A MODIFICATION TO THE "CHELEX" DNA EXTRACTION METHOD FOR CASEWORK SAMPLES.

M.J.Greenhalgh

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

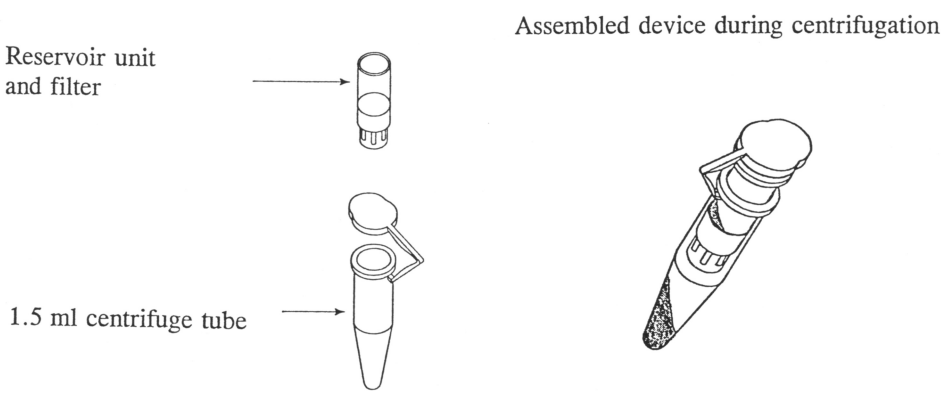
Introduction.

DNA profiling using multiplex amplification of four tetrameric STR loci is routinely used in casework at the Metropolitan Police Laboratory.

The loci used are:	HUMVWA31
(Kimpton et al.1994)	HUMTHO1
	HUMF13A1
	HUMFES

DNA is extracted from stain material using "Chelex 100" as described by Walsh et al. (1991). The problem encountered at the Metropolitan Police Lab was that the DNA from a significant number of stains, extracted using this method would not amplify. This was especially noticeable with semen and saliva staining. Organic extraction can be useful but it is a lengthy process and involves the use of hazardous reagents. One of the advantages of "Chelex" is that it is an extremely rapid method. Any solution to the problem could not be allowed to increase the extraction time significantly.
A method has now been introduced which filters the aqueous DNA extract produced by the "Chelex" method using a microconcentrator with a molecular weight cut off of 30,000 Daltons. This retains the majority of the DNA whilst allowing low molecular weight inhibitors of PCR to pass through. The DNA can then be rehydrated and the PCR process repeated. The "Microcon" range of miniconcentrators have been used though similar devices are available from other manufacturers.

Description of the Microcon Device:



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The Microcon miniconcentrator consists of a sample reservoir with an integral filter which connects to a 1.5 ml microcentrifuge tube. The membranes are available in a range of pore sizes. The reservoir can accept a maximum volume of 500 μl.

MPFSL Microcon Protocol:

This method is used on DNA solutions produced by the "Chelex" extraction method which should have sufficient DNA to give a result yet have produced no profile.

1) Assemble the filter unit in the microcentrifuge tube provided and label with the appropriate identifier.

2) Load the sample (max vol 500 μl) into the reservoir and close the cap.

3) Centrifuge at 14,000 g for 12 minutes. (the majority of the solution passes through the filter into the lower tube).

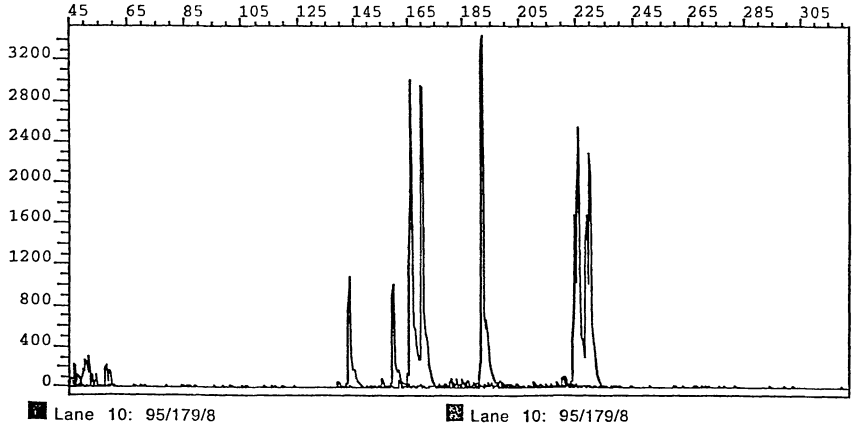
4) Add 300μl of TE buffer to the reservoir and centrifuge again as above.

5) Add 75μl of TE buffer to the reservoir, mix by pipetting up and down 2-3 times and then pipette all the solution from the reservoir into a sterile tube.

6) This sample is assayed and the PCR reaction repeated using 1-3 ng of target DNA.

An example of results following Microcon treatment.

This item was vaginal swab stained with semen. A preferential extraction was performed and 3ng of DNA amplified. The initial result was negative. Following the use of the Microcon, the profile below was obtained.



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Discussion:

Initially the Microcon procedure was used on samples that contained DNA yet which failed to amplify. Approximately 40% of these samples gave a full or partial profile when repeated. A significant proportion of these samples were semen or saliva stains and now all these types of stain are given the treatment as routine. It is inevitable that some of the DNA cannot be recovered from the device and it advisable to perform an assay before proceeding to the PCR stage.

The manufacturers recommend that after the filtration spin, the unit is inverted into a fresh tube and spun briefly to remove the retentate. Unfortunately after a 12 minute spin at high speed the two parts are firmly wedged together. Separating them requires some force and runs the risk of contaminating the operator's gloves with DNA or creating an aerosol of DNA solution. This DNA is concentrated and pure - An ideal substrate for PCR and therefore a possible contamination risk. At the Metropolitan Police Lab the procedure has been modified to avoid this. Buffer is added to the upper chamber, mixed and then pipetted into a fresh tube.

Conclusion:

Microconcentrators are a useful tool for additional purification of DNA extracts prior to PCR. They are quick to use and make a significant difference to the success rate of DNA STR profiling.

References:

Kimpton et al. Evaluation of an automated DNA profiling system 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 based typing from forensic material. Biotechniques 1:91-98.

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