

# Fast Track DNA Analysis Suite for human identification

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This paper details the development of a portable 'Lab on chip' DNA analyser that was developed to facilitate rapid analysis of DNA samples for 'at scene of crime' and in custody suite situations where human identification is required rapidly. This system was proven to work with human DNA for 3 loci, namely VWA, D21 and D18 taken from raw sample through PCR separation to detection within 90 minutes. Once the sample was loaded onto the microfluidic chip which in turn was loaded into the instrument no further human interaction took place. This paper details the approach to the biochemistry, hardware before going on to give results proving the proof of principle and then the authors' conclusions.

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## Abstract

This paper details the development of a portable 'Lab on chip' DNA analyser that was developed to facilitate rapid analysis of DNA samples for 'at scene of crime' and in custody suite situations where human identification is required rapidly. This system was proven to work with human DNA for 3 loci, namely VWA, D21 and D18 taken from raw sample through PCR separation to detection within 90 minutes. Once the sample was loaded onto the microfluidic chip which in turn was loaded into the instrument no further human interaction took place. This paper details the approach to the biochemistry, hardware before going on to give results proving the proof of principle and then the authors' conclusions.

## Introduction

This Paper details the development of a lab on chip approach to providing a solution to the need for fast track DNA analysis along with the instrument design that supports the analysis. In contrast to other emerging devices described in the literature [1-4] the system described here embodies design simplicity which reduces cost and improves reliability.

The system is portable and can be powered by battery. It is designed to accept buccal, blood and hair follicle samples which currently make up over 90% of routine forensic and medical DNA analysis. By building a system that accepts these sample types it greatly simplifies the architecture of the micro fluidic chip. This in turn greatly reduces and simplifies hardware required to support the analysis. The current system uses only a single laser and interrogates three loci tagged with FAM dye. As the system uses a single fluorescent tag for all the loci analysed it significantly simplifies the optics allowing for future miniaturisation and substantial cost saving when manufacturing commercial systems. Even with the three loci multiplex demonstrated for this article it has the ability to discriminate between

individuals with an accuracy of 5 million to 1. If the multiplex is extended to 4 or possibly 5 loci this would increase to 8.5 million and 1 billion respectively. The current system can definitely eliminate an individual from an enquiry whilst also having a significant chance of positively identifying an individual. This positions this machine perfectly as a front line system which would allow for evidence to retain a suspect so he can have more rigorous DNA checks made that require longer time scales.

Protocols used in the macro environment to extract DNA and prepare for separation are often not required in all instances for example PCR'ed DNA can be directly separated without heating it to 94°C in formaldehyde to keep it single stranded. The Urea in the separation matrix is capable of doing this. Early work looked at extraction methods on chip using monoliths and glass beads to extract DNA and multiple channels to prepare the DNA prior to PCR [5]. This however is only required when trying to extract DNA from difficult sources such as ancient bones or a contaminated sample. By opting to concentrate on the samples mentioned above a fast track approach that is capable of receiving 90% of current samples has developed.

The chip was designed to be a single use item and there for all hardware requirements such as thermo-cycling and detection were built into a portable analyser that the chip is put into for analysis. This was to ensure the chip cost was kept to a minimum and by allowing them to be single use they can be kept to archive the DNA samples that remain on them. All requirements for hydrodynamic pumping were eliminated further reducing the complexity of the system. All the reagents required to carry out a full analysis are stored on the chip only requiring the DNA sample to be analysed to be added. Interfacing chips with such pumps for an end user is always problematic also requiring relatively large dead volumes between reservoirs and on chip structures. All movement on chip is electrophoretic which is carried out via conductive polymer electrodes interfacing high voltage power supplies contained within the analyser.

