Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography A, 1049 (2004) 9–16
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Automated solid-phase extraction for purification of single nucleotide polymorphism genotyping products prior to matrix-assisted laser desorption/ionisation time-of-flight mass spectrometric analysis

Sascha Sauer[a,*], Pamela Kepper[a], Anett Smyra[a], Andreas Dahl[a], Falk-Thilo Ferse[b], Hans Lehrach[a], Richard Reinhardt[a]

[a] *Department Lehrach, Max-Planck-Institute for Molecular Genetics, Ihnestrasse 73, 14195 Berlin-Dahlem, Germany*
[b] *Waters GmbH, Hauptstrasse 87, 65760 Eschborn, Germany*

Received 17 May 2004; received in revised form 29 July 2004; accepted 30 July 2004

Abstract

In this article, we describe the application of a novel micro elution solid-phase extraction method for purification of short stretches of DNA products of single nucleotide polymorphisms (SNPs) prior to MALDI mass spectrometry analysis. An important feature of our method is that the purification columns containing a copolymer of divinylbenzene and *N*-vinylpyrrolidone can be used several times thereby significantly reducing costs. We implemented this DNA purification technology into a fully automated procedure including molecular biology, MALDI sample preparation, automated mass spectrometric analysis and SNP allele calling by software. Due to the facile purification protocol, the methodology shown could furthermore be used for other applications where efficient medium to high-throughput purification of nucleic acids is required.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; Single nucleotide polymorphism; Solid-phase extraction; Genotyping; Purification

1. Introduction

During the last two decades, mass spectrometry has become a very popular tool within life science. For the detection of biomolecules matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) [1] has been introduced, and is particularly useful for the analysis of peptides and DNA markers such as single nucleotide polymorphisms (SNPs) [2]. SNPs are in general biallelic polymorphisms and the most abundant DNA markers in the human genome occurring at a frequency of one in every 500–1000 nucleotides [3]. The main advantage of MALDI-TOF-MS over other DNA analysis techniques for SNP detection is its signal accuracy, speed, and automation capability [4].

The common approaches for genotyping SNPs by MALDI-TOF-MS are similar. After an initial step of complexity reduction and amplification of the genomic DNA, usually by polymerase chain reaction (PCR), most methods use primer extension for allele distinction [5]. The primer extension reaction is robust, flexible, and generates fairly small products. A DNA polymerase extends a primer upstream of the SNP of interest with a set of deoxynucleotides (dNTPs) and/or dideoxynucleotides (ddNTPs) on a PCR product resulting in allele-specific products. The DNA polymerase elongates the 3′-end of the primer by specifically incorporating nucleotides that are complementary to the DNA template. The extension reaction stops at the first nucleobase in the tem-

Abbreviations: MALDI-MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; SNP, single nucleotide polymorphism; SPE, solid-phase extraction

* Corresponding author. Fax: +49 30 84131565.
E-mail address: sauer@molgen.mpg.de (S. Sauer).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.07.069

plate where a nucleotide occurs that is complementary to one of the ddNTPs in the reaction mix. Products of the primer extension reaction are then analysed by MALDI-TOF-MS. The different masses of the DNA products detected represent the different alleles. Prominent assays are the PROBE assay (more recently called MassEXTEND assay), the PinPoint assay, the GenoSNIP assay, and the GOOD assay [6–9].

For the analysis of DNA, the main problem consists of the negatively charged sugar-phosphate backbone. In solution phosphate residues of nucleic acids constitute a site of negative charge [4,9]. Therefore, cations such as sodium and potassium, which are used in buffers for PCR and primer extension reactions, cause adduct-formation with phosphate linkages significantly reducing MALDI signal quality. As a logical consequence prior to DNA analysis by MALDI, purification procedures have been implemented in sample preparation protocols. The protocols are mainly reversed-phase purification procedures and magnetic bead separation optionally combined with streptavidin–biotin binding. Magnetic bead technologies are efficient for desalting but difficult to automate. Reversed-phase purification procedures are powerful methods for purifying peptide and oligonucleotide samples but a number of such procedures are only available in a rather large format. Small formats such as micro-ZipTips™, however, are expensive and require several aspiration, washing and dispensing steps. We observed that they are only suitable for single use due to potential problems such as cross-contamination and plugging.

