

## MULTIPLEX GENETIC TYPING OF CSF1PO, TPOX AND HUMTH01 LOCI IN FORENSIC SAMPLES

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### INTRODUCTION

Due to their abundance, polymorphic nature and simplicity of amplification by PCR, microsatellite DNA loci (Small Tandem Repeats), represent a rich source of markers for genetic identification. Edwards et al. (1992) introduced the analysis of 3-4 bp repeat unit STRs which enabled the analysis of even 0.1 ng DNA samples. Moreover, these STR systems are suitable for the analysis of ancient or badly preserved specimens, which only contain very degraded DNA (Hagelberg et al. 1991; Gill et al. 1992; Jeffreys et al. 1992).

The 3 loci chosen for this work, CSF1PO, TPOX and TH01, have the advantage that can be analyzed in approximately 24 h, as they can be amplified and typed simultaneously. To evaluate the applicability of these systems to forensic casework, we have examined several samples such as hair-roots, semen stains, vaginal swabs and blood stains, each of these of varying ages. We could type unambiguously 30 day old hair-roots, up to 6 months old phenol-chloroform or Chelex 30%-extracted semen-stains and up to 13 year old blood-stains, showing the high applicability of this Multiplex system.

### MATERIALS AND METHODS

**Extraction of DNA from whole blood:** DNA extraction from 700µl of whole blood was performed using the phenol-chloroform method and the concentration adjusted to 30 ng/µl.

**Extraction of DNA from hair-roots:** the DNA from hair roots was extracted by rapid lysis. The hair root was washed with sterile milli-Q H<sub>2</sub>O and incubated in 200 µl of the lysis buffer (0.01M Tris-HCl pH 8, 0.09% Triton X-100, 35 mM DTT, 0.35 mM NaAc pH 5.2) for 30 min at 56°C. After boiling for 10 min and mixing for 10 s, it was spun at 12000 rpm and the supernatant recovered.

**Extraction of DNA from semen stains:** the DNAs from semen stains were extracted using phenol-chloroform or Chelex resin (Jung et al. 1991). With the aim of increasing the concentration of the DNA extracted, the following modifications were made: a 30% stock solution of Chelex resin was prepared and 30 µl of this were added to the sample.

**Extraction of DNA from vaginal swabs:** a differential lysis was performed followed by a phenol-chloroform extraction.

**Extraction of DNA from blood stains:** the extraction procedure was as Chelex resin above described.

**Quantification of the human DNA:** the quantities of DNA extracted were measured by slot-blot using the probe D17Z1 (Gibco-BRL) following the manufacturer's protocol.

**Multiplex amplification:** amplification were performed in a Perkin-Elmer 2400 thermocycler, using the multiplex system GenePrint™ (CSF1PO, TPOX y TH01) (Promega, USA) with minor modifications: increase to 0,75 U of Taq polymerase per sample and addition of 1 µl of BSA (10 mg/ml). Amplifications were checked by electrophoresis in 2% agarose gels stained with ethidium bromide.

**Typing of the amplification product:** electrophoresis was performed in acrylamide denaturing gels (4%, 7 M urea) in a Cambridge Electrophoresis sequentiation chamber; bands were visualized by silver staining as Budowle et al. (1991), except that the ethanol step was omitted.

## RESULTS

The analysis of CSF1PO, TPOX and TH01 loci in 161 individuals from the resident population of the Basque Country showed heterozygosities of 0.78, 0.64 and 0.72. The probability of two individuals sharing the same genotype with each of these loci is 0.08, 0.21 and 0.13 respectively, being their combined value 0.002.

The hair roots from 5 different individuals showed identical results when analyzed 7 and 30 days after pulled out.

The semen stains from 4 individuals were extracted by the methods of phenol-chloroform and Chelex at the end of 1 day and 6 months. Good results were obtained with these samples; only one of the 6 month old samples extracted with Chelex showed faint bands.

Three 1 h post-coital and three 10 h post-coital semen samples obtained through vaginal swabs were also analyzed. It was possible to obtain the male genetic profile in all the samples taken in the first hour, while, of the samples taken after 10 h or more, one of them could be typed for the three loci, another one only showed faint bands in loci TH01 and TPOX, and the last one did not show spermatic bands at all.

Twenty blood stains of 13 years old stored at environmental conditions were studied. The human DNA concentrations ranged between 0.2 to 1 ng/µl. A 72.5 % of the blood stains whose human DNA concentration was greater than 0.4 ng/µl could be directly typed. The remaining percentage could not be amplified in a first assay, despite showing a DNA content higher than the detection limit of the applied technique. To eliminate any possible inhibitor, every sample was microfiltrated by Microcon 30, removing simultaneously small DNA fragments that can interfere in the amplification reaction. Following this procedure, it was possible to type 95% of the analyzed samples.

## DISCUSSION

One of the characteristics that an identification protocol should include is rapidity. Multiplex analysis considerably reduces the time required to obtain the genetic typing. Moreover, a protocol of extraction based on Chelex-100 can be used as PCR amplification does not require highly purified DNA, though it has to be taken into account that DNAs from whole blood or blood stains extracted by Chelex resin must be amplified as soon as possible because the continuous release of Fe<sup>++</sup> from the hemo group interferes with the amplification process as was also described by Prinz and Schmitt (1994).

Another characteristic is that the analyzed loci must show enough power of discrimination. The probability of genotype matching with the 3 loci CSF1PO, TPOX and TH01 that we have calculated for the population residing in the Basque Country is  $2 \times 10^{-3}$ . Therefore a multiplex genetic identification protocol of STR loci with high probability of discrimination can be proposed that should allow, in a first step, to straightforwardly reduce the number of cases of no exclusion that must be analyzed further employing a high number of loci.

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## LITERATURE

Edwards A, Hammond H. A, Jin L, Caskey C. T, Chakraborty R. (1992). Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics*. 12:241-253.

Gill P, Kimpton C. P, Sullivan K. M. (1992). A rapid polymerase chain reaction method for identifying fixed specimens. *Electrophoresis* 13:173-175.

Hagelberg E, Gray I. C, Jeffreys A. J. (1991). Identification of the skeletal remains of a murder victim by ADN analysis. *Nature* 352:427-429.

Jeffreys A. J, Allen M. J, Hagelberg E, Sonnberg A. (1992). Identification of the skeletal remains of Josef Mengele by ADN analysis. *Forensic Sci. Int.* 56:65-76.

Jung JM, Comey CT, Bair DB, Budowle B (1991) Extraction strategy for obtaining DNA from blood stains for PCR amplification and typing of the HLA DQ $\alpha$  gene. *Int J Leg Med* 104:145-148.

Prinz and Schmitt (1994). Effect of degradation on PCR based DNA typing. *Advances in Forensic Haemogenetics*, 5: 375-378