types, including murders, thefts, sex crimes and vandalism. Forensic scientists need to be able to process the complete range of sample materials. For each sample, genomic DNA is extracted from the cells, purified and quantified prior to amplification of short tandem repeats (STRs) by PCR. Sequencers are then used for fragment analysis of STRs, using capillary electrophoresis, and a genetic fingerprint is formed.

In 2005, Dr Kai Vollack and his colleagues Dr Barbara Haak and Dr Ralph Schwenzer, the automation integration team at the LKA-BW, started looking for a semi-automated method for processing their samples in order to cope with the demanding workload. The laboratory had a number of stringent requirements that the automated system needed to meet. It needed to be able to handle a wide range of biological sample types and volumes; to provide the maximum DNA yield and highest analysis sensitivities; to ensure minimal risk of DNA contamination; to incorporate barcode-based sample identification; and to integrate real-time PCR using human-specific primers and performing PCR of STR DNA sequences.

The members of the automation integration team of the LKA-BW (from left to right: Dr Barbara Haak, Dr Werner Pflug, Anja Gruber, Angelika Jelinski, Dr Ralph Schwenzer, Jessica Loidl, Marga Kugler and Dr Kai Vollack).

Another requirement of the LKA was based on the pioneering work, started in the early nineties by Dr Werner Pflug, the head of the DNA division, using evidence from perpetrators' skin cells for DNA profiling. "When a person touches an object, he leaves single particles from his skin on that object," explained Dr Vollack. "It was originally thought that these particles were dead cells with totally degraded DNA but we now know that this is not the case and we often have enough DNA in these particles to create a complete genetic fingerprint." For this reason, it was particularly important that the set-up could separate epithelial cell samples that do not contain any DNA from those that do contain DNA at an early stage within the processing workflow. No available, off-the-shelf system could meet these requirements so the project went to public tender and Tecan was chosen for a number of reasons.

"The main advantage of choosing Tecan was the flexibility of the whole system," explained Dr Vollack. "We

needed a platform that was open to new developments and compatible with devices and reagent kits from different companies. We had seen a Tecan system in action at Professor Scheithauer's laboratory at the department of legal medicine, Medizinische Universität Innsbruck, and we appreciated its outstanding performance, particularly with the sample identification provided by the integrated barcode reader and the automated data exchange with the LIMS. We were also invited by Tecan to see the automated genetic sample processing system that was recently created for the South African Police Service and we were very impressed with this."

The forensic science division at the LKA-BW now has its own fully operational system provided by Tecan which has semi-automated sample analysis. The first step of the process involves manual separation of the cell lysate from the stain carrier, which could be a skin particle, piece of tissue or swab, for example. The lysate is transferred to a Freedom EVO 150 workstation, which is equipped with

Manual separation of the cell lysates from the stain carriers and preparation of the crime scene samples for the ChargeSwitch® Technology magnetic bead extraction of genomic DNA.

an 8-channel liquid handling (LiHa) arm, two Te-MagS™ magnetic bead separation modules and a PosID™ barcode sample identification device. Samples undergo magnetic bead-based DNA extraction and purification using the ChargeSwitch® Technology from Invitrogen and eluted DNA is pipetted into 96-well PCR plates. These plates are transferred to the second Freedom EVO 150 workstation for the PCR set-up for DNA quantification using the Quantifiler® Human DNA Quantification Kits from Applied Biosystems. Samples undergo real-time PCR using an integrated Applied Biosystems 7500 Real-Time PCR System and data are exported to the LIMS and imported into the second Freedom EVO workstation for STR PCR set-up of samples containing sufficient DNA, then sent to an Applied Biosystems 9600 Thermal Cycler. The samples are prepared for capillary electrophoresis by the third Tecan liquid handling system, a single-channel MiniPrep 75, and finally subjected to capillary electrophoresis with a 16-capillary Applied Biosystems 3130*xl* Genetic Analyzer.

The system includes a number of extra safety precautions in the workflow, including several that are thought to avoid cross-contamination of samples. For example, the liquid handling arms do not travel across open wells or tubes other than their targets, and using the Te-MagS module instead of a shaking device for mixing prevents the formation of potentially contaminating aerosols. The forensics division at LKA-BW has a 'four-eye' policy, where a second member of staff must confirm every sample

transfer, which is labor-intensive as well as time-consuming, but incorporating Tecan's automated sample identification processes within the workflow has helped to free up personnel for other tasks.

"We are really happy with the system, it is very sensitive and we have already tested a wide range of possible samples, including the more difficult skin particle samples," said Dr Vollack. "The automated processing also reduces operational error and, therefore, improves the quality of our data."

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Preparation of single epithelial particles from the butt of a pistol used as a murder weapon.
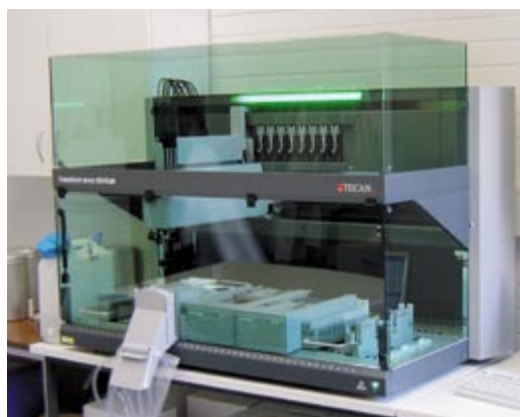
The Tecan Freedom EVO 150 liquid handling workstation with two Te-MagS magnetic bead separation modules for DNA extraction of crime scene samples using the ChargeSwitch® Technology from Invitrogen.