

A Fully Integrated Robotic System for High Sample Throughput Within a DNA Databasing Unit

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ABSTRACT

High throughput production of DNA profiles has now become routine by coupling multiplex amplification of STR loci with automated fluorescence-based detection of the PCR products during electrophoresis. However, to maximise the efficiency of production it is necessary to consider the streamlining, and automation of sample and data handling at all stages of the DNA profiling process. This issue has been addressed within the UK National DNA Database Unit by developing a microtitre plate-based robotic process for the manipulation of samples prior to electrophoresis. Buccal scrapes submitted to the unit for analysis are entered into the system using a custom built sample logging station, and DNA extraction is subsequently performed in deep-well microtitre plates. Automated quantification, dilution and PCR set-up are undertaken by a fully integrated robotic system comprising a 9 channel pipetting station, an articulated robotic arm on a linear 3 metre track, plate hotels, and a fluorimeter for DNA quantification using a PicoGreen-based assay. Following amplification, the PCR products are aliquotted by a second pipetting station and then loaded into an automated DNA sequencer by multi-channel pipette. Sample tracking and all data flow throughout the process are controlled by system supervisory software integrated with a bespoke laboratory information management system. This approach, coupled with newly developed expert systems for analysis of the output data, enables very high throughput of samples to be achieved, together with a significant reduction in manpower requirements and processing costs.

INTRODUCTION

The UK National DNA Database (NDNADB) was launched in April 1995 as a result of changes in legislation which now enables non-intimate samples, namely buccal scrapes and plucked hairs, to be taken by the police from any individual arrested on suspicion of having committed an indictable offence. Furthermore the new legislation allows DNA profiles generated from these Criminal Justice (CJ) samples to be entered on to, and challenged against, an intelligence database containing profiles generated from stains recovered from scenes of crime for which the perpetrator is undetected.

In the first 29 months of operation over 200,000 samples from suspects have been analysed together with 20,000 stains recovered from scenes. Comparison of these profiles has identified over 8000 matches of stains to suspects, demonstrating the enormous value to the police of the intelligence database. The success of the database is fueling demand for ever increasing numbers of samples to be analysed, and this is being addressed by the development of high throughput automated systems described below.

To date, CJ samples have been analysed using a manual process comprising extraction of DNA by boiling in Chelex resin, fluorescence-based DNA quantification [1], followed by a heptaplex STR amplification [2] in combination with AmpliTaq[®] DNA polymerase. Resolution of the amplified products on ABD 377 automated sequencers, followed by analysis with Genescan[®] software enables alleles to be accurately designated and ultimately results in complete DNA profiles being entered on to the NDNADB. The tracking of individual samples throughout the manual procedure is accomplished by electronically scanning individual sample bar-codes at each stage to prevent errors in sample handling. Overall however, the manual processing of CJ samples is highly repetitive, labour intensive work and, as such is ideally suitable for automation.

THE AUTOMATED SYSTEM

Automation Overview

Automation within the NDNADB has been achieved by the development of a sample logging machine and two robotic systems (Table 1). The latter are controlled by Overlord, a supervisory software package which allows the execution of both DOS and Windows[®]- based packages plus integration with a Laboratory Information Management System (LIMS). This integrated system forms the basis of an automated high throughput DNA process. As with the original manual approach, samples are extracted, quantified, amplified, separated by electrophoresis and analysed. However, processing is streamlined by the use 96-well microtitre plates in conjunction with sample tracking based on uniquely bar-coded microtitre plates and position within these plates. Completed STR profiles are added to the database whilst samples requiring further work are flagged in the LIMS and exported to the appropriate robotic system for re-processing.

The LIMS

All data relating to solutions used in DNA extraction, quantification, PCR, gel pouring and gel running are manually entered into the LIMS. Details of sample movements from logging to completion within the automated systems are automatically exported by the robotic procedures and imported into the LIMS. Any manual actions such as microtitre plate storage, transfer to thermal cyclers, gel pouring, and gel loading are recorded on Psion Workabouts and downloaded to the LIMS. Consequently, a full sample history can be produced which gives all information relevant to the sample processing. Following resolution of the fluorescently-labelled PCR-products, gels are analysed and the results are input directly into the LIMS. Any requirements for re-analysis can then be exported to the robots for re-processing. The LIMS affords the database complete sample tracking and handling continuity for individual samples and/or batches of samples.