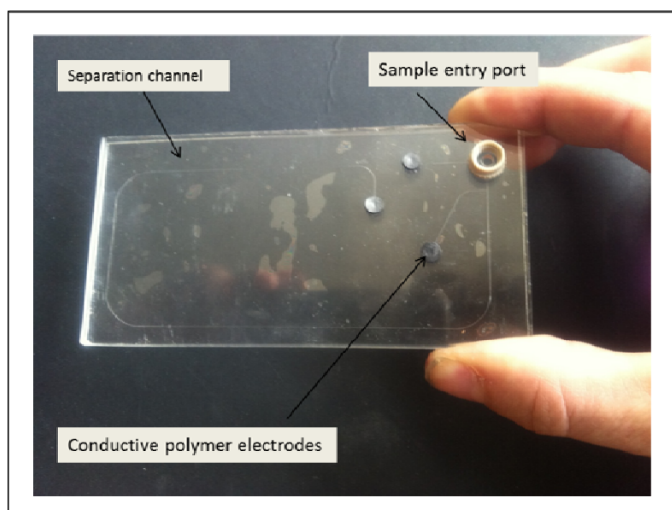


Figure 1 the Fast track DNA Analyser chip.

To further reduce the requirement for valves on chip flow is controlled by head pressure. The reagents stored in the reservoirs at the ends of all the channels are always maintained at a slightly higher level than the level in the PCR chamber ensuring all reagents stay in the PCR chamber during the PCR procedure and movement of the DNA afterwards is only by controlled electrophoretic injection and separation. Many chip designs endeavour to have a remote PCR chambers and to electro-kinetically load the sample into the separation channel. It was found empirically that the post amplified DNA could not be reliably moved in this way as the amplified fragments have different mobility's and there for injection varied with each separation. It was for this reason that the system was developed with the current direct sample injection from the PCR chamber. This eliminated this problem and by empirically determining there was no need for any post PCR processing allowed for a system that directly injected into the separation column. By using the a simple side arm mechanism the DNA is pulled into the channel past the T intersection (figure 2a) and then the side arm electrode becomes the negative electrode and the electrode behind the PCR chamber has a negligible positive voltage applied to ensure the rest of the sample is retained in the PCR chamber (figure 2b). Other methods of retaining the PCR reagents in the PCR chamber during thermo-cycling included having a mechanical valve and also PCRing whilst applying a holdback voltage were assessed. The first would of added significant cost to the Chip design and subsequently proven unnecessary and the later inhibited PCR believed to be due to the fact the reagents contained within the mix have different charges so the bias would of held them apart hence stopping them interacting.

Due to the simplicity of the chip design it can be made from two plates of crown BS270 glass. The channels are etched in 1mm plate using standard lithography techniques [6] and wet etching and the ports drilled in 3mm glass plate and the two bonded together. By ensuring the PCR chamber was above a critical size it allowed for the glass to require no additional processing only filling with separation matrix and PCR reagents.

## The Injection Methodology

This chip's architecture was characterised using 'blue loading dye' used when DNA is analysed on an open slab gel to give the operator a visual indication the separation has worked. The dye is actually manufactured from two dyes bromophenol blue and xylene cyanol which mimic the movement of 300 and 3000bp DNA being separated in a gel. This allowed for the optimization of the voltages required to inject sufficient DNA and a holdback voltage that would stop additional DNA being injected without inhibiting separation.

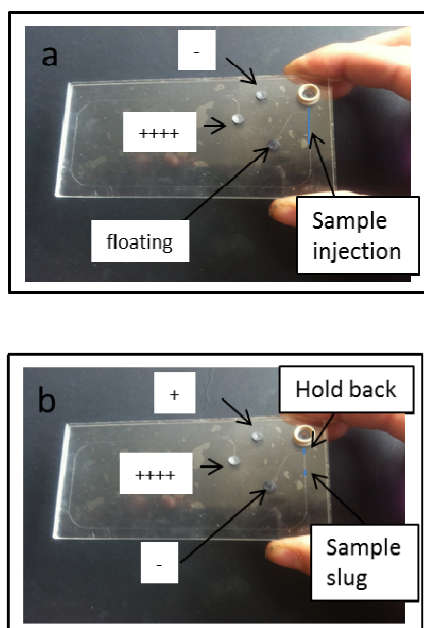


Figure 2 injection sequence

The sequence used to carry out the injection and the separation can be seen in Figure 2. In Figure 2a a voltage is applied between the electrode behind the PCR chamber and the end of the separation channel to draw sample into separation channel. Figure 2b shows how the electrode at the end of the side arm becomes the negative electrode and a separation voltage is applied at the end of the separation channel an a reduced positive voltage applied

behind the PCR chamber to hold back any further introduction of PCR product. The mechanism was confirmed to work with injection of DNA and is repeatable proven by the reproducibility of the results that it consistently yields with real DNA separations.

