International Congress Series 1261 (2004) 9–11

ELSEVIER

www.ics-elsevier.com

Detection of genetic variation by MALDI-TOF mass spectrometry: rapid SNP genotyping using the GENOLINK system

D. Niemeyer[a], T. Elssner[a], K. Fahr[a], D. Peters[a], T. Wenzel[a], E. Petkovski[b,c], M. Kostrzewa[a,*]

[a] *Bruker Daltonik GmbH, Permoserstrasse 15, D-04318 Leipzig, Germany*
[b] *CODGENE Laboratory, Strasbourg, France*
[c] *Institute of Legal Medicine, Louis Pasteur University, Strasbourg, France*

Abstract. Single nucleotide polymorphisms (SNPs) are the most common form of genetic variation in the human genome. Because of their low mutation rate, SNPs have the potential to be as discriminating as loci of higher polymorphism, e.g. for forensic approaches such as paternity tests or human identification in general. To balance the lack of polymorphic information the number of markers which are tested has to be increased. Here, we introduce a new automated platform for SNP genotyping by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), easy to use as well for higher DNA sample throughput as also for the analysis of an increased number of SNPs.

Keywords: SNP genotyping; Human identification; MALDI-TOF MS; Automated sample preparation

1. Introduction

In forensic casework, single nucleotide polymorphisms (SNPs) are of considerable interest because of the capability to genotype very short amplified fragments [1]. Therefore, SNPs may get of increasing importance for analysis of strongly degraded samples. In forensic routine casework methods for SNP genotyping have to achieve highest quality requirements. The limited discriminatory nature of biallelic SNPs can be balanced by increasing their number per analysis. A set of about 50 different SNPs is necessary to obtain the discrimination power of existing STR multiplexes. Nowadays SNP databases already contain numerous suitable and validated loci [2].

We present the analysis of nine autosomal SNPs, located in non-coding regions, which might serve as forensic markers. The analysis was performed using GENOLINK, a SNP genotyping platform for functional genomic studies [3]. GENOLINK is the combination of

* Corresponding author. Tel.: +49-341-4912-170; fax: +49-341-4912-179.
E-mail address: km@bdal.de (M. Kostrzewa).

0531-5131/
doi:10.1016/S0531-5131(03)01836-3

Table 1
PEX-primer/product sequences and masses

	PEX-primer sequence 5′-3′	PEX-primer	PEX-products
PCI	Rx: bio-ctg ggg gca gcg gLa gcc aag	2194 Da	ddA: 2492/ddG: 2507 Da
C1r	Fx: bio-gtg ctt ccc gLa gca aat	2194 Da	ddC: 2467/ddT: 2481 Da
C8a	Fx: bio-gtt cta caa ctt Ltg taa gg	2240 Da	ddA: 2538/ddC: 2514 Da
DNaseI	Fx: bio-gtt gca ggg atL ctg ctc c	2112 Da	ddA: 2410/ddG: 2426 Da
WI 1126	Rx: bio-caa gaa ata aaa gLc aag tcc	2146 Da	ddA: 2443/ddG: 2459 Da
WI 1306	Rx: bio-aag gta gcc cLc aga agc	2195 Da	ddC: 2468/ddT: 2483 Da
WI 1349	Fx: bio-cag aaa ata tga ttL gaa aaa g	2243 Da	ddA: 2539/ddC: 2515 Da
WI 1888	Rx: bio-gtt gtc ttt tct Ltg taa tg	2216 Da	ddC: 2489/ddT: 2504 Da
WI 867	Fx: bio-tct ttc cct ttL cat ctc c	2096 Da	ddA: 2394/ddG: 2410 Da

a novel method for SNP genotyping, genoSNIP [4], dedicated hardware for automated sample preparation and analysis, and a bioinformatic package.

2. Materials and methods

2.1. DNA amplification

PCR was performed in singleplex and multiplex reactions. Amplicons of 64–194 bp were generated. Residual primers and nucleotides were disabled by incubation with Shrimp Alkaline Phosphatase (SAP) at 37 °C for 45 min. After deactivation of SAP (90 °C, 10 min), this reaction mixture was directly used for the following primer extension reaction (PEX).

2.2. Primer extension reaction

Primers with a biotin label at the 5′ end and a photocleavable building block at position 8 or 9 from the 3′ end were used for single base extension (Table 1). The PEX mixture contained 1 U of PEX-Polymerase and 2 mM of each ddNTP.