Sample Logging and DNA extraction

Samples are loaded into a 96-well microtitre plate format using a Hamilton WELLstar, a purpose built single-plane robotic device which acts a loading guide. WELLstar generates a CSV format file which provides irrefutable identification of a specific sample within a defined well in a specific microtitre plate. The device is designed such that a single well of a microtitre plate is accessible through a sample-loading hole. To prepare a batch of samples for extraction, the bar-code of a 2.2 ml deep-well microtitre plate is scanned into the software as prompted and the plate is placed onto the plate carriage. The required sample layout is selected and WELLstar positions the extraction plate such that the first well designated as CJ/QA is made available to the user through the loading guide. The user is prompted to scan the sample bar-code. When this is done, the operator is prompted to add the sample directly to the plate. A third prompt requires the sample bar-code to be re-scanned to verify the samples identity. WELLstar then repositions the microtitre plate to make available the next designated well for CJ/QA sample addition. When all the samples and controls have been deposited, 20% Chelex-100 at 80°C is automatically added to all wells. The plate is then heat sealed manually and incubated at 56°C for 2 hours prior to centrifugation to pellet the Chelex®.

Pre-PCR Robotic System

Both the pre-PCR and post-PCR robotic systems process samples in a 96-well microtitre plate format, and employ a liquid handling system which utilises fixed Teflon-coated steel cannulae, rather than disposable tips. This format was found to be the most suitable for accurate and precise liquid handling at small volumes, with the cannulae being vigorously washed between samples. The pre-PCR system comprises a Hamilton ML2200 pipetting station fitted with a Multi-probe head, a Labsystems Fluoroskan Ascent plate reader, plate hotel, plate stacks and a bar-code reader (BCR). Microtitre plates are moved between these components via the BCR by a Hamilton ML R16 robotic arm on a 3 metre track. Overlord is used to execute the Eclipse software (Dos) for the ML2200, the Ascent software (Windows) for the fluorescent plate reader and Roboforth (Windows) for the ML R16 robot (Figure 1). At runtime, Overlord prompts the operator to place the required labware, reagents and sample plates in specific positions of the system. The standard run protocol first makes up a DNA concentration curve using a PicoGreen[®] fluorescence-based assay. The first extraction plate is then placed onto the ML2200 along with a standard U-bottom microtitre plate (DNA plate). The steel cannulae are used to pierce the foil lid of the extraction plate and an aliquot of supernatant DNA is carefully transferred to the DNA plate. A flat-bottomed microtitre plate (Q-plate) is then placed onto the ML2200 into which a 5ml aliquot of DNA from the DNA plate is dispensed for quantification. The Q-plate is placed into the Fluoroskan Ascent fluorimetric plate reader which dispenses the required amount of PicoGreen[™] into the plate and reads the fluorescence of each sample. The Q-plate is then discarded. Two ng of each DNA sample is then removed from each well of the DNA-plate and diluted to the required concentration for PCR (0.16 ng/ml). Twelve ml of this diluent is transferred, after mixing, to a 96-well PCR plate containing SGM multimix and TaqGold[®]. The PCR plate, the DNA plate and the extraction plate are replaced onto the plate hotel and the dilution-plate is discarded. The next extraction plate is then placed on the ML2200 and the process repeated until all batches for that run are complete. Up to 8 batches can be processed in a single run.

In addition to the standard run, protocols have been developed to allow either whole batch or mixed batches can be assembled for re-PCR. With the latter, individual samples in a batch requiring re-PCR are flagged in the LIMS. A "PCR factor" is used to designate a requirement to amplify more, or less template DNA compared to the original template volume. This information is exported to the robot along with the DNA-plate ID and the well position for that sample. The operator is prompted to load the required DNA-plates onto the system by Overlord. The dilution-plate and PCR plate are loaded onto the ML2200 and the bar-code of the first DNA plate is scanned. The data relevant to that DNA-plate is imported into Eclipse and the appropriate volumes of DNA and water are dispensed to the dilution-plate using a single probe. All samples required from that plate are aliquotted to the dilution-plate before the DNA-plate is removed. All subsequent DNA plates are treated similarly until the dilution-plate is complete. The diluted DNA is then mixed and aliquotted to the PCR-plate.

