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SNP genotyping with single base extension-tag microarrays

B. Sobrino, M. Lareu*, M. Brion, A. Carracedo

Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, University of Santiago de Compostela, San Francisco 15782, Santiago de Campostela, Spain

Abstract. During the last few years, there is an increasing interest in the use of Single Nucleotide Polymorphisms (SNPs) for forensic purposes as an alternative to STR analysis. At this moment, development of SNP genotyping technologies to analyse several markers in the same reaction with high accuracy, simplicity and reasonable cost is the key to progress in SNP typing for forensic genetics. A promising approach for this purpose is DNA microarrays. We have developed a microarray for typing a set of 29 Y-chromosome SNPs for European populations. Single Base Extension and Tags (SBE-Tag) has been the strategy selected. © 2004 Elsevier B.V. All rights reserved.

Keywords: Single nucleotide polymorphism (SNP); Y-chromosome; Microarrays; Single base extension (SBE)

1. Introduction

Single Nucleotide Polymorphisms (SNPs) represent the most abundant source of sequence variation in the human genome. This feature together with their low mutation rate and their simplicity make these markers suitable for forensic applications. Many methods are currently available for characterizing known SNPs [2]. The strategy selected for typing a set of 29 Y-chromosome SNPs is SBE-Tag [3]. With this method, SNPs are genotyped by minisequencing using primers with a 5′ tag sequence. Each SNP has its own specific tag. The multiplex SBE reaction is performed in solution and is analysed after hybridisation to a generic tag array. The use of tags allows us to use the same tag array to genotype different sets of SNPs for different purposes. The ‘‘array of arrays’’ concept [4] allows the simultaneous analysis on different samples in the same slide, saving money and time.

2. Material and methods

Oligonucleotides complementary to the tags of the SBE primers, with a stretch of 15 dT residues followed by a NH2 group at the 3′ end, were spotted onto CodeLink slides

* Corresponding author. Tel.: +34-981582327; fax: +34-981580336.
E-mail address: apimllar@usc.es (M. Lareu).

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(Amersham Bioscience) with the Affymetrix 417 Arrayer. After spotting, the slides were prepared as described in the product protocol.

The 29 Y-SNPs were divided in 4 multiplexes. Each PCR reaction was performed in 25 μl using 1 × PCR buffer, 300 μM of dNTPs, 2 mM of MgCl2, 0.1–0.5 μM of primers, 2U of AmpliTaq Gold DNA polymerase (Applied Biosystem) and 10 ng of DNA. The PCR conditions were 95 °C for 10 min, 32 cycles of 94 °C for 30 s, 59 °C for 30 s, 70 °C for 30 s and a final extension at 65 °C for 15 min. The sequences of PCR and minisequencing primers are available from the authors upon request. The four PCR products were mixed and evaporated to a final volume of 20 μl to concentrate the sample. 4 μl of Exo-SapIT (Amersham Bioscience) were added to 10 μl of mixed PCR products and incubated at 37 °C for 15 min followed by 85 °C for 15 min.

2.5 μl of the treated PCR product was added to 10 μl of a SBE reaction mix containing 1X Thermosequenase buffer, 0.25 μmol of each TAG-SBE primer, 2U of Thermosequenase (APB) and one of the four JOE-labelled ddNTP and the other three ddNTPs unlabelled at a concentration of 0.5 μmol. The SBE reaction consisted of 30 cycles of primer extension (96 °C for 30 s, 50 °C for 30 s and 60 °C for 30 s).

The hybridization was performed as described on the slide protocol. The volume involved in the hybridization reaction was 7 μl of SBE products mixed with 8 μl of hybridization buffer. Arrays were scanned using the Affymetrix 418 Scanner, and analysed using ImaGene 4.1 (BioDiscovery).

3. Results and discussion

We selected 29 Y-chromosome SNPs, which appear to be the most important for European populations [1]. These SNPs were divided into 4 multiplex PCR reactions. The four PCR products were pooled in order to be used as template for the multiplex SBE reaction. The use of SBE-Tag strategy allows us to use a standard array of generic tags, which eliminates the need to design and manufacture custom arrays for specific sets of markers. The tags are immobilized covalently on microscope slides in an "array of arrays" format (Fig. 1) [4].

Fig. 2 shows the results of a sample. Four SBE reactions were performed per sample, one for each labelled ddNTP because only one fluorescent label for ddNTPs was used. We obtained results for 24 of 29 SNPs, although the signal of some SNPs needs to be

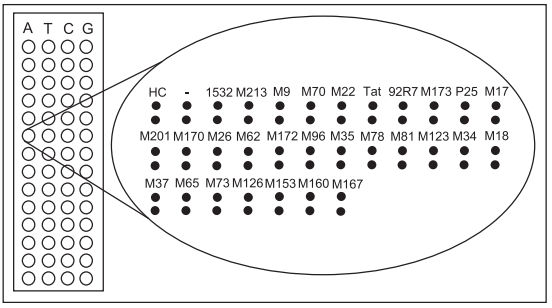

Fig. 1. Representation of an "array of arrays" format [4].

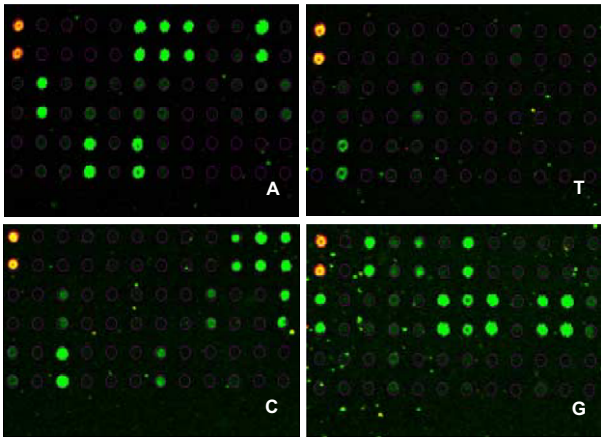

Fig. 2. Results of a sample.

increased. P25 shows signal in panels A and C because it is duplicated. However, the G signal of M22 is because of self-complementary of the primer.

In the light of the results, the SNP genotyping with SBE-Tag microarray is a promising strategy for different applications where a large number of SNPs are required such as forensic genetics. Nevertheless, much work remains to be done to validate SNP typing methods before they can be used in routine forensic analysis.

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