International Congress Series 1239 (2003) 21–25

Typing Y-chromosome single nucleotide polymorphisms with DNA microarray technology

M. Lareu [a,*], B. Sobrino [a], C. Phillips [a,b], M. Torres [a], M. Brión [a], A. Carracedo [a]

[a] *Institute of Legal Medicine, Unit of Forensic Genetics, Faculty of Medicine, University of Santiago de Compostela, C/ San Francisco, 15705 Santiago de Compostela, Galicia, Spain*
[b] *Department of Haematology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, UK*

Abstract

We are developing DNA microarrays for Y-chromosome single nucleotide polymorphism (SNP) typing in forensics using the Affymetrix 417 arrayer and the Affymetrix 418 scanner. The advantages of using Y-chromosome SNPs include the low mutation rates (in deficiency paternity testing), the sensitivity of Y-chromosome SNP typing in mixed stains when the male component is in a very low proportion and the possibility of using high-throughput methods for typing. A total of 32 Y-chromosome SNPs distributed in four PCR multiplexes were selected including the most interesting ones in western European populations. The SNPs selected include some classical ones and some recently reported Y SNPs. Low-density DNA microarrays are sufficient for forensic applications. Spotting probes on slides, followed by hybridization, are in general adequate for SNP typing. Different spotting systems are available and the pin-and-ring system that we have selected is robust and reproducible. Evaporation has its greatest potential impact on the samples in the wells waiting to be spotted and its control is the main problem for reproducibility. The type of slides used, hybridization strategies and typing strategies will be shown and discussed. The advantages and disadvantages of the methods used to increase typing accuracy are discussed as well as the PCR multiplex strategies, which must necessarily be combined with microarray technology, at least in criminal casework applications.

Keywords: Y-chromosome; SNPs; Microarray

* Corresponding author. Tel.: +34-981-582327; fax: +34-981-580336.
E-mail address: apimllar@usc.es (M. Lareu).

0531-5131/03 © 2003 Elsevier Science B.V. All rights reserved.
PII: S0531-5131(02)00495-8

1. Introduction

Single nucleotide polymorphisms (SNPs) represent the most abundant form of genomic sequence variation, they are very robust, and are suitable for analysis using high-throughput technologies. All these characteristics make these markers very useful for a variety of different applications such as forensic genetics, anthropology, clinical genetics, and pharmacogenetics [1,2].

The choice of SNP markers used, depends on the application. In paternity testing, autosomal SNPs without linkage or association with disease provide the best approach. In criminalistic casework, mitochondrial and Y-chromosome SNPs markers are often the only real option for solving problematic cases. Y-chromosome SNPs have the additional advantage that can be used to type forensic samples of mixed origin, when a male component is in a very low proportion.

It is now clear that SNP typing on a large scale is and will be of prime importance in human genetics, and the choice of the method to analyse these polymorphisms must be a high-throughput technique that can be easily applied in molecular labs. Microarray technology has become one of the most promising approaches for analysing SNPs. However, in order to make microarray technology a realistic and manageable option the construction of large multiplexes (40–50 loci) is essential.

2. Material and methods

A total of 32 Y-chromosome SNPs distributed in four multiplexes were selected including some of the most interesting ones in western European populations (Jobling, personal communication): *Core multiplex* (PN25, SRY-1532, M45, M9, M89, YAP), *YAP+ multiplex* (SRY-8299, PN2, DYS391, PN1, sY81, M35, M123, M81, M78) *M89 Multiplex* (12f2, M62, M172, M170, M26, M52, M201) *PN25 multiplex* (SRY-2627, M153, M160, M126, M73, M65, M37, M18) and two singleplexes LLY22 g and Tat. To create microarrays, we used an Affymetrix 417 Arrayer at 25–27 °C and 62–65% relative humidity. Reproducibility, background noise, attachment efficiency and signal intensity were checked using different spotting solutions: ArrayIt Microspotting solution (TeleChem) and SSC, and two different kinds of orientated slides: SuperAldehyde slides (TeleChem) and 3D-Link slides (Motorola). Two different strategies were used to type the SNPs for the microarray: allelic specific oligonucleotides (ASO) hybridization and minisequencing with single base extension (SBE)–TAGs [3].

