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Multiplex Y chromosome SNP genotyping using MALDI-TOF mass spectrometry

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Abstract. Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS) offers a reliable, inexpensive and time-saving method for SNP genotyping. The method is based on weight determination of single base extended (SBE) primers. Here, we present a MALDI-TOF protocol for typing 17 Y chromosome SNPs using biotin-labelled ddNTP in the SBE reaction and solid-phase avidin capture purification of extended primers. All 17 fragments were amplified in a multiplex reaction and all 17 primers were extended in a single SBE reaction.

Keywords: Y chromosome; SNP; MALDI-TOF

1. Introduction

One of the most promising techniques for SNP typing is the matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS). This technique is fast, accurate and easy to automate. The most widely used MALDI-TOF SNP typing strategy is based on single base extension (SBE) [1].

The smallest difference in mass between the four dideoxynucleotides (ddNTPs) is 9 Da between A and T. It is very difficult to distinguish two peaks differing by only 9 Da using primers of usual length (16–25 bases) and mass (5000–8000 Da) [2]. However, by using modified biotin-labelled ddNTPs in the SBE reaction, the smallest difference in mass is 16 Da between G and A, and two peaks differing by 16 Da can be separated by MALDI-TOF using primers of usual mass (5000–8000 Da) [3]. Here, we present a protocol for simultaneous detection of 17 Y chromosome SNPs by SBE and MALDI-TOF MS analysis using biotin-labelled ddNTPs in the SBE reaction.

2. Materials and methods

2.1. PCR and SBE

Seventeen SNPs placed on 17 different PCR fragments were selected from a 35 Y-SNP package [4]. The multiplex SBE primers were designed based on criteria previously

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described [4]. The mass was calculated using Mongo Oligo Mass Calculator v 2.05. (http://www.medlib.med.utah.edu/masspec/mongo.htm by Jef Rozenski, 1999). No mass overlap between extended and un-extended primers was allowed. The minimal difference in mass was 13 Da. The length of the primers varied from 17 to 31 bases.

The multiplex PCR conditions were as previously described [4].

Cycle SBE was performed in an 8-μl reaction volume with 0.4 μl of the purified PCR product (10–20 fmol of each product) or 50 fmol doped template (Fig. 1) using 0.6 μl thermosequenase reaction buffer, 0.4 μl ddNTP or biotin-ddNTP (PerkinElmer and Roche), 0.75 μl 3 units Themosequenase (Amersham Biosciences) and 0.6 μl primers (0.005–0.07 μM). The following programme was used: 35 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 30 s.

2.2. Purification of SBE products and MALDI-TOF analysis

Drop dialysis was performed as previously described [5].

Solid-phase reverse phase columns were in-house fabricated as described [6] with the exception that we used Poros OligoR3 (Applied Biosystems) column material and eluted the samples with 2 μl 25% acetonitrile/15mM triethyl ammonium acetate.

The solid-phase avidin column was based on a modification of an existing protocol [3] using 5 μl monomeric avidin (Pierce). Washing was done two times with 30 μl 0.1 M NH_4HCO_3 pH 7.0. Rescue of biotin-labelled DNA was done directly on the target by 3 μl 5% triethyl amin pH 12.5. The samples were prepared by the dry droplet method [6]. Samples were analysed on a Voyager DE-PRO (Applied Biosystems) Instrument.

3. Results and discussion

Since very pure DNA is needed for MALDI-TOF MS analysis, we investigated the efficiency of two purification approaches: drop dialysis and in-house fabricated reverse phase microcolumns. Our results showed that the recovery by both purification protocols were approximately 85%. Samples purified by either protocol resulted in high quality MALDI-TOF spectra, demonstrating that the purification protocols were very efficient.

We used a doped template to analyse the separation of the four possible alleles on the MALDI-TOF using conventional ddNTPs and mass-tagged ddNTPs (biotin-labelled ddNTPs) in the SBE reaction. Fig. 1 shows spectres with four peaks, one peak from each alleles. Our results showed insufficient separation between the A and T peaks in the mass area 5000–10,000 *m/z* when conventional ddNTPs were used (Fig. 1a). However, by using modified biotin-labelled ddNTPs (Fig. 1b), it was possible to obtain acceptable separation between all peaks in the mass range 5000–10,000 *m/z*.

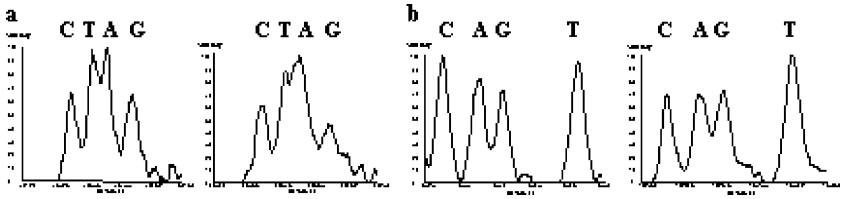

Fig. 1. Separation of peaks by MALDI-TOF. A doped DNA template containing a random nucleotide in the SNP position, and either conventional ddNTPSs (a) or biotin-labelled ddNTPs (b) was used in the SBE reaction.

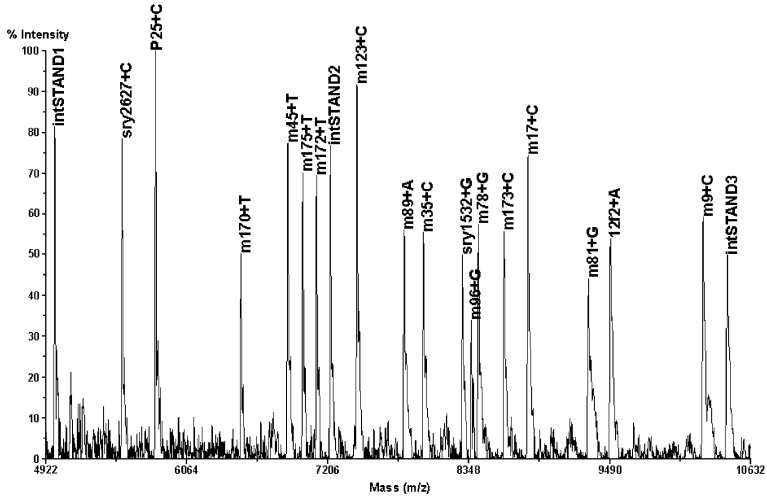

Fig. 2. MALDI-TOF results from a 17 SNP SBE reaction using biotin-labelled ddNTPs.

To avoid problems with separation of the A/T alleles, we continued to use biotin-labelled ddNTPs in the SBE reaction and we combined this approach with our newly developed one-step purification protocol for biotin-labelled DNA. The recovery from the solid-phase avidin capture assay was slightly lower then the recovery from the drop dialysis and the reverse phase purification. Seventeen primers were designed for detection of 17 SNPs selected from a 35 Y-SNP package [4]. Fig. 2 shows the SBE results obtained from a single male sample using the 17 Y-SNP package. The spectrum displays 20 peaks, three internal biotin-labelled standards and 17 extended primers, whereas un-extended primers and one internal standard without biotin label are not displayed, demonstrating the specificity of the solid-phase avidin capture purification assay.

In conclusion, the combined use of biotin-labelled ddNTPs in the SBE reaction and the solid-phase avidin capture purification assay results in better resolution of the peaks and in a much more simple spectrum than the spectrum obtained from conventional SBE.

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