## Reagents

The chip uses a commercial separation gel supplied by Applied Biosystems known as 'Performance Optimized Polymer (POP6)' to be injected into all the channels. The ports at the ends of the channels that contain the electrodes also contain reservoirs of the buffer solution used with the POP6 in commercial systems. The PCR chamber contains all the reagents to accept a raw DNA sample and PCR it to amplify three loci used with the current Forensic DNA database VWA, D21 and D18. These reagents for long term storage are contained in a 2% agarose gel which will melt on the first 94degree step of the PCR cycle. The preloaded chips have to be stored at 4°C to maintain the integrity of the separation matrix [7] and to maintain the integrity of the PCR reagents that are stored in the gel with potentially an indefinite life time [8]. The positive head pressure stops any diffusion of reagents into the channel during the thermocycling and the only movement from the PCR chamber is by capillary electrophoresis.

## The Analyser

The hardware required for the analyser included high voltage power supplies, peltier system for thermo-cycling and a means of excitation and detection. These needed to be housed in a system which could integrate the control to allow for the full analysis to be automated. The flow diagram of a typical analysis can be seen in figure 3

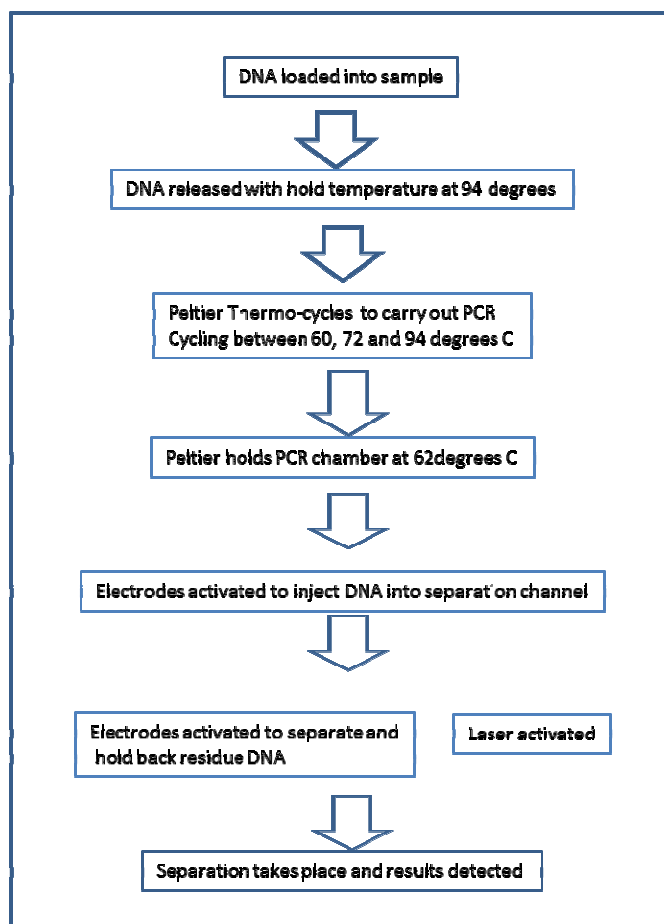


Figure 3 flow diagram of automated analysis.

The system contains 3 10kv powers supplies from Emco in the USA part number H101p. These can supply 0- 10Kv with a maximum current of 1.5mA. Input voltage required is 24v and requires 0-5v control voltage which is proportional to the output voltages. Switching the power supplies would be more complex than just having three dedicated ones. As their dimensions are so small and solenoids and additional hardware that would have been required would of need additional space this was the best solution. Figure 3 shows one of the three power supplies. Note the twenty pence for scale.



Figure 3 H101p 10kv power supply

## Peltier Thermocycling System

The parts for the peltier system were bought from Quick ohm in Germany and consist of a peltier chip, cooling fins, and a controller that allows thermo-cycling by switching voltages and polarity appropriately according to feedback it receives via a thermocouple. Initially, this system was configured via a bespoke Labview program run on a laptop. This allowed initial thermo cycling and PID control loop configuration. Once the system had been optimised, it was integrated into the analyser and controlled by its own software. Figure 4 shows the hardware and a screenshot from the Labview software used to configure the system.