The primer extension products were purified using genostrep kits (Bruker Daltonik). The biotin-labelled oligonucleotides were specifically bound to the streptavidin-coated cavities of special microtiter plates. Salts and other impurities were removed by

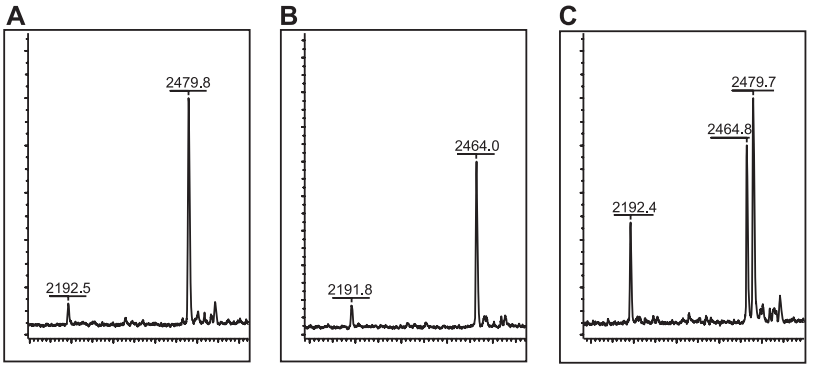

Fig. 1. Three example spectra for SNP WI 1306. *X*-axis—mass/charge ratio; *Y*-axis—relative intensity. (A) Homozygous person + ddC. (B) Homozygous person + ddT. (C) Heterozygous person.

washing. After adding the elution buffer the plate was exposed to UV light (366 nm, UV-unit CL-366, Bruker Daltonik). The primers were cleaved and the specifically extended 3′ end was eluted and directly supplied to matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) analysis. All pipetting steps were performed by the puredisk robot (Bruker Daltonik).

2.3. MALDI-TOF MS

Samples were transferred onto an AnchorChip target 400-384 and measured in linear mode in an autoflex mass spectrometer (Bruker Daltonik). The matrix was 3-hydroxypicolinic acid (20 mg/ml 3-HPA + 1 mg/ml di-ammoniumhydrogen-citrate). The spectra were automatically analysed using the software genotools 2.0 (Bruker Daltonik). The analysis implied the genotype assignment and a quality check of the spectrum/result.

3. Results

All SNPs of interest could be genotyped successfully. Fig. 1 shows the results of the assay for three different anonymous DNA samples, representing the three different genotypes for SNP WI 1306.

To demonstrate multiplex capability, several SNPs were successfully combined in duplex or triplex reactions (Clr/WI1349, WI867/1126, WI867/Clr/WI1348, data not shown). Automated screening runs, using the puredisk for all pipetting were performed for a 96-microtiter plate containing 93 anonymous DNA samples and 3 water controls.

4. Discussion

GenoSNIP delivered reliable genotyping results. The PEX-primers hybridise directly adjacent to the polymorphic sites and are converted into allele specific products by single nucleotide extension. The photo-cleavable building block in their sequence enables the elution after purification. As the released fragments consist of only 8–10 nucleotides, they are measured with high precision and sensitivity, in particular because of a decreased affinity to salt ions. The slightest mass difference between two nucleotides (9 Da between A and T) can steadily be detected in this mass range.

The mass spectra were translated into genotypes by the genotools 2.0 software. The samples were transferred from the 96-raster to the 384-raster and therefore each sample was measured four times. Analysis of four spectra per sample gave equal results and therefore verified the reliability of analysis. Summing up the GENOLINK system comprises a complete solution for automated, highly accurate SNP genotyping by MALDI-TOF MS.

References

[1] P. Gill, An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes, Int. J. Leg. Med. 114 (4–5) (2001) 204–210.

[2] D. Sachidanandam, et al., A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms, Nature 409 (2001) 928–933.

[3] W. Pusch, et al., MALDI-TOF mass spectrometry based SNP genotyping, Pharmacogenomics 3 (2002) 537–548.

[4] T. Wenzel, et al., GenoSNIP: SNP genotyping by MALDI-TOF MS using photocleavable oligonucleotides, Nucleosides Nucleotides Nucleic Acids 22 (5–8) (2003) 1579–1581.