Recently, the use of columns containing a copolymer of divinylbenzene and *N*-vinylpyrrolidone has been shown to be useful for purification of synthetic oligonucleotides [10]. The hydrophobic divinylbenzene moiety provides reversed-phase interaction, whereas the more hydrophilic *N*-vinylpyrrolidone increases the sorbent water wettability, which even preserves analyte binding when the sorbent runs dry. The performance of this material is maintained after the sorbent has been processed hours after cartridge conditioning or sample loading. To put simply, purification is not affected by drying during extraction. Additional advantages of this system are that the support has no silanol activity thus reducing cation-exchange retention so that secondary retention of alkali ions and their potential coelution is efficiently minimised.

In this short communication, we show the implementation of the purification procedure described in the previous paragraph in an automated method for facile genotyping of SNPs by MALDI-TOF-MS.

2. Experimental

Oligonucleotides were obtained from MWG (Ebersberg, Germany). dNTPs and ddNTPs were purchased from Roche Diagnostics (Mannheim, Germany). Taq Polymerase was produced by Roman Pawlik (Max-Planck Institute for Molecular Genetics, Berlin, Germany). Thermosequenase and shrimp alkaline phosphatase (SAP) were obtained from Amersham Buchler (Braunschweig, Germany). Chemical reagents were purchased from Aldrich (Steinheim, Germany). The MALDI matrix (3-hydroxy-picolinic acid), target and the GenoTools software are available from Bruker Saxonia (Leipzig, Germany). The thermocycling procedures were carried out in a MJ PTC 200 Thermocycler obtained from Biozym (Hess Oldendorf, Germany). Plasticware was obtained from Eppendorf (Hamburg, Germany). Oasis® μElution plates (HLB) were obtained from Waters (Eschborn, Germany). A robotic workstation from QIAGEN (BioRobot 9600) including a liquid handling device and a vacuum station was used for sample purification. A Savant Speed Vac plus (SC210A) was used for lyophilisation. We used the CybiWell robot (Cybio, Jena, Germany) for sample preparation and MALDI target preparation.

2.1. PCR

Anonymous DNA samples were prepared as previously described [11]. For the amplification of a stretch of genomic DNA containing SNPs of the opioid receptor, mu1 (OPRM1) gene. The SNPs were extracted from the NCBI dbSNP database. For SNP rs607759 we used primers AAGCTCTAAAACATGGAAAGGAAA and TCATGCAATGAAGGGGTCTTAT and for rs3778152 we used primers GTCCCCAAGCTCCAGAACACAA and AGAGGTCACCAGTGGTTCAAGC. The following protocol was applied: 1.5 pmol of the forward and 1.5 pmol of the reverse primer were used in 40 mM Trisbase, 32 mM $(NH_4)_2SO_4$, 50 mM KCl and 2 mM MgCl at pH 8.8 with 200 μM dNTPs and 0.2 U Taq DNA polymerase (produced in-house) in a 3 μl volume. The reaction was denatured at 95 °C for 4 min, then thermocycled for 15 s at 95 °C, 30 s at 56 °C and 60 s at 72 °C, repeating the cycle 40 times.

2.2. Shrimp alkaline phosphatase digestion

We added 0.25 μl (1 U/μl) of shrimp alkaline phosphatase (SAP) and 1.75 μl 50 mM Tris base (pH 8.0) to 3 μl of the PCR and incubated for 75 min at 37 °C. The SAP was denatured for 10 min at 90 °C.

2.3. Primer extension

We added 5 pmol extension primer, 1.6 U Thermosequenase, 4 nmol $MgCl_2$, 250 pmol of each dNTP and ddNTP to the PCR in a final volume of 7 μl. The following cycling regime was used: 45 cycles of 15 s at 95 °C, 1 min at 50 °C, +0.3 °C/cycle, and 10 s at 72 °C. The following extension primers were used: AATTGAATGGCTCTAGGAC (for SNP rs607759), and AATGTGATATTTTAAAGGGC-CCT (for SNP rs3778152). For rs607759 we used dTTP, ddCTP and ddGTP as substrates, while for rs3778152 we employed dGTP, ddATP and ddTTP.