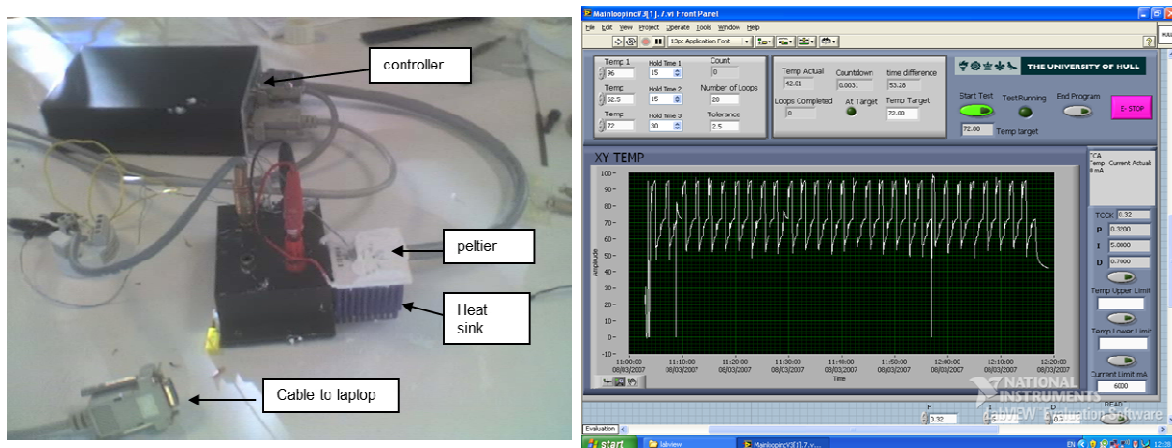


Figure 4 Thermo-cycling hardware and screenshot from Labview control software

## Optics excitation and Detection

The system uses a 488nm 25mwatt JDSU laser for excitation. The laser is used at this higher power as it negates the need for lensing and fine alignment where lower powers can be used. The amount of light energy hitting the channel remains the same. This is launched at the separation channel via a bifurcated optical fibre. The fibre carrying the light to the channel splits to form a ring formation illuminating a larger area of the chip. The fibre that collects the light passes down a single central fibre back to the detection system. As the system draws its sample neat from the PCR chamber rather than with more typical system where the DNA fragments are diluted in formaldehyde to a ratio of 22:1 there is much more



signal to detect. This has the added advantage that simpler optics can be used. In this case the light passes through a Semrock 488 cut off filter into a standard USB 2000 spectrometer from Ocean optics (See figure 5).

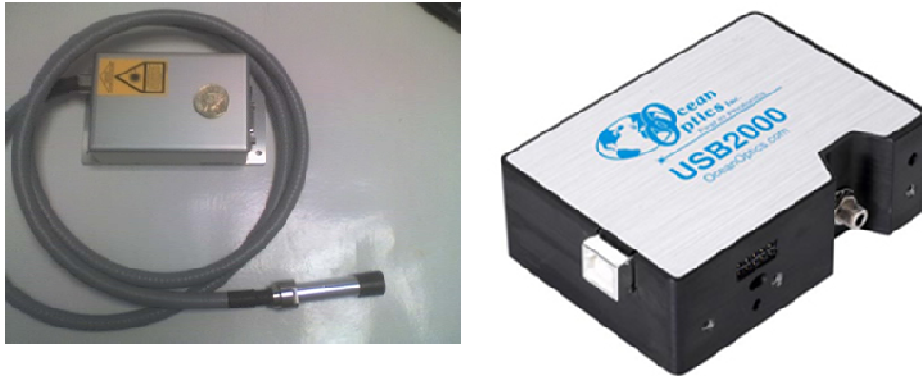


Figure 5 JDSU 488mwatt laser and the USB2000 spectrometer used for detection.

