# AUTOMATION OF PATERNITY TESTING

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## INTRODUCTION

The past five years have witnessed the widespread adoption of polymerase chain reaction (PCR) based short tandem repeat (STR) technologies by forensic science laboratories. The need to analyse small amounts of highly degraded template DNA was the major driving force behind the application of PCR in the forensic setting. Other equally important reasons for the switch from agarose gel based single locus probing (SLP) systems to PCR are the ability to automate the processes of analysis, and the ability to build large criminal databases.

In the paternity laboratory, where template quantity and quality are rarely an issue, we still benefit greatly from the potential for automation which STR-PCR offers. Furthermore, the comparison of discrete alleles has allowed the development of computer software which compares, matches and analyses paternity trios in a highly efficient manner.

During 1998, Cellmark Diagnostics completed a detailed assessment of the potential benefits of using STR-PCR as the backbone of our paternity testing service. The project (called *STR*ident) studied and validated extraction, amplification and analysis processes. Transfer of the majority of our testing from SLP analysis to STR-PCR analysis was successfully implemented and turn around time of samples was immediately and dramatically reduced. The established technology of SLP analysis is retained in order to unravel more complex relationships than simple mother-child-father trios. These complex relationships are not as amenable to analysis using STR technology.

*STR*ident culminated in the successful launch of a new system integrating commercially available components with proprietary software solutions.

### Automated Processes

STREAMLINED CUSTOMER SUPPORT

As the largest paternity testing laboratory in Europe, Cellmark Diagnostics requires a highly efficient customer services team. The efficiency of the team is maximised through use of a sophisticated case tracking and scheduling system called *Solomon*. This system enables the arrangement of the clients' appointments with the doctor who will take the sample, and the tracking of the sample. *Solomon* also generates paper correspondence with the clients, including their final report.

AUTOMATION OF TEMPLATE SAMPLING

Pivotal to the automation of our analysis was the adoption of FTA (Fitzco, MN, USA) as the bloodstain collection medium. This porous paper is impregnated with a strong detergent, buffer and a free radical trap to minimise DNA degradation. Upon addition of fresh peripheral blood, the lymphocytes are instantaneously lysed and the DNA is liberated. Once dried, the bloodstain can be sampled (a small disk is cut from the stain) and the contaminants removed using a series of wash steps.

Initially, the sampling of the bloodstains was a manual process which involved a 1.2mm (diameter) disk being excised from the stain using a Harris Micropunch (LTI, Glasgow, UK). This device retained the paper disk within the body of the punch until it was ejected into the appropriate well of a 96 well microtitre plate. This critical step is witnessed by a second operator in order to ensure sample identification and delivery to the correct well. The manual system also suffered from static charge on the plastic microtitre plate. The

static charge could cause the dry 1.2mm punch to be expelled from the plate after delivery. This problem was eradicated by bathing the working surface with ionised air from an ion fan (RS Components, UK).

In order to automate this process, we assessed the DBS puncher (Perkin Elmer Wallac,), an instrument which is widely used in laboratories studying protein polymorphism in neonates. Modification of the cutting tool reduced the size of the disk generated from a minimum 3mm diameter to 1.5mm diameter. Even at this size, the 1.5mm disk will contain 1.56 fold more DNA than the comparable 1.2mm disk. This increase in template DNA destabilises the PCR reaction, resulting in preferential over-amplification of the smaller PCR products. This was effectively dealt with by reducing the number of PCR cycles.

Using the DBS puncher, a single operator is able to scan bar code labelled FTA bloodstains into the computer. The punching instrument then cuts a disk from the bloodstain and delivers this to a known well on the 96 well plate. The computer records the sample unique reference number (URN) and the well into which the sample was delivered.

Validation of the punching instrument concentrated upon accuracy of punch delivery and carry over contamination from one sample to the next. 960 disks were cut into 10 plates, and there were no instances of the disk being delivered to the wrong well. Contamination of a sample with material from the previous sample was never observed, but as a precaution, two blank disks are punched between samples. The fact that the plate is not being handled means that there is a smaller likelihood of static build up. However, as a precaution, 50µl of the extraction buffer was dispensed into each well of the plate prior to loading on the DBS puncher. This small volume of buffer acts as a liquid trap, holding the punched disk in the appropriate well.