2.1. ASO

The allele-specific oligonucleotides contained a 5′ amino group for covalent immobilization on SuperAldehyde or 3D-link slides. Hybridization conditions: 4 μl of multiplex PCR product was denatured at 96 °C for 3 min and mixed with 16 μl of UniHyb (ArrayIt) hybridization solution pre-warmed at 65 °C for 30 s with SuperAldehyde slides. With 3D-link slides, PCR products were resuspended in 5 × SSC, 0.1% SDS, 0.1 mg/ml salmon sperm DNA and heated in a boiling water bath for 2 min.

2.2. SBE–TAG

Generic oligonucleotides (cTAGs) were spotted on SuperAldehyde and 3D-link slides. After spotting, the slides were prepared as described in the product protocols. Mini-sequencing reaction: After multiplex PCR, 15 μl of the PCR products was added to a mixture containing 5 U of shrimp alkaline phosphatase (APB, Uppsala, Sweden) and 2 U of exonuclease I (APB) in 1 × SAP buffer was incubated at 37 °C for 60 min followed by 96 °C for 15 min. A 10 μl of the treated PCR product was added to 5 μl of a SBE reaction mix containing 1 × Thermosequenase buffer, 1 pmol of each TAG–SBE primer, 2 U of Thermosequenase (APB) and 5 pmol each of labeled-ddNTPs. The SBE reaction consisted of 30 cycles of primer extension (96 °C for 30 s, 50 °C for 30 s and 60 °C for 1 min). The SBE products were digested with 1 U of SAP at 37 °C for 60 min followed by 96 °C for 15 min. Hybridization conditions: 15 μl of SBE products was mixed with 7 μl of hybridization solution, added to the microarray and hybridized at 50 °C for 4 h.

2.3. Washing

The microarrays were washed in 2 × SSC for 10 s at room temperature, followed by a brief rinse with H_2O.

2.4. Scanning and quantification

Arrays were scanned using an Affymetrix 418 Scanner, and analysed using software Affymetrix Pathways from Affymetrix and ImaGene from BioDiscovery.

3. Results and discussion

Microarray technology is an important approach for typing SNPs, although a lot of work still needs to be done to use this technique as a realistic option in forensic genetics. To realise the full potential of microarray technology, large-scale multiplexes are an essential pre-requisite. Initial PCR experiments with a limited number of SNPs in small

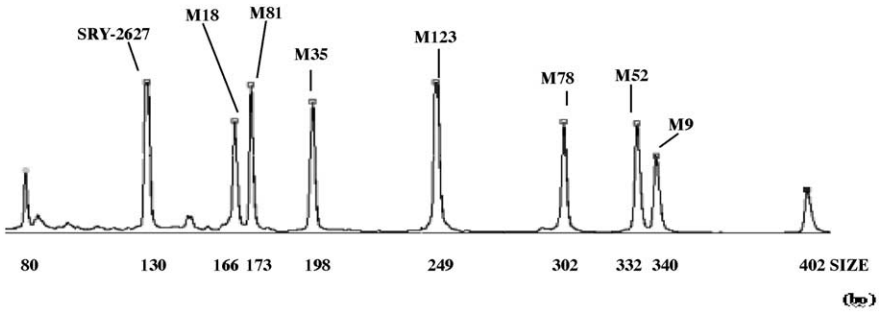

Fig. 1. Octoplex Y-chromosome SNPs.

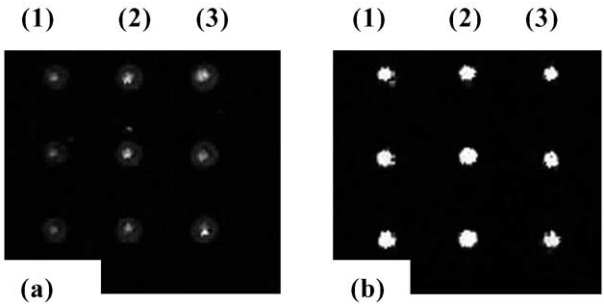

Fig. 2. Comparison between SuperAldehyde slides (a) and 3D-link slides (b). In both slides, we can see a mixture of the two alleles for the M9 Y-SNP. Row 1—positive control. Row 2—allele C. Row 3—allele G.

multiplexes of 8–9 loci gave good results (Fig. 1), but much larger combinations of 40–50 SNPs become very difficult to optimise.