2.4. Purification of oligonucleotides

The solid-phase extraction was performed using the appropriate manifold kit for Oasis® HLB purification from Waters (Eschborn, Germany) or the vacuum station of the BioRobot 9600 (QIAGEN). Vacuum was adjusted to 507,957 HPa/step. We used 96-well micro-elution plates packed with 5 mg Oasis® HLB resin (Waters, Eschborn, Germany). We wet the resins with 200 μl methanol and equilibrated them with 200 μl deionised water. We filled up our sample to a final volume of 40 μl with 0.1 M triethylamine, pH 7, washed the sample with 50 μl 100 mM triethylamine/5% methanol and eluted the samples in two steps with 60% methanol, first with 25 μl and after that with 20 μl. Subsequently, we washed the resins with 250 μl 100% methanol, and we lyophilised the pool eluate and finally resuspended it in 10 μl deionised water.

2.5. MALDI target preparation and analysis

As matrix we used 50 mM 3-hydroxypicolinic acid in 3.3 mM ammonium citrate in deionised water. In solution ammonium exits as a NH_4^+ counterion, whereas in the gas-phase NH_3 is readily lost, leading to a reduced alkali ion-oligonucleotide adduct formation. For manual operation, 0.25 μl of the purified reaction products were spotted onto 0.25 μl of pre-crystallised matrix on an anchor chip target 400/384 from Bruker Daltonik. The mixture was allowed to dry at room temperature. For automatic operation using the Cybiwell robot, we recommend firstly aspirating 0.5 μl of the matrix into the tips, followed by seamless aspiration of 0.5 μl of the sample, and finally eject 0.5 μl of the matrix-sample mixture onto the anchor chip targets. Spectra were recorded on a Bruker Daltonik Biflex III time-of-flight mass spectrometer. The mass spectrometer is equipped with a 384 Scout MTP™ ion source with delayed extraction. Spectra were recorded in positive ion linear time-of-flight mode with 20 kV acceleration voltage and 18.50 kV IS/2 potential, accumulating on average 50 laser shots. For delayed extraction, the extraction delay was 300 ns. For automatic analysis of MALDI data, we used the Bruker Daltonik software GenoTools [12], which calculates masses of allele-specific primer extension products, performs genotyping analysis, and displays results. For GenoTools analysis, we applied the default parameters of the parameter set termed "soft" but increased the relative intensity threshold from 0.2 to a value of 0.4; the absolute intensity threshold was set to a value of 50. As mass accuracy, in particular in automatic detection mode, can shift about 10 Da in the mass range of oligonucleotides using tendentially inhomogeneous 3-hydroxypicolinic acid preparation on anchor targets, the calibration tolerance was set to ±20 Da.

3. Results and discussion

In this communication, we present the implementation of solid-phase purification plates, containing resins that consist of divinylbenzene and *N*-vinylpyrrolidone copolymers, in a medium to high-throughput SNP genotyping procedure based on MALDI-TOF-MS. The molecular biological procedure consists of PCR, shrimp alkaline phosphatase digestion and primer extension. We subsequently purify samples using a workstation equipped with liquid handling devices and a vacuum manifold, and finally prepare the samples for subsequent MALDI analysis (Fig. 1).

