Building a case of precision pipetting

The DNA Section of the Orange County Crime Laboratory has recently taken delivery of its first Air LiHa equipped Freedom EVO® workstation for DNA quantitation and amplification reaction set-up. Complementing the laboratory's existing liquid displacement Freedom EVO platforms for DNA extraction, this new instrument will help to streamline the workflow and improve pipetting precision.

Forensic scientists Jennifer Jarrett, Robert Binz and Heather Pevney (left to right) with one of the DNA Sections' Freedom EVO workstations

"Although we are only in the validation phase at the moment... the pipetting precision of the Air LiHa is very good."

The Orange County Crime Laboratory (OCCL) in California, USA, is a full service, internationally accredited laboratory providing forensic analyses to law enforcement and fire protection agencies throughout Orange County. OCCL's DNA Section collects and profiles DNA from a variety of sources – including samples taken from victims or suspects and DNA trace evidence from items – processing around 15,000 samples a year. To help deal with this large caseload, OCCL has invested in a number of automated laboratory solutions, including two Freedom EVO 150 workstations for DNA extraction. Robert Binz, a Forensic Scientist at OCCL, explained their approach:

"We purchased our first Freedom EVO in 2010 to speed up DNA extraction, and originally chose the HID EVOlution™ because it was already optimized and validated for use with the Applied Biosystems PrepFiler® Forensic DNA Extraction Kit (Life Technologies). Using this platform, we can process up to 80 samples per run – to match our downstream processes – and it was so successful that we purchased a second platform within a year."

"More recently, we began looking to acquire a new system to replace the third-party workstations used to automate the quantitation, normalization and amplification steps of our workflow. As we do not use the Applied Biosystems Quantifiler® Duo chemistry for the quantitation step, the HID EVOlution package was not an option. We decided that an air displacement liquid handling system was the best fit for our needs, providing accurate and reliable low volume pipetting with disposable tips. Several manufacturers offered such systems, but none of the others offered the versatility of the Tecan instruments, making a Freedom EVO workstation equipped with an Air LiHa the obvious choice. Although we were not familiar with the Air LiHa itself, our existing relationship with Tecan was good, so we were optimistic that the system would offer the precision and reliability we needed."

Robert continued: "The workstation itself is fairly simple, with just the Air LiHa pipetting arm and carriers for tubes, plates, troughs and pipette tips on the deck of the instrument. The key consideration for our quantitation and amplification protocols is accurate liquid transfers at low volumes, allowing us to pipette from 1 to 15 microliters with high precision. Quantitation is performed in 96-well microplates, into which we transfer 14 µl of master mix reagent and 10 µl of buffer, followed by 1 µl of sample from either a tube or microplate. We need to do this for up to 80 samples per plate, plus standards and controls, so reliable and reproducible transfers are vital to avoid false negatives which could potentially impact on a criminal case. The quantitation plates are then transferred to an Applied Biosystems 7500 for analysis, while the sample tubes or plate remain on the Freedom EVO. The results of the quantitation are automatically uploaded to our LIMS, which then instructs the Freedom EVO to perform sample normalization and amplification as required via a worklist."

"Amplification set-up is the most complex part of our liquid handling workflow. Initially, 15 µl of master mix reagent is transferred to the amplification plate. The workstation then cherry-picks the samples for amplification – avoiding those extracts with insufficient DNA for typing – and adds between 1 and 10 µl of either neat or normalized DNA to the amplification plate based on the quantitation results. This is followed by the addition of reciprocal volumes of TE buffer to make the total volume in each well up to 10 µl. Again, accurate liquid transfers are vital for this application, as the amplification process is very sensitive to the amount of DNA present."

"Although we are only in the validation phase at the moment, we have performed numerous calibration protocols, and the pipetting precision of the Air LiHa is very good. For 1 µl transfers we are achieving CVs of 1-5 %, dropping to 1-3 % when the volume was increased to 10 µl, which we are extremely happy with."

Robert concluded: "When the new workstation was first installed, we worked with Tecan's application development team to set up some of the basic scripts for our two major protocols, then continued the development of these scripts in house. A colleague and I have both attended the Freedom EVOware® Standard and Liquid Handling Basic training course at Tecan's North Carolina site, and this, combined with our previous experience of the liquid displacement Freedom EVO platforms, has enabled us to exactly match the system to our workflow and LIMS requirements."

To find out more on Tecan's Air LiHa, visit **www.tecan.com/airliha**

To learn more about the Orange County Crime Lab, go to **www.occl.ocgov.com**

The Air LiHa offers precision liquid handling down to 1 µl