## AUTOMATION OF EXTRACTION

Once a full 96 well plate has been prepared for extraction, a series of washes are used to elute the PCR inhibitors from the FTA paper whilst the DNA remains associated with the paper matrix. The washes were comprised of 3x FTA Extraction Reagent followed by 2x TE buffer. Manually, these washes were extremely tedious and prone to inadvertent removal of the FTA disk from the well.

Transfer of the extraction process to an automatic liquid handling robot was a necessity, but given the small size of the punched disk, we anticipated that there may be a proportion of the disks inadvertently aspirated during the washing steps. However, through careful programming of the Beckman Biomek® 2000 instrument, we have achieved a robust, walk away extraction system which suffers no disk loss at all. Careful control of the tip position relative to the bottom of the microtitre plate well, and a controlled rate of solution delivery and aspiration were key to achieving a loss-free system. This system was developed using the 1.2mm Harris Punch cut disks, and the adoption of the larger DBS Punched disks makes removal even more unlikely.

## PCR SET-UP

Once the PCR contaminants have been removed from the cut disks, the PCR reactions are set-up using a multi-channel pipette to dispense sterile water and PCR multimix. Using an automated system for this part of the process would of course be possible, but cannot be justified for an operation which takes less than 10 minutes manually.

During our assessment of commercially available kits we examined PE Biosystems AmpF/STR™ Profiler™ Plus, Profiler™ and Cofiler™, and PowerPlex™ version 1.2 from Promega. We found that all these kits, which were developed for forensic use, would tolerate a reduction in the final volume of the PCR reaction. This is due to the abundance of high quality template DNA we have at our disposal in the paternity testing laboratory. At reduced volumes however, it becomes critical to remove all traces of the final TE wash buffer used to clean the FTA paper. The removal of traces of TE buffer is most efficiently carried out manually; another compelling reason for not automating the PCR set-up.

AUTOMATION OF ANALYSIS

The PE Biosystems 377 automated DNA sequencing and fragment analysis platform has the potential to run 96 samples at once. The launch of the upgraded 377-96 coincided with our move to analyse samples in a microtitre plate format. The loading of the instrument is facilitated by the use of a gantry of eight steel tipped 10µl micro syringes (Kloehn, Las Vegas, NV).

Analysis of the results utilises standard GeneScan® and Genotyper® software, with two operators carrying out all stages of the analysis independently. The tabular results of the Genotyper® analysis are then loaded into a second piece of proprietary software called *PatCalc*.

The *PatCalc* system compares the submitted allelic designations of operator 1 to those of operator 2, and highlights any discrepancies. The two operators then resolve these discrepancies and the corrected data is re-loaded into a repository of completed sample information. The information held by the *PatCalc* sample database links the URN and Case Number with the full STR profile.

Once a gel has been completely analysed, the paternity trios may be re-assembled from the *PatCalc* Build Screen (Figure 1). In this screen the operator specifies the relevant case number, test performed and frequency database to be used. The sample URNs of mother, child and alleged father are then specified. The system checks that the specified URNs all match the specified case number and that there are no obvious errors of the sex of the specified mother and father. As the URN and case number are unique, there is no requirement for the profiles held on the sample database to have originated from the same gel. Upon successful completion of these checks, the system retrieves the appropriate profiles from the sample database, and selects the appropriate statistical formula to be used for each STR locus. Having identified the correct formula and paternal (or shared) allele(s), it then retrieves the appropriate frequency from the specified ethnic database, and generates a likelihood ratio. Having completed this for each of the STR systems in the specified test, the LRs are combined to give an overall LR.

The Probability of Paternity screen (Figure 2) displays the comparisons and calculations performed and allows manual checking to ensure that the system has performed effectively. A basic electropherogram can also be generated to allow comparison of the figures calculated by *PatCalc* with the pictorial representation of the paternity trio, should this be desired.

MUTATION FREQUENCY

The efficient system described above has, with one instrument set (DBS/Biomek®/377-96), the capacity to analyse 360 samples plus controls per day. This equates to 120 paternity trios per day.

Using this system, we have rapidly amassed a pool of data which has allowed us to estimate the apparent rate of meiotic mutation observed at the common STR loci we utilise. These figures are presented in Table 1. These figures can be further broken down to estimate the relative level of maternal versus paternal mutation at each of the loci (Table 2) although the number of observations are still relatively small. In common with the SLP systems, it appears that the rate of paternal mutation is higher than the rate of maternal mutation. We are currently analysing a larger body of data to confirm this observation.