The issue whenever detecting DNA separations is the huge discrepancy between the light that needs to be detected when compared to the light required to excite it. This is why typical optics in a commercial system such as an ABI 310 are in the region of £30k [9]. The optics are bulky and not suitable for true portability. They require the light to be split using an optical grating so the laser light is completely removed therefore allowing for Avalanche photodiodes or photo-multiplier technology to be used. The simplified optics used in this system can be seen in Figure 6.

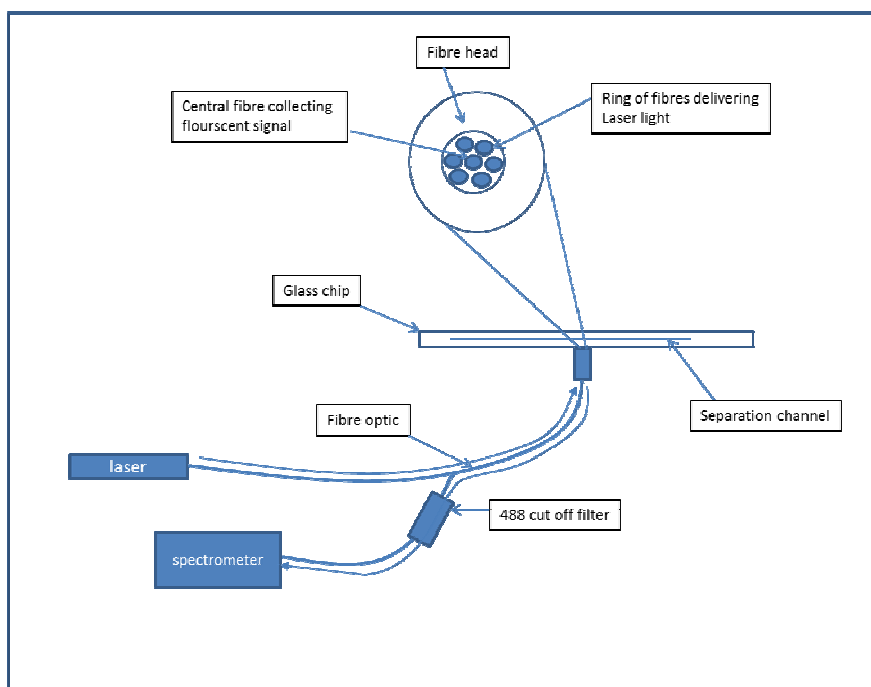


Figure 6 optical set up

By delivering the excitation and detecting the fluorescence through a single fibre head the alignment between laser light and detection is always maintained.

## The analyser

After ensuring all the component parts worked correctly the parts were integrated into the complete portable system by JLS Designs. This was controlled via a touch screen built into it. The output is currently read directly by a laptop that processes the raw data. This prototype system was built to be controlled using programmable logic controllers (PLC's) and the user interface and control system runs using OMRON software. For future more portable commercial systems the control could be run on a laptop and the control electronics be miniaturized onto bespoke circuitry now less flexibility is required now the system is fully working.

