SEQUENTIAL MULTIPLEX AMPLIFICATION (SMA) IN CASES WITH MINIMAL AMOUNTS OF DNA

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INTRODUCTION

Typing polymorphic loci at the DNA level has become a routine procedure in the identity testing field. DNA samples are usually amplified by PCR and subsequently typed; the remainder of the untyped PCR is generally not used or discarded.

To obtain more genetic information from a sample several distinct loci can be amplified in one PCR simultaneously by a process known as multiplex PCR (Edwards et al. 1992).

We proposed an alternate multiplex approach called *SEQUENTIAL MULTIPLEX AMPLIFICATION (SMA)*. SMA enables the typing of several loci using only one DNA sample without requiring all the loci to be amplified under one set of PCR conditions (Lorente et al, 1994a).

MATERIALS AND METHODS

The *general procedure* for SMA typing is as follows:

1. Extract DNA (organic or chelex).

2. Determine the quantity of the DNA with a slot-blot procedure using a human specific alphoid probe.

3. Amplify and type DNA for the D1S80 locus according to the method of Budowle et al (1991).

4. Recover the genomic DNA from the remaining D1S80 PCR solution by filtration through a Microcon-100 (Amicon, Beverly, Ma).

5. Amplify and type the recovered DNA for the HUMTH01 locus (Lorente et al. 1994b).

6. Repeat step 4 and amplify and type for HUMVWA (Lorente et al. 1994c).

7. Repeat step 4 and amplify and type for HLA-DQx (Saiki et al. 1989).

Following the SMA procedure, we have reconsidered 62 cases (44 from previous research programs; 18 from casework samples) that were studied (PCR-based technology) in our Laboratory between 1989 and 1995. In all cases, only one locus could be amplified

at that time (usually D1S80 or HLA-DQA1), because the amount of DNA was not enough to try further loci.

The 62 samples considered came from blood-stains (24), semen-stains (8), hairs (11), cigarette butts (9), stamps or envelopes (5), and bones (5).

All of these samples had been frozen at -40°C during the last 1 to 6 years, and for this study were thawed in the refrigerator(+4°C). Since the whole genomic DNA extracted were used for the amplifications performed some years ago, SMA procedure started by step #4 of the Materials & Methods.

RESULTS and DISCUSSION

SMA has enabled amplification of further loci up to 6 years after the extraction and initial amplification. Therefore, a higher degree of discrimination was thus obtained from a small quantity of sample.

Out of 62 samples, 60 (96.7%) could be positively amplified after SMA for a second locus; for a third locus, positive amplification and correct typing was possible in 32 (53.3%) out of the 60 cases that yielded a positive third amplification; furtherly, 12 (32.5%) samples out of 32 were amplified for an additional fourth locus or set of loci, as Polymarker® (Perkin Elmer Corporation, Norwalk, CT).

As an average, the probability of discrimination increased from 0.934 (HLA-DQA1), 0.921 (D1S80), 0.933 (HUMTH01) or 0.995 (Polymarker®) to 0.9995, considering all the loci together. Considering a database of the Spanish population, probabilities can be increased from 1 in 2.500 to 1 in 3.000.000, depending on the allele frequencies in each case.

When using the SMA procedure with the Perkin-Elmer-Cetus's Polymarker or HLA-DQA1 kits, special attention should be given to the interpretation of the *control-dots* if the same sample had been previously analyzed with any of these kits, since the controls appear more intense as regularly. This usually happens with old samples that had been previously submitted to HLA-DQA1 analysis and now can be processed through SMA and be ready for Polymarker®. (Hochmeister et al, J Forensic Sci, July 1995)

In any case, the SMA procedure can be useful for situations where the quantity of template DNA is limited or to amplify additional loci from DNA templates that have been previously used and that were adequately frozen or refrigerated.

With this procedure it is also possible to recover template DNA from PCR tubes that yielded no amplification product (e.g. DNA from evidentiary samples that might be degraded for a given locus, but that could be amplified for some other loci).

The SMA approach is compatible with any of the multiplex amplification approach, and we've got good results by using the Promega® Triplex (CSF, TH01, TPOX) after a D1S80 amplification and just before using the HLA-DQA1 kit.

Special care should also be taken during the SMA procedure, since amplified materials are handled; we strongly recommend to proceed in post-amplification rooms and using specific set of primers, dNTP's and Taq, following the same rules and cautions that are usual when re-amplifying allelic ladders.

As a general rule, it is recommended that amplification should proceed as follows: length polymorphisms first, in descending order by size, followed by sequence polymorphisms (currently detected by sequence-specific oligonucleotide probes).

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