**CONCLUSION**

The adoption of STR-PCR has enabled our laboratory to drastically reduce the turn around time for a paternity test. The automation of repetitive and potentially error prone manipulations has been embraced, whilst maintaining human control over elements of the process which require human dexterity or assessment of success. The use of *PatCalc* enables the accurate and rapid analysis of data. The automation of laboratory processes has prompted a greater degree of automation in the customer services department, and the use of our *Solomon* software has enabled more efficient customer support.

The rapid analysis of samples has allowed us to assess the level of meiotic mutation observed at a number of commonly used STR loci, indicating that in general, these STRs demonstrate a meiotic mutation rate of around $1 \times 10^{-3}$. Our expansive software will allow us to automatically build upon the figures we have already

generated. We will continue to report these apparent mutation rates in the literature as our database expands.

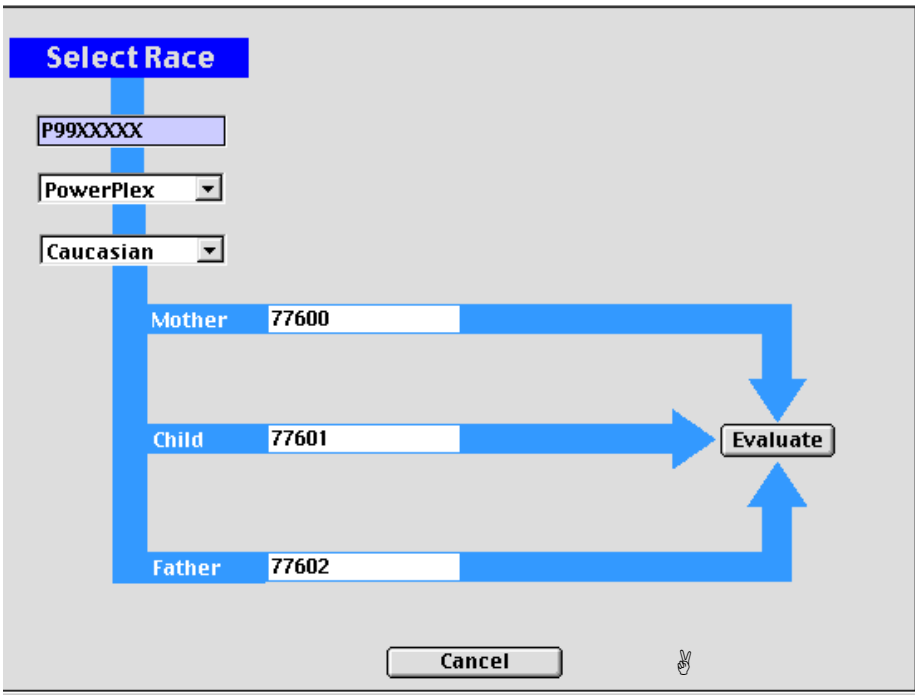


Figure 1 The PatCalc Build Screen

This screen allows the determination of the case number, test performed and the ethnic database to be used. Typing the URNs of the three individuals into the appropriate field retrieves the STR profile from the sample database, and reconciles the case number associated with each, and the sex of the specified individual.