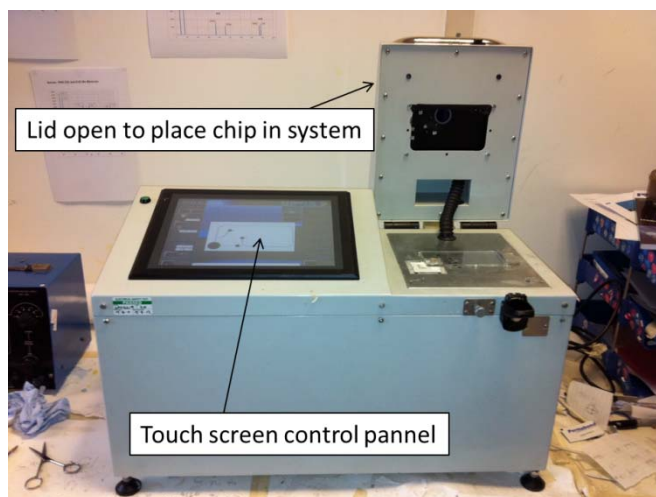


Figure 7 The Analyzer

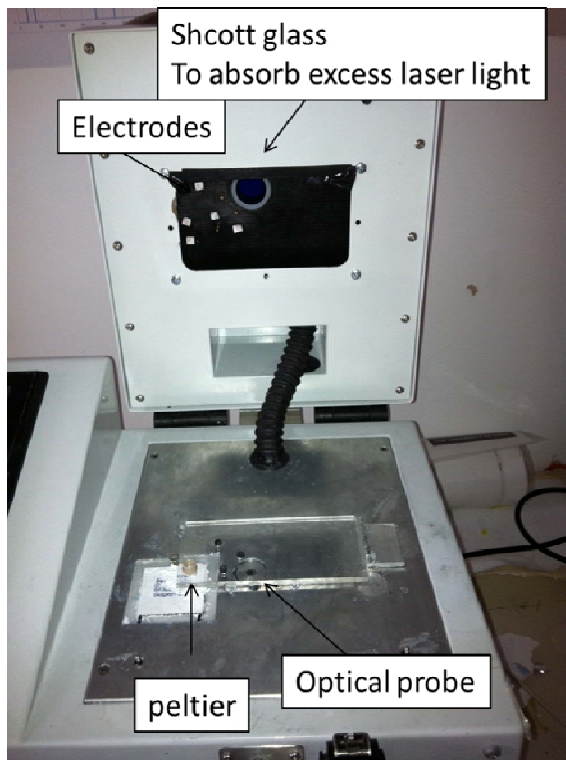


Figure 8 Chip/analyser interface

## Results

The initial system had its architecture tested using plasmid DNA. Primers were designed to give DNA fragments of 154,200 and 312bp [9]. As the plasmid DNA is only 400,000bp long as appose to human DNA that is 3billion base pairs long there is much more target for the primers to amplify per nanogram of DNA. The process of amplification is also more efficient as it is not amplifying sites on the genome containing errors (short tandem repeats). The subsequent signal from these fragments is a lot brighter and ultimately gave proof of concept to the separation processes. On proving the holdback, separation and detection was possible on chip the full process was carried out. The PCR multiplex for the plasmid DNA is very robust so ideal for system proof of concept. The output for this complete run through on the system can be seen in figure 8.

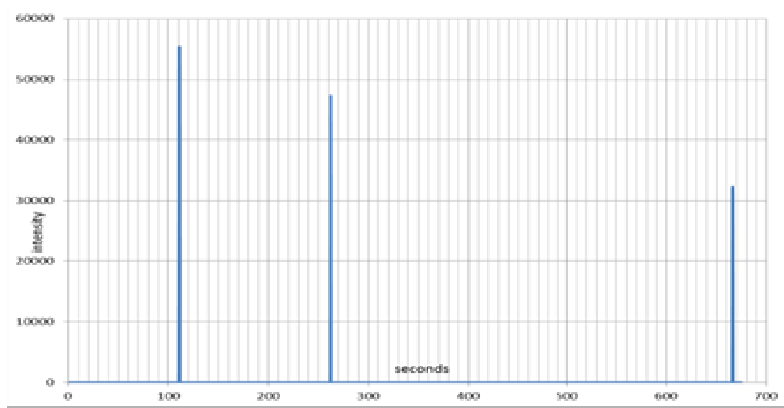


Figure 8 The result of a complete test of the system using Plasmid Dna amplifying 3 loci 154, 200 and 312bp

Once the system had been proven the same test was carried out using Human DNA loaded into a preloaded chip PCRed separated and detected and the results of which can be seen in figure 9. This DNA was analysed using the conventional route and showed the alleles at each loci to be VWA 10bp, D21 18bp and D21 to be 4bps apart. The full process with bench top clean up, pcr and separation took two days. This complete process was achieved on chip in the system in 90minutes.