At the end of each of these processes, all the plates are removed from the system and sealed. The PCR plates are placed on a PE 9600 thermal cycler manually for amplification. All required data is recorded by a Psion for transfer to the LIMS.

The Post-PCR Robotic System

The post-PCR system prepares amplified DNA samples for electrophoresis through an acrylamide gel. It comprises a Hamilton ML2200 pipetting station fitted with a Multi-probe head, a plate hotel, and a bar-code reader (BCR). Microtitre plates are moved between these components via the BCR by a uni-point Hamilton ML R16 robotic arm. As with the pre-PCR system, Overlord prompts the user to place the appropriate labware and PCR-plates in designated positions of the system. The standard run protocol transfers two 48-well microtitre plates and a PCR plate to the ML2200. Dextran blue/formamide/GS350 size marker is aliquotted to each of the 48-well microtitre plates. The PCR products from the PCR plate are subsequently transferred to these 48-well plates by the robotic system. The plates are replaced on the hotel and stored at room temperature prior to loading onto an acrylamide gel. Data files produced are imported to the LIMS and used to produce sample sheets for electrophoresis. Up to 8 PCR plates can be processed in a single run of the system.

In addition to the standard run, other programmes have been developed to enable whole and mixed batches to be re-electrophoresed. For the latter, the operator is prompted to load the required plates onto the system. A 48-lane denaturation-plate is transferred to the MP2200 into which Dextran blue/formamide is aliquotted. The first PCR plate is picked up and identified by the bar-code. The correct well and the volume of PCR product and water required for the re-run are identified from a LIMS data file, and the sample aliquotted directly to the denaturation plate. This is repeated until all re-run samples from that plate have been aliquotted. The PCR plate is then replaced with the next plate and the procedure is repeated then assembly is complete.

Gel-loading

The samples on the denaturation plates are heat-denatured and loaded in 3 passes using 8-channel Hamilton pipettes into a 48-well denaturing 4% acrylamide gel. The products are detected during electrophoresis using fluorescence detection with ABD 377 XL upgrade and associated software. Sample sheets are imported directly into the 377 collection software from the LIMS system. Gel loading details are imported to the LIMS via a Psion.

Data Analysis

The data is analysed using Genescan[®] Analysis Version 2.1 and Genotyper[®] Version 1.1. Full profiles are denoted as such in the LIMS and submitted to the NDNADB. The remaining samples are denoted as either re-PCR if the sample is a partial profile or as re-run if the sample has been over-loaded on the gel or any other gel problem is identified. The LIMS then exports files for mixed batch re-PCR or mixed-batch re-run to allow reprocessing of individual samples as part of a new batch under new conditions designated by the analyst.

REFERENCES

1. Hopwood A., Oldroyd N., Fellows S., Ward R., Owen S., Sullivan K. Rapid quantification of DNA samples extracted from buccal scrapes prior to DNA profiling, *Biotechniques* 1997; 23 (1): 18-20.
2. Urquhart A., Oldroyd N., Kimpton C.P., Gill P. Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. *Biotechniques* 1995; 18 (1): 116-121.