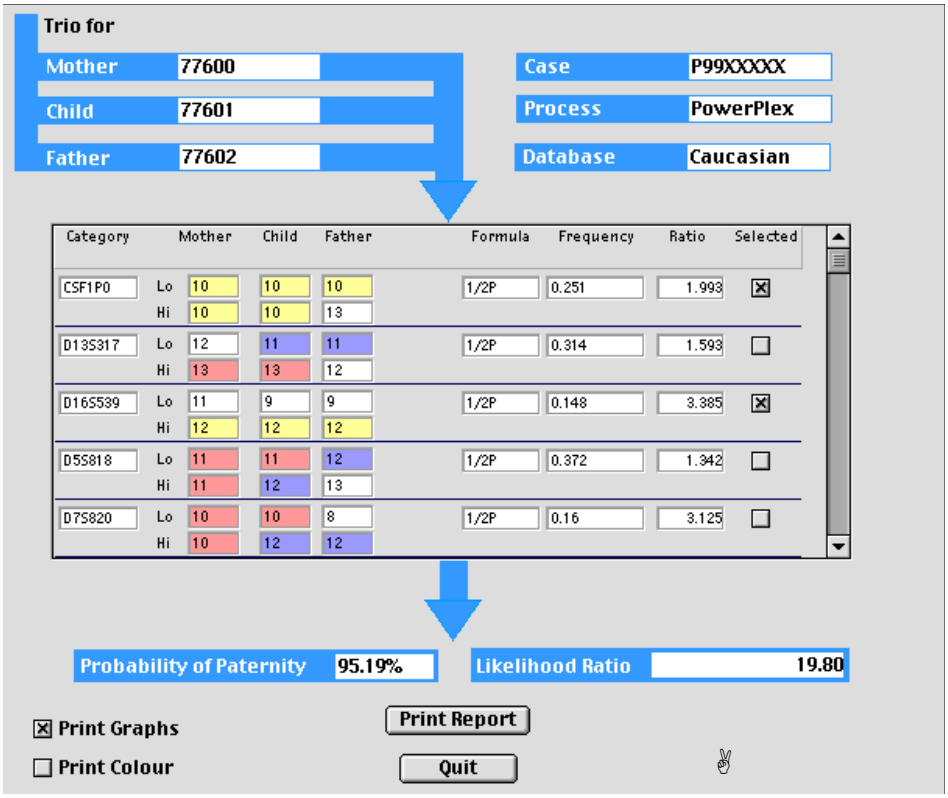


Figure 2 The Probability of Paternity Screen

This screen displays the retrieved data in tabular form, and highlights the obligate paternal, or shared alleles. The appropriate formula and frequency retrieved from the appropriate database are also shown, together with the calculated LR for each individually selected system. The Probability of Paternity and combined LR are displayed at the bottom of the sheet, together with printing options. In this example only four of the possible eight STR loci have been selected.

| Locus | Frequency | | Locus | Frequency |
|---|---|---|---|---|
| D3S1358 | $1.3 \times 10^{-3}$ | | D13S317 | $0.8 \times 10^{-3}$ |
| vWA | $1.4 \times 10^{-3}$ | | D7S820 | $0.8 \times 10^{-3}$ |
| FGA | $1.4 \times 10^{-3}$ | | | |
| D8S1179 | $0.7 \times 10^{-3}$ | | THO | 0 |
| D21S11 | $0.9 \times 10^{-3}$ | | TPOX | 0 |
| D18S51 | $0.9 \times 10^{-3}$ | | D16S539 | $1.6 \times 10^{-3}$ |
| D5S818 | $0.7 \times 10^{-3}$ | | CSF1PO | $0.6 \times 10^{-3}$ |

Table 1 Meiotic Mutation Rates

The frequency of apparent meiotic mutation observed at a number of common STR loci are shown. These figures were derived from *c.*9000 events for the Profiler™ *Plus* loci and *c.* 2000 events for the PowerPlex™ 1.2 loci (TH01, TPOX, D16S359 and CSF1PO).

| Locus | No. Observations | Maternal (%) | Paternal (%) | Unassigned (%) |
|---|---|---|---|---|
| D3S1358 | 12 | 33.33 | 58.33 | 8.33 |
| vWA | 14 | 14.28 | 57.14 | 28.57 |
| FGA | 14 | 7.14 | 92.85 | 0 |
| D8S1179 | 7 | 0 | 83.33 | 16.66 |

| | | | | |
|---|---|---|---|---|
| D21S11 | 9 | 44.44 | 33.33 | 22.22 |
| D18S51 | 9 | 11.11 | 88.88 | 0 |
| D5S818 | 8 | 0 | 50.00 | 50.00 |
| D13S317 | 7 | 0 | 85.71 | 14.28 |
| D7S820 | 7 | 14.28 | 71.43 | 14.28 |
| TH01 | 0 | 0 | 0 | 0 |
| TPOX | 0 | 0 | 0 | 0 |
| D16S539 | 3 | 0 | 66.66 | 33.33 |
| CSF1PO | 1 | 0 | 0 | 100 |

Table 2 Attribution of Meiotic Mutation

It should be stressed that the number of observed mutations are still relatively small, but there appears to be a greater occurrence of mutation attributed to the father rather than the mother in the majority of loci studied (D21S11 excepted).