It is obvious that microarray quality depends on the uniformity and consistency of the spots, which depends in turn on spotting technique, spotting conditions (temperature and humidity), spotting solutions, slide surface, etc.

Temperatures between 25 and 27 °C and humidity levels of 62–65% gave us the best results. Using SuperAldehyde slides with ArrayIt spotting solution gave better results that SSC, but the best results were obtained using 3D-link orientated slides with the manufacturers' conditions (Fig. 2).

The strategy chosen to type SNPs with microarrays is equally important. The hybridization and consistency of results across a range of different loci must be optimal when working with multiplexes. Using ASO hybridization methods, we obtained good results when working with singleplex SNPs but the results were weaker and more inconsistent when used with multiplexes. In agreement with other authors, we think that it is very difficult to analyse several SNPs simultaneously because the efficiency of hybridisation and the stability of the formed hybrid depend on the flanking sequences.

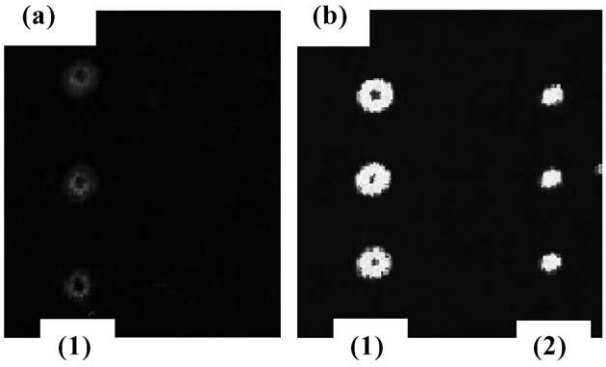

Fig. 3. 92R7 (sample with allele T) analysed by minisequencing SBE–Tags (a) Green laser—Joe ddCTP. (1) Positive control. (b) Red laser—Cyanine ddUTP. (1) Positive control, (2) allele T.

Minisequencing is gaining increasing acceptance as the strategy for typing SNPs in microarray assays, using the protocol for SBE–TAGs described above provides good signal strength, more balanced spot intensities and good discrimination between alleles (Fig. 3). It is a good strategy to type SNPs particularly when analysing large SNP multiplexes.

4. Conclusions

Microarray technology is a promising approach for typing large numbers of SNPs in single reaction analyses.

The spotting conditions including spotting solutions and slide surface are very important for the quality of the microarray.

Minisequencing with SBE–TAGs is a good strategy to type SNPs particularly when analysing large SNP multiplexes.

The creation of large multiplexes of SNPs is a big challenge and is essential to make microarray technology a real option for the forensic genetic laboratories.

Acknowledgements

This work was supported by grants from Xunta de Galicia (PGIDT01PXI20806 PR) and Ministerio de Educación y Ciencia (BIOT 2000/PC092). We want to thank Dr. Denise Syndercombe Court and Meli Rodriguez for their help. B.S. has a fellowship from the Ramón Areces Foundation.

References

[1] P.A. Underhill, L. Jin, A.A. Lin, Q. Mehdi, T. Jenkins, D. Vollrath, R.W. Davis, L.L. Cavalli-Sforza, P.J. Oefner, Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography, Genome Res. 7 (1997) 996–1005.

[2] T. Zerjal, B. Dashnyam, A. Pandya, A. Pandya, M. Kayser, L. Roewer, F.R. Santos, W. Schiefenhövel, N. Fretwell, M.A. Jobling, S. Harihara, K. Shimizu, D. Semjidmaa, A. Sajantila, P. Salo, M.H. Crawford, E.K. Ginter, O.V. Evgrafov, C. Tyler-Smith, Genetic relationships of Asians and Northern Europeans, revealed by Y-chromosomal DNA analysis, Am. J. Hum. Genet. 60 (1997) 1174–1183.

[3] J.N. Hirschorn, P. Sklar, K. Lindblad-Toh, Y.-M. Lim, M. Ruiz-Gutierrez, S. Bolk, B. Langhorst, S. Schaffner, E. Winchester, E.S. Lander, SBE–TAGS: an array-based method for efficient single nucleotide polymorphism genotyping, PNAS 97 (22) (2000) 12164–12169.