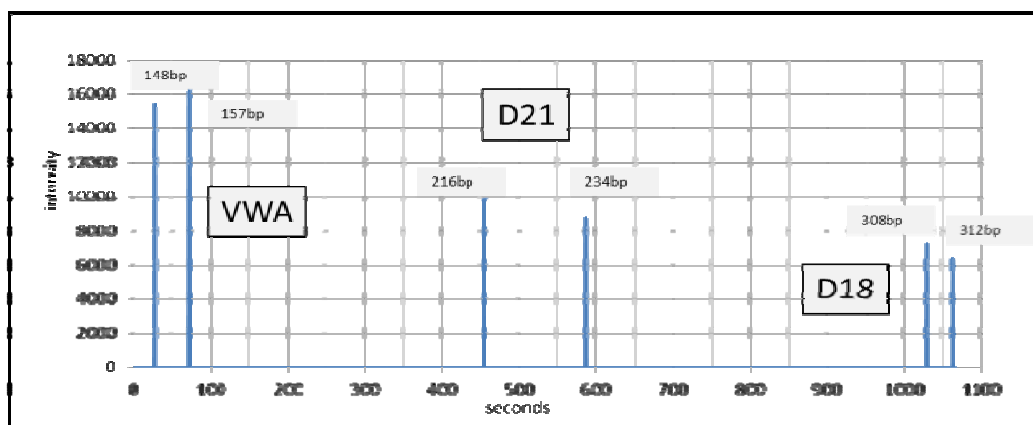
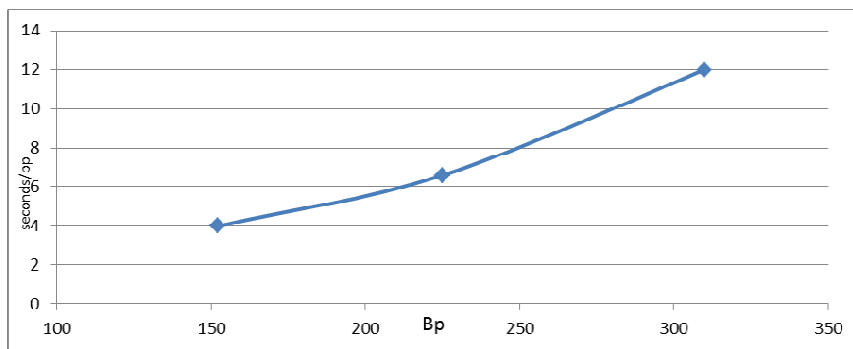


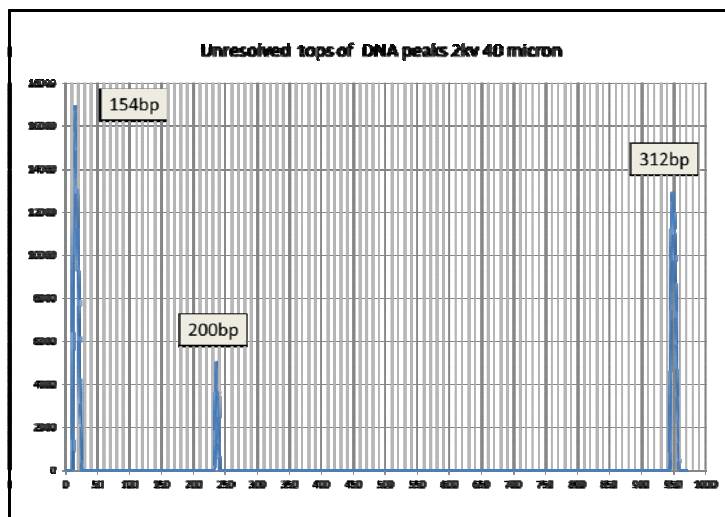
Figure 9 VWA D21 and D18 full analysis on chip.

To achieve the ultimate resolution on the footprint of the chip the separation channel was maximised to a length of 24cm and the channel depth reduced to the minimum it could be and allow for ease of channel filling and then separation voltage reduced to 90 volts per cm. The peak broadening expected at the low separation voltage was countered by reducing the CSA of the channel to give recognisable peaks less than ten seconds wide when separation between bps ranges between 4 and 12 seconds depending on fragment size see figure 10.



*Figure 10 base pair separations according to fragment size*

From the figure 11 the resolution of the peaks from the raw data can be seen when a sample is run using the above stated conditions. It can be clearly seen the peaks are much smaller in their width than the separation between them at this separation configuration. Further refinement could see the system achieve base pair resolution which would allow the system to be used for sequencing.