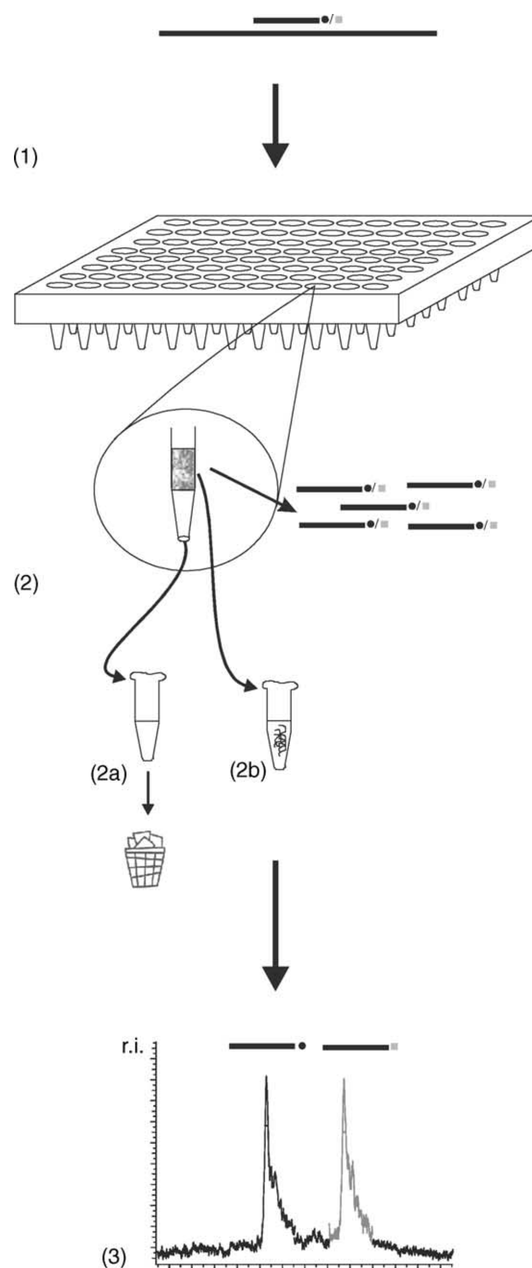

Fig. 1. Principle of the procedure. Allele-specific products (symbolised by circles and quadrates) are generated by primer extension reaction on a PCR product. The resulting products are purified by automated solid-phase extraction consisting of only three steps; loading of the sample, washing, and elution of DNA products. By using our method the allele-specific DNA products of SNPs are concentrated and subsequently analysed by MALDI mass spectrometry.

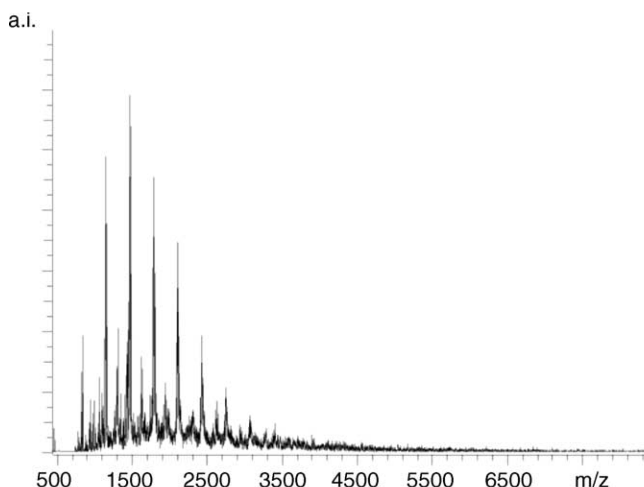

Fig. 2. A typical mass spectrum of SNP genotyping product samples prior to purification is shown.

Fig. 2 shows a spectrum of an unpurified SNP genotyping product sample. As expected product signals cannot be detected; in contrast a number of peaks deriving from detergents that are contained in enzyme buffers can be identified. Fig. 3 shows the result of a typical SNP genotyping experiment using our approach. The three genotypes of SNP rs607759 could be unambiguously analysed using three different, appropriate DNA samples. We compared the results obtained with the GOOD assay [9] and detected the same genotypes. Using the approach shown here we detected DNA products of SNPs generated by the primer extension reaction with an approximate amount of 1–5 pmol that are contained in 10 μl sample solution after purification of which we took 0.25 μl for MALDI preparation. In case of inefficient PCR, for example when only faint bands of PCR products are detected in agarose gels, there is generally too less template for primer extension. In our experience, more than 1 pmol of PCR products gained in a 3 μl reaction is required for efficient primer extension and subsequent procedures including detection. The use of genomic DNA in picogram range (from small number of cells) would generally be feasible using our procedure if PCR produces mentioned required quantities. It was shown in the past that using only 3 pg of the human genome for PCR, which represents one copy of the human genome, is sufficient for amplification and leads to a molecular haplotyping method based on optical detection [13]. In particular if low quantities of DNA are used, its quality becomes crucial for PCR, and depending on the degree of degradation amplification efficiency varies significantly.

