International Congress Series 1288 (2006) 43–45

www.ics-elsevier.com

Application of Nanogen microarray technology for forensic SNP analysis

SNP*for*ID Consortium[1]

M.K. Balogh [a,*], K. Bender [a], P.M. Schneider [a,b]

[a] *Institute of Legal Medicine, Johannes Gutenberg University of Mainz, Germany*
[b] *Institute of Legal Medicine, University of Cologne, Germany*

Abstract. The NanoChip® Molecular Biology Workstation using electronic microarrays is an approach for rapid and high throughput analysis of SNPs. This instrument is fully automated and uses a microchip for electronic addressing of capture probes to specific array sites followed by electronic hybridisation of the single stranded PCR products, and passive hybridisation of fluorescently labelled reporter probes. Discrimination is achieved by applying thermal stringency to denature the mismatched reporters. 48 SNP assays have been designed using the 'capture down' assay which applies a thermal 'touch down' strategy to obtain the best reporter probe discrimination.

Keywords: Electronic microarray; SNP typing; Microchip technology; Hybridisation

1. Introduction

For high throughput forensic identification of individuals using SNPs, it is essential to assess and validate suitable DNA typing platform for reliable and accurate multiplex SNP typing. A very promising microarray format is the electronically activated NanoChip (Nanogen Inc.), a hybridisation-based genotyping platform that theoretically offers simultaneous analysis of genetic polymorphisms [1]. The main purpose of the present work was to evaluate the sensitivity, the accuracy and the multiplex capability of this platform by using 48 autosomal SNPs chosen from 52 non-coding SNPs, previously

* Corresponding author.
E-mail address: balok000@students.uni-mainz.de (M.K. Balogh).
[1] www.snpforid.org.

0531-5131/
doi:10.1016/j.ics.2005.10.047

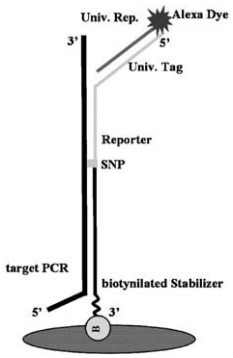

Fig. 1. Capture down assay.

selected by the SNP*for*ID consortium. Forensic typing requires rapid multiplex analysis from limited samples under high throughput conditions. Our preliminary data seem to indicate that the capture down method may fulfill the expectations for a high throughput SNP typing technology.

2. Material and methods

The Nanogen Workstation is a fully automated instrument and uses a proprietary semiconductor microchip for rapid electronic addressing of capture probes to specific array sites, followed by electronic hybridisation of single-stranded PCR products, and passive hybridisation of labelled reporter probes in a single reaction (Fig. 1). Discrimination is achieved by applying thermal stringency to denature the mismatched reporter probes [2,3]. PCR primer sequences to amplify 48 autosomal SNPs were used which were previously designed for a 52 SNP multiplex PCR reaction and the SNaPshot reference method (J.J. Sanchez et al., unpublished). The PCR products must be properly desalted after amplification, as the presence of salt will strongly inhibit the microarray hybridization. The purification was successfully carried out using the MSB Spin PCRapace kit (Invitek GmbH). The Nanogen technology requires target-specific fluorochrome-labeled reporter oligonucleotide probes, for both wild-type and mutant sequences. To eliminate the need for large numbers of expensive labeled reporter probes, only two universal reporters were used for all assays. The universal reporting system utilizes a non-labeled SNP-specific discriminator oligonucleotide combined with a universal recognition sequence complementary to one of the two reporter probes. With

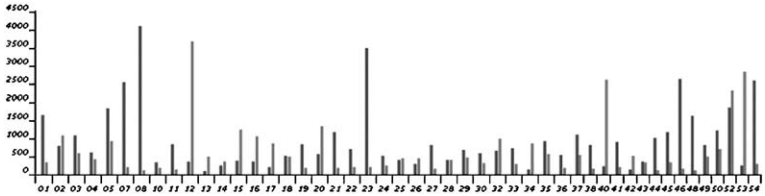

Fig. 2. Typical result of one sample obtained for 48 SNPs simultaneously at 41 °C scan.

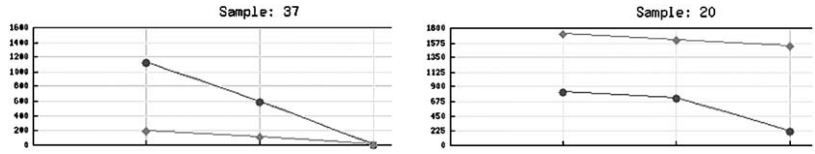

Fig. 3. Trending graphs for two SNPs (SNP 37 and SNP 20) for three different scans, detected at 38 °C, 41 °C and after washing with low salt buffer.

this approach, only one pair of fluorochrome-labeled probes is required for analyzing all SNPs in the multiplex assay (Fig. 1).

3. Results and discussion

Fig. 2 depicts a typical result obtained simultaneously for all 48 SNPs from one amplified DNA sample bound to the capture array, hybridized to the reporter probes, and scanned at 41 °C. It has to be noticed that there were some SNPs, which gave poor fluorescent signals, e.g. SNPs 10 and 14. To overcome these problems, asymmetrical reamplification of the PCR products by using only one primer to increase the number of single stranded DNA molecules for hybridization improved the results for these SNPs. The trending graph for three different scans (Fig. 3), detected at 38 °C, 41 °C, and after washing with low salt buffer indicates that SNP 37 worked well at 38 °C while the reporter was lost at a higher temperature or after stringent wash. In contrast, other SNPs such as the wild-type SNP 20 could reliably be discriminated after washing with low salt buffer (Fig. 3). Thus we succeeded in getting most of the 96 reporter probes for 48 SNPs to discriminate at only three different conditions. However, 9 SNPs from 48 did not work well. However, we are confident that redesigning the reporter probes for these SNPs will help to overcome these difficulties. Alternatively, problematic SNPs can be replaced with others more suitable for the design requirements. Further studies are needed to optimize the reporter oligo design for obtaining the same discrimination temperature for all SNPs. Our experience shows that not all of the selected SNPs can be easily typed using the NanoChip platform because some will probably fail the oligo design criteria—accordingly a larger contingent of SNPs are needed for selection to develop and validate a standardized capture probe array. Overall, the NanoChip platform is a suitable technology for SNP multiplex typing. It will be further expanded and evaluated for forensic casework.

Acknowledgments

Supported by a grant from the European Community, GROWTH programme, contract G6RD-CT-2002-00844. Special thanks to Bjoern Hihn from Nanogen for helpful discussions, and to Nanogen Europe B.V. for support.

References

[1] H.A. Behrensdorf, et al., Nucleic Acids Res. 15 (30) (2002) (14):e64(30(14)):e64.
[2] L.H. Eckardt, et al., Nature 420 (6913) (2002) 286.
[3] R. Sosnowski, et al., Psychiatr. Genet. 12 (41) (2002) 81–92.