CASEWORK EXPERIENCES WITH A MULTIPLEX STR SYSTEM.

M.J.Greenhalgh

Metropolitan Police Forensic Science Laboratory,
109 Lambeth Rd. London SE1 7LP
United Kingdom.

INTRODUCTION

DNA profiling using Short Tandem Repeat (STR) Loci has been used in casework at the Metropolitan Police Laboratory since August 1994. The system used is that devised by Kimpton et al.(1994). This uses multiplex amplification of four loci, (HUMVWA31, HUMTHO1, HUMF13A1 & HUMFES). Casework data have been collected over the first nine months of operation and a summary of the results is given in this paper.

METHOD

1) DNA extraction of stains by boiling with "Chelex" resin. (Walsh et al. 1991)
2) Quantification of the DNA using a slot blot which is hybridised to the Human specific probe D17Z1.
3) Multiplex PCR amplification using fluorescently labelled primers. 1-3 ng of target DNA where possible.
4) The PCR products are analysed using denaturing acrylamide gel electrophoresis on an ABI 373 Sequencer.
5) Sample details, processing details (gel number etc.) and final results are stored in a computer database and a report form is automatically generated for the Reporting Officer.

RESULTS

During a period of nine months from August 1994, 885 cases were analysed. This represents more than 5000 individual samples as each case often contains several crimestains. Reference blood samples are duplicated for QA purposes. In all 1497 blood reference samples were completely duplicated and no discrepancies were observed. A second sequencer is now in operation and additional staff are being trained for a planned increase in work. Estimates have been made that one sequencer and five staff could produce STR profiles from approximately 10,000 samples per year. It would be possible for fewer individuals to achieve this work rate for short periods of time but it has been found at the MPFSL that a considerable reserve of staff is required to cope with holiday absences, training and quality assurance activities.

Types of Reference samples:

SAMPLE TYPE	*NUMBER*	*SUCCESS RATE (FULL PROFILES)*
BLOOD	1497	> 95%
SALIVA	121	79%
HAIR	30	66%

Blood samples were the most numerous and the most successful samples in giving results at all four loci. (a full profile). Liquid saliva, mouth swabs and hairs were more variable samples and much depended on the manner in which the sample was taken. eg. some hair samples had no discernable roots and there was very little cellular material present on some of the mouthswabs.

Types of Cases Analysed:

CASE TYPE	*STR CASES(%)*	*SLP CASES(%)*
ASSAULT	26	4
SEXUAL ASSAULT	24	76
MURDER	21	16
ROBBERY	19	*
MISCELLANEOUS	10	4

* Results for SLP robbery cases were recorded under miscellaneous.

The sensitivity of the STR method has enabled it to be used in a much wider range of cases than Single Locus probe (SLP) analysis. The normal amount of target DNA for this STR system is between 1 and 3 ng however full profiles have been obtained with as little as 150 pg of DNA. Care has to be taken in interpreting results from such small amounts of DNA because of the increased dangers of contamination with extraneous DNA and possible loss of some of the loci. No instances of allelic "dropout" have been observed with these low amounts of target DNA. No attempts are made to amplify samples which have given a negative DNA assay.

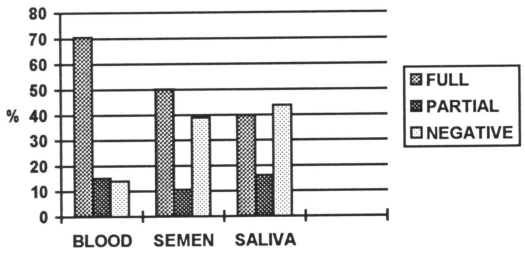
At the Metropolitan Police Laboratory the relatively rapid turn round time of STR analysis and its sensitivity has meant that conventional blood grouping systems have been discontinued. STR analysis is used as an initial screening technique in complex cases prior to SLP analysis and it is often the only technique used where very small or degraded stains are encountered. Although SLP remains the system of choice if sufficient DNA is present, there has been a reduction in the number of samples submitted. This is probably because it is more important to get rapid results in some cases rather than powerful statistics. (The average frequency of occurrence of a Caucasian Quad STR profile is approx 1 in 10,000)

Types of Bodyfluid Analysed

BODY FLUID	*% of ITEMS*
BLOOD	77
SEMEN	8
VAGINAL EPITHELIA	7
SALIVA	3
OTHER	5 (hair, skin, bone etc)

Many of the assault cases involve the analysis of very small bloodstains which would be unsuitable for SLP. These were formally analysed using conventional markers such as EAP and PGM. STR profiles can also be obtained from the small amounts of saliva staining on items such as stamps,envelope flaps, cigarette butts and face masks with a reasonable chance of success. Items such as these, which are unlikely to work using SLP analysis could cause a considerable increase in the STR caseload as forensic scientists and police officers become more aware of the capability of the system.

Relative Success Rates of Different Types of Stain.



The overall success rate for STR profiling is high, especially for bloodstaining. There are indications that the lower success rates obtained with semen and saliva are due to contaminants which are not removed by the simple "Chelex" extraction method. From the survey data it can be seen that the higher the concentration of DNA in the stain extract, the greater the chance of obtaining a full profile, even when the same final amount of target DNA is analysed. Presumably this is because only a small volume of concentrated extract is required to give 3ng. in the reaction mix. This will cause any contaminant present to be diluted. If the DNA extract is dilute, a much greater volume of the extract and any contaminants present, will be required to give 3ng of DNA.

Mixture Profiles

Mixtures of bodyfluid have been detected in a wide range of STR profiling results. Approximately 7% of all STR profiling results showed the presence of a mixture of bodyfluids. When only cases with semen and saliva staining are considered this figure rises to 14% even though a method to preferentially separate semen from epithelial cells is used routinely. The relatively low number of alleles in the STR loci can be a problem as components from the different individuals are much more likely to overlap. Mixtures are relatively easy to detect if the components parts are present in approximately equal amounts. Where one profile is present as a minor component there can be difficulties in distinguishing the alleles from background noise and care needs to be taken.

CONCLUSION

The Quad STR multiplex is robust and reliable in casework operation giving results from a wide range of bodyfuids.

Its greater sensitivity than SLP anaysis will mean that more case types are suitable for DNA analysis.

References

Kimpton et al. Evaluation of an automated DNA profiling sustem employing multiplex amplification of four tetrameric STR loci. Int J. Leg Med (1994) 106: 302-311

Walsh et al. Chelex 100 as a medium for the simple extraction of DNA for PCR typing from forensic material. Biotechniques (1991) 1:91-98.