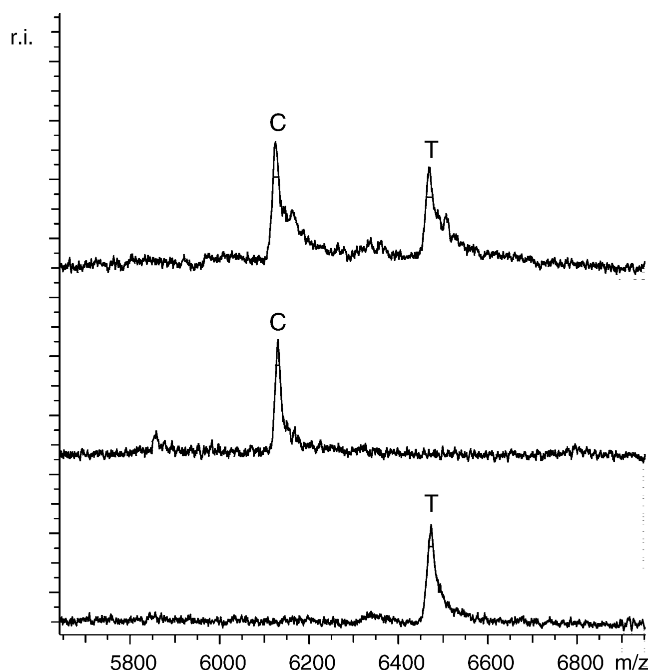

Fig. 3. For the SNP rs607759 primer AATTGAATGGCTCTAGGAC was used. Trace (a) shows the analysis of heterozygous DNA while in trace (b) the analysis of DNA homozygous for C is shown. In trace (c), the analysis of DNA homozygous for T is displayed. The spectra were not smoothed or otherwise manipulated.

Furthermore, we evaluated for robustness of the procedure shown here performing SNP genotyping in higher throughput and checking the well-to-well reproducibility of the purification plate. As an example, we genotyped two SNPs (rs607759 and rs3778152) on three different DNA samples, each representing a different genotype, thereby performing 16 experiments in parallel for each DNA sample. In this way, we performed enzymatic sample preparation and purification in 96-formats. As can be seen from Fig. 4, we estimated the quality of the spectra using GenoTools software [12]. Two of 96 samples could not be determined due to poor quality of spectra, 9 of 96 measurements resulted in spectra of medium quality and 85 of 96 samples were of good quality according to GenoTools. However, medium quality according to the software and its parameters used is still good enough for accurate allele calling. As can be seen in Fig. 3, in some cases, we observed up to 30% counterion adduct formation, mainly due to potassium ions that were used in PCR buffers. However, a limited degree of adduct formation does not pose a problem using GenoTools software so that we consider the purification method being efficient enough for SNP typing. Beyond salts MALDI sample preparation is also significantly inhibited by the presence of low amounts of detergents commonly used in enzyme buffers. Consequently we observed that non-purified samples performed bad co-crystallisation with MALDI matrix and therefore lead to suppression of the MALDI desorption process, resulting in typical signal ladders of detergent peaks in the low mass range. Matrix preparation was significantly improved by our purification so that we observed efficient desorption of matrix and analyte molecules, while detergents did not interfere with the analysis.

We also checked the genotypes and found consistent spectra for each DNA sample displaying the correct genotype in each case. As was shown for the example displayed in Fig. 3, the genotypes obtained matched completely with the results

of the GOOD assay. The total number of correct genotypes detected was 94. With the method presented here we observed similar call rates for a number of additional DNA samples and SNPs. This is not surprising since once the optimal molecular biological conditions have been found, the final results of automated SNP analysis usually fluctuate slightly when performed in high-throughput. Fluctuation can depend on several factors such as DNA template quality, pipetting accuracy, transfer of solutions, efficiency of enzymatic reactions, thermocyclers, MALDI preparation, and last but not least on the purification procedure prior to MALDI. For showing the robustness of our automated purification protocol and subsequent MALDI sample preparation, a limited number of DNA samples were used to compare the results in detail. Furthermore, we used master mixes of reagents in all molecular biological reactions. It is conceivable from the accumulation of spectra of lower quality in the same rows (Fig. 4) that the quality of spectra might have partly suffered from slight inconsistencies in liquid handling. However, the analysis shown in Fig. 4 represents a typical result of a SNP genotyping experiment using our procedure in high-throughput mode with a sufficient degree of efficiency for genetic application. As can be seen from Fig. 4, the purification method shown is reliable, reproducible and robust. Furthermore, it represents a facile procedure that requires only one loading, washing and elution step.