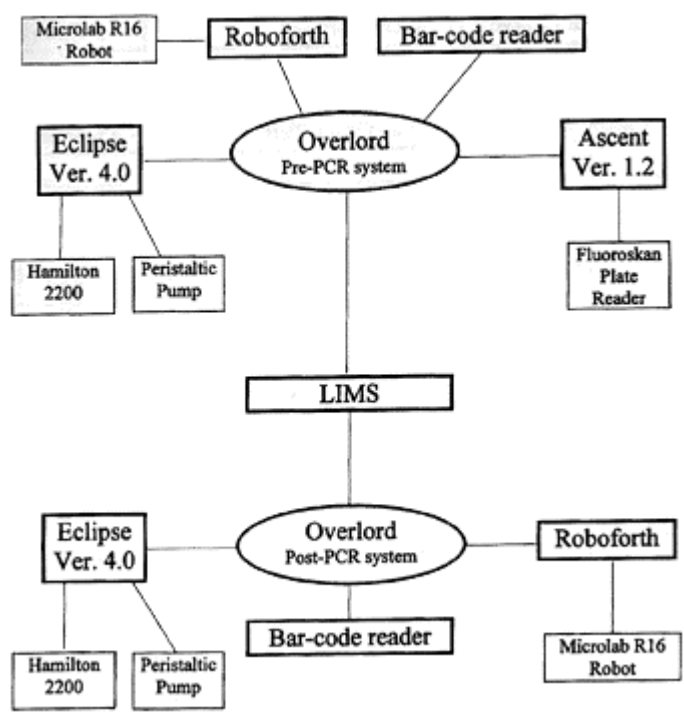


Figure 1. Overview of Overlord integration within the NDNADB systems

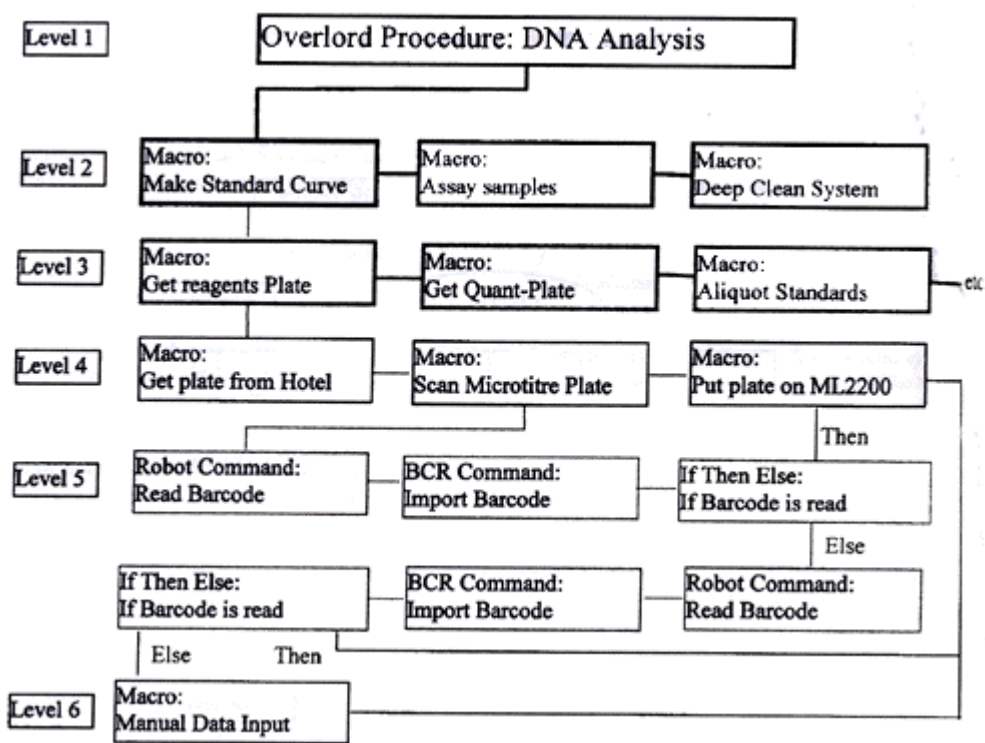


Figure 2: Structure of Overlord Programming

Table 1: Component Parts of the Pre-PCR and Post-PCR Systems

Equipment	Pre-PCR System	Post-PCR System
Overlord	+	+
Microlab R16 robot	+	+
Robot track	3m	-
Hamilton ML 2200 + Eclipse Ver 4.0	+	+
Low volume Multi-probe head (8+1 format)	100 ml syringes	50 ml syringes
Peristaltic pump	+	+
Fluoroskan Ascent fluorimetric plate reader + Ascent Ver.1.2	+	-
Laser bar-code reader	+	+
Plate Hotel	72 plate capacity	38 plate capacity
Plate stack system	3	-
Plate disposal system	+	-