

## ANALYSIS OF STR LOCI IN OLD BLOOD STAINS USING AUTOMATED AND MANUAL GENOTYPING SYSTEMS

J. Thomson, C. Phillips, D. Beckett, O. Summerfield and P. Lincoln.

Department of Haematology, London Hospital Medical College, London, UK.

### INTRODUCTION

PCR based analysis of polymorphic short tandem repeat (STR) loci is becoming widely used in forensic identity testing (Lygo *et al.* 1994). These systems offer a number of important advantages over the Southern blot analysis of VNTR loci using single locus probes; the technique which has been predominantly used over the past decade. These advantages are principally, (1) increased sensitivity; (2) the ability to analyse highly degraded DNA; and (3) the ability to co-amplify a number of loci simultaneously, so reducing the overall time for the analysis.

Various methodologies have been developed for the identification of the different length alleles amplified by PCR. These have included high sieving agarose with ethidium bromide staining; polyacrylamide electrophoresis under both denaturing and non-denaturing conditions combined with ethidium bromide or silver staining; capillary electrophoresis; and detection of fluorescently labelled PCR products using an automated DNA sequencing apparatus and appropriate analysis software.

This study investigates the suitability for STR typing of aged blood stains, dating from 1968 to 1994, from four individuals. The STR locus chosen for this analysis was humTH01. The aim of the study was threefold. To investigate the effect of increasing age of blood stain on STR profiles from the same individual; to investigate the relationship between the size of the blood stain and the STR profile obtained; and finally to assess the relative suitability of manual MetaPhor agarose electrophoresis and an automated fluorescent detection system in genotyping these old and small stains.

### MATERIALS AND METHODS

**Samples:** Three blood stains on cotton cloth from each of four individuals dating from 1968, 1975 and 1994. Two further fresh blood stains on cotton cloth from staff members. DNA was extracted using a 5% chelating resin (Lareu *et al.* 1994). Samples of the stained material 3mmx3mm were used in the investigation of increasing age of stain. To investigate the suitability of small stains, single cotton threads were removed from the stained material and lengths of thread of 0.5mm, 1mm, 4mm and 12mm were used as starting material.

**PCR:** PCR was carried out in 20µL reaction volumes with 8µL of DNA extract, 200mM dNTP, 0.25µM of each primer and 0.4U of Taq polymerase. PCR conditions were 94°C x 45 secs; 54°C x 60 secs; 72°C x 60 secs for 26 cycles.

**Automated fluorescent detection:** 1µL of PCR product was mixed together with 2µL of formamide/dextran blue and 0.3µL of internal size standard GS2500 (labelled with 6-carboxy-X-rhodamine [ROX] dye). Samples were denatured at 95°C for 5 min before loading into a 36 well 6% polyacrylamide, 8M urea denaturing gel (Sequagel-6, National Diagnostics). Electrophoresis was carried out in an ABI 373 DNA Sequencer at 30W for 3 hours. Fragment sizes were analysed automatically using the 672 GeneScan software.

**Agarose / ethidium bromide detection:** 20 $\mu$ L of each PCR product were run in 4.5% MetaPhor agarose (FMC) gels as previously described (Lareu *et al.* 1994). Gels were visualised and photographed under UV

## RESULTS

### Automated fluorescent detection

**1. Increasing age of stain.** Samples from four individuals dating from 1968, 1975 and 1994 were typed for TH01 by automated fluorescent detection. TH01 was successfully typed for all ages of stain in at least three of the four individuals. The results of this typing are summarised in table 1.

Sample	Fluorescence Peak Height		
	27 years	20 years	1 year
PL	60	150	3500
SW	50	0	2100
BD	0	240	6100
MW	40	160	3900

*Table 1. Mean peak height fluorescence for TH01 alleles detected in blood stain material of varying age.*

**2. Decreasing size of stain.** Single bloodstained cotton threads of 12mm, 4mm, 1mm and 0.5mm from four individuals were typed for TH01. Control stains of 3mmx3mm were also typed. The 0.5mm threads gave no visible product for any of the samples. However, threads of 1mm, 4mm and 12mm were all typed successfully in at least two of the individuals. These results are summarised in table 2.

