SEQUENTIAL MULTIPLEX AMPLIFICATION (SMA) WITH THE POWERPLEX™ 16

Jose A. Lorente[1], Miguel Lorente[1], J. Carlos Alvarez[1], Carmen Entrala[1], Bruce Budowle[2], and Enrique Villanueva[1]
[1]*Lab. of Genetica Identification, Legal Medicine Dept., University of Granada, Spain*
[2]*Laboratory Division, FBI Academy, Quantico, VA*

Lorente, et al. [1, 2] previously proposed an alternate approach to multiplex PCR known as *Sequential Multiplex Amplification (SMA).* SMA utilizes the same template DNA recovered from a previous PCR in a subsequent PCR amplification. Basically, the DNA template is amplified at a particular target sequence; but during the PCR the original template DNA remains intact. After typing the locus, the remaining PCR sample is washed using a Microcon-100 filtration device and the recovered genomic DNA then is used for amplification of another locus or loci. Theoretically, the process can be repeated several times, if desired.

Originally when SMA was developed, HLA-DQA1, Polymarker and the D1S80 loci were being used routinely. Combining these loci with a couple of STR loci (i.e., TH01 and vWA) in the SMA was the only way to obtain very high levels of characterization with very limited template DNA samples. Because today's STR typing systems employ fluorescent detection systems, non-fluorescently labeled amplified product cannot interfere with subsequent typing. Thus, the SMA approach would be facilitated. However, more powerful multiplex systems exist today that enable simultaneous amplification of up to 15 STR loci. Thus, the need for SMA would seem unnecessary today.

However, there are at least two situations where SMA still can be useful. The first is some old cases (prior to STR typing) where the amount of template DNA permitted only a single amplification, and the amplified product has been stored. It is possible that a few old cases may be revisited and more genetic information might be needed. The second is to use the SMA approach to recover the template DNA on a limited sample where an initial PCR failed, yet the data in the case suggests that there was sufficient template DNA for an analysis. This second application may be practical in some PCRs because with current STR typing only a small portion of the amplified product is consumed after PCR. Thus, most of the original template DNA remains in the PCR.

To test the application of SMA with multiplex STR kit technology (PowerPlex™ 16 kit, Promega Corp.) 43 old PCR samples that were previously typed for the HLA-DQA1 and/or D1s80 locus, and which had been stored for at least 6 years, were subjected to SMA. 24 of these samples were from paternity cases (thus the original template DNA typically was a high quality), and 19 of these samples were from forensic evidence (thus the quality and quantity of the original template DNA varied). Using the PowerPlex™ 16 kit, 92% (22/24) of the paternity cases were typable for all loci, 53% (10/19) of the casework samples yielded typable results at all loci, and 21% (4/19) of the casework samples yielded partial typable profiles.

While the SMA approach can be used on old samples, limitations such as manipulating amplified DNA in pre-PCR areas could prove problematic for most QC/QA protocols. However, for those laboratories that no longer type the loci HLA-DQA1, Polymarker, and/or D1S80, use of SMA may not be considered a violation of QC/QA protocols. Bringing into the pre-PCR area amplified product of loci no longer analysed would not affect STR typing and thus can be employed maintaining the current QC/QA requirements for PCR-based typing.

In conclusion, SMA using multiplex STR kits to obtain more genetic information has proven to be useful in typing genomic DNA contained in stored PCR samples and analysis of samples of limited quality and/or quantity for previous PCRs.