*Figure11 Resolution of raw data peaks.*

## Conclusion

This paper details the successful development of a lab on chip approach to the fast track analysis of DNA for genetic fingerprinting for identification or for medical applications. The novel approach taken in this work is to simplify what is required to carry out the analysis leading to a final system that would actually lend itself to miniaturisation and ultimately

true portability. Other workers in the literature have concentrated on replicating all the steps carried out in the macro lab. The system proposed in this paper would lend itself to significantly lower manufacturing costs and development to produce for a mass market.

The system also shows a good fundamental starting point for the minimum requirement for what is required to carry out a full DNA micro-satellite analysis. With careful design of the on chip architecture the need for complex valves was eliminated and the signal required for detection greatly improved significantly improving the signal to detect reducing the requirement for bulky costly optics. By determining the critical size chamber you could carry out PCR without salinization the need for surface treating the chip was also eliminated. The approach has minimized hard ware requirements, chip processing and biological steps more than capable with separating 4bp alleles routinely (which is what commercial machines will guarantee with current commercial systems) with the potential to achieve 1bp. This would make the system potential useful for sequencing applications such as genetic barcoding. The system has proven to be reliable, repeatable and a good solution to the need for fast DNA analysis in a system that can become truly portable

## References

- [1] Bienvenue, Joan M.; Legendre, Lindsay A.; Ferrance, Jerome P, 'An integrated microfluidic device for DNA purification and PCR amplification of STR fragments', FORENSIC SCIENCE INTERNATIONAL-GENETICS Volume: 4 Issue: 3 Pages: 178-186 APR 2010
- [2] Peng Liu, Tae Seok Seo, Nathaniel Beyor, Kyoung-Jin Shin, James R. Scherer, and Richard A. Mathies, 'Integrated Portable Polymerase Chain Reaction-Capillary Electrophoresis Microsystem for Rapid Forensic Short Tandem Repeat Typing', *Anal. Chem.*, 79 (5), 1881-1889, 2007
- [3] Daniel J. Ehrlich, Paul Matsudaira, 'Microfluidic devices for DNA analysis', Trends in biology Volume 17, Issue 8, 1 August 1999, Pages 315-319
- [4] Peng Liu, Stephanie H.I. Yeung, Karin A. Crenshaw, Cecelia A. Crouse, James R. Scherer, Richard A. Mathies, 'Real-time forensic DNA analysis at a crime scene using a portable microchip analyzer' Forensic Science international: Genetics, Volume 2, Issue 4, September 2008, Pages 301-309
- [5] Shaw, KJ; Joyce, DA; Docker, PT; Dyer, CE; Greenway, GM<sup>1</sup>; Greenman, J; Haswell, SJ, 'Development of a real-world direct interface for integrated DNA extraction and amplification in a microfluidic device' LAB ON A CHIP, Volume: 11 Issue: 3 Pages: 443-448 Published: 2011
- [6] McCreedy T 'Fabrication techniques and materials commonly used for the production of microreactors and micro total analytical systems', TRAC-TRENDS IN ANALYTICAL CHEMISTRY Volume: 19 Issue: 6 Pages: 396-401 June 2000
- [7] Yoshinobu Baba, Toshiko Matsuura, Kyoko Wakamoto, Mitsutomo Tsuhako, 'A Simple Method for the Preparation of Polyacrylamide Gel Filled Capillaries for High Performance Separation of Polynucleotides by Using Capillary Gel Electrophoresis', Chemistry letters Vol 20 (1991) No3 P371-374
- [8] Jonas Høgberg,<sup>1</sup> Thanner Christine,<sup>2</sup> Cuong Cao,<sup>1</sup> Laouenan Florian,<sup>3</sup> Maria Agirregabiria, 'Gelification-A simple and efficient method of storing on chip reagents: Towards Lab on chip systems for point of care

diagnostics', 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences October 2-6, 2011, Seattle, Washington, USA

[9] Verbal communication with DSTL Porton down Feb2011

[10] M J H Van Oppen, C. Rico, J C Deutsch, G F Turner, G M Hewitt, 'Isolation and characterisation of microsatellite loci in the cichlid fish *Pseudotropheus zebra*', Molecular Ecology 1997, 6, 387-388