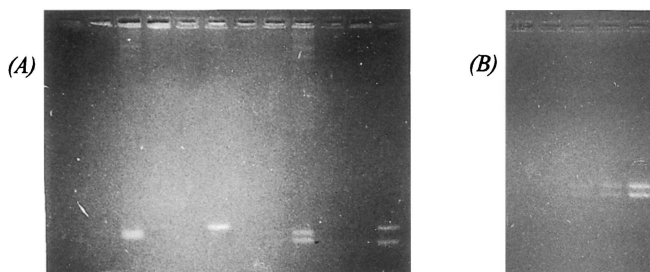
Sample	Fluorescence Peak Height				
	0.5mm thread	1mm thread	4mm thread	12mm thread	3x3mm control
DB fresh	0	100	150	200	2500
JT fresh	0	50	40	280	1500
BD 1 yr old	0	0	0	210	4700
MW 1 yr old	0	0	140	450	1400

*Table 2. Mean peak height fluorescence for TH01 alleles detected in varying lengths of blood stained thread from four individuals*

### MetaPhor agarose / ethidium bromide detection

PCR product from TH01 typing of both the old and small stains was also analysed using MetaPhor agarose and ethidium bromide staining. Photographs of these analyses are shown in fig. 1.

From these results it is evident that ethidium bromide staining will reliably provide results only for recent (one year old) stains, although some signal is visible in one of the 1975 stains (lane 8) and possibly in one of the 1967 stains (lane 4). The minimum stain size giving a readable result is the 4mm thread for the one individual analysed.



*Fig.1. Results of 4.5% MetaPhor agarose / ethidium bromide typing of (A) old stains from four individuals (MW, BD, SW, PL) and (B) varying sized stains from one individual. (A): Lane 1: MW 27yrs old; 2: MW 20yr; 3: MW 1yr; 4: BD 27yrs; 5: BD 20yrs; 6: BD 1yr; 7: SW 27yrs; 8: SW 20yrs; 9: SW 1yr; 10: PL 27yrs; 11: pl 20yrs; 12: PL 1yr. (B): Lane 1: JT 0.5mm; 2: JT 1.0mm; 3: JT 4mm; 4: JT 12mm; 5: JT 3mmx3mm control.*

## DISCUSSION

This study helps to confirm the advantages of using STR analysis combined with an automated fluorescent detection system in forensic analyses.

The availability of stains dating back to 1967 from four different individuals, prepared and stored under identical conditions allows the effect of ageing to be studied in isolation from any other environmental factors. The results show clear quantitative trends, even between the 20 year old stains and the 27 year old stains, indicating that DNA quality continues to decrease over a long period of time. Despite this, successful typing of 3mmx3mm samples of three of the four 27 year old stains confirm the important potential of this technique in investigations involving aged materials.

The sensitivity of PCR based systems are central to their widespread adoption in forensic science. The experiment using short lengths of stained cotton thread confirms that samples previously untypable using Southern blot techniques are now viable substrates for STR analysis. 1mm of thread removed from a stain corresponds to approximately 0.025 $\mu$ L of whole blood. This represents about 125-200 white cells which theoretically contain approximately 0.6-1ng of DNA. The amount of DNA recovered from these stains was not measured in this study but this theoretical yield corresponds closely with the minimum quantities of 0.5-1ng reported previously (Lygo *et al.* 1994).

The results from the agarose gel analysis of the same PCR products confirm that this form of manual electrophoretic analysis is only useful in cases where a relatively large quantity of PCR product can be produced. In this study, this is limited to stains up to one year old of 3mmx3mm in size, or fresh stains down to 4mm of thread. In cases where sample size or condition are not limiting, MetaPhor agarose analysis could offer a viable alternative to automated fluorescent detection.

## REFERENCES

- Lareu MV, Phillips CP, Carracedo A, Lincoln PJ, Syndercombe Court DS and Thomson JA. (1994) Investigation of the STR locus humTH01 using PCR and two electrophoretic formats: UK and Galician caucasian population surveys and usefulness in paternity investigations. *For. Sci. Int.* **66** 41-52.
- Lygo JE, Johnson PE, Holdaway DJ, Woodroffe S, Whitaker JP, Clayton TM, Kimpton CP and Gill P. The validation of short tandem repeat loci for use in forensic casework. *Int. J. Leg. Med.* (1994) **107** 77-89