A microTAS DNA Analysis System: Sample to Match in 2.5 Hours.

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In urgent cases, control samples such as buccal swabs for DNA analysis can be processed in the laboratory in as little as 6-8 hours. More typically, processes allow for the routine analysis of DNA samples from controls such as buccal swabs in 24-72 hours and require that the sample taken from a suspect is transported to a laboratory for processing, adding additional time to the overall process. Hence the suspect is likely to have been released from custody whilst the sample is processed. Frequently, where the suspect believes they will be subsequently charged with an offence, additional crimes are committed between the release from custody and re-arrest following DNA database intelligence reports. The implementation of a rapid system whereby a control sample can be processed within the police custody area would be of value to the law enforcement community: a suspect's DNA sample could be processed and compared to a database of crime sample DNA profiles whilst the individual remains in custody. Rapid elimination of an individual from an investigation can also be achieved, releasing resources to investigate alternative leads in a case.

A crude lysate of a DNA sample is added to the microfluidic cartridge-based system. The sample is purified using a Chargeswitch®-based extraction process which provides DNA at a concentration of 0.86 ± 0.41 ng/µL (n=27). The DNA extract is transferred to a 10 µL PCR chamber (9.96± 0.21 µL n=20) which has pre-loaded PCR multimix stored within. The PowerPlex® ESI 16 reagents are packaged and stabilised in a Reax™ bead (stability data shows the multimix is stable for at least 12 weeks at room temperature) and activated by heating prior to amplification. The amplification is performed using peltiers both front and back of the cartridge, allowing amplification in approximately 1h 30 min. DNA solution in excess of the 10 µL PCR reaction is stored in an archive chamber and can be accessed by pipette should further investigations of the sample be required.

After thermal cycling, the amplified sample is collected from the PCR chamber by flushing with formamide and ILS, collected in a chamber and denatured at 95°C for 3 minutes prior to pumping to the CE chip for resolution of the STR alleles and detection using laser induced fluorescence. Data collected from the CE is processed with FSS developed software and the DNA profile is recorded in a format compatible with the data requirement for submission to the UK National DNA Database. All fluidic movement is controlled using embedded actuators and sensors, avoiding the need for complex fittings and fixings to integrate the cartridge with the instrumentation, and significantly reducing the potential for contamination of the sample. The whole system is designed to allow simple loading of a control DNA sample to the cartridge, and robust walk-away processing of the sample, to give an STR profile which is fully compatible with the National DNA Database®.

Resolution of 1.25 bp has been achieved with the glass microchip for CE, and the maximum variation between runs of allelic ladders was 0.2 bp at the TH01 locus, 0.4 bp at the FGA locus and 0.7 bp at the D22 locus. More recently, work has investigated the macro to micro interface (how to get the sample into the microfluidic system) and a lysis tool has been designed to take the buccal swab, process it through lysis and inject the lysate directly to the purification and PCR cartridge. With a view to complete integration in a single injection moulded part, the work on CE has progressed to deliver separation in plastic chips and resolutions of 1.2 bp have been achieved with a separation length of 67mm

The whole process takes approx 2 hours 30 minutes from provision of the DNA sample to delivery of a DNA profile in a format suitable for loading to the National DNA Database. The concepts required to enable database searching and reporting of any matches in real time to realise the benefit of rapid sample processing have been demonstrated.

Forensic DNA, STR, cartridge, rapid, microTAS