Important questions with regard to purification are costs and automation capability. Therefore we tested if we could use the micro elution plate several times. An exemplary experiment is shown in Fig. 5. In our experience, if the resins are washed with 100% methanol each time after the elution, they could be used at least four times without any adverse effect for purification of SNP products with approximate amounts of 1–5 pmol. However, from time to time signal intensities decrease slightly and we do not recommend using the same resins more than four times. No contamination was observed by MALDI using different samples containing 1–5 pmol oligonucleotides. Furthermore, no residual

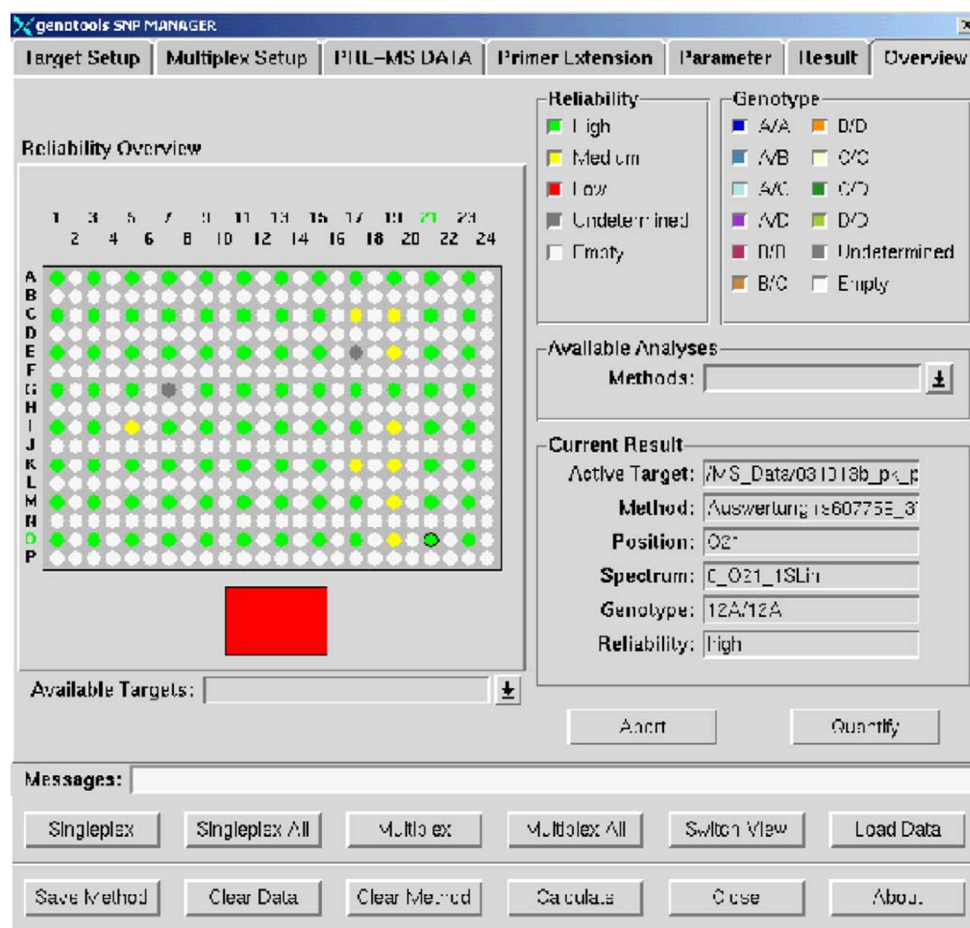

Fig. 4. For automatic analysis we used the GenoTools software. This figure represents an overview of the genotype analysis of the described experiments for SNPs rs607759 and rs3778152 in the OPRM1 gene. We used three DNA samples (DNA 1 in column 1, 3, 13, and 15; DNA 2 in column 5, 7, 17 and 19; DNA 3 in column 9, 11, 21 and 23), and performed 16 experiments in parallel. On the left half of the target we spotted samples for SNP rs607759 and on the right half samples for SNP rs3778152. Green points correspond to spectra with high quality and yellow points with medium quality. White points are empty positions and grey points indicate undetermined results due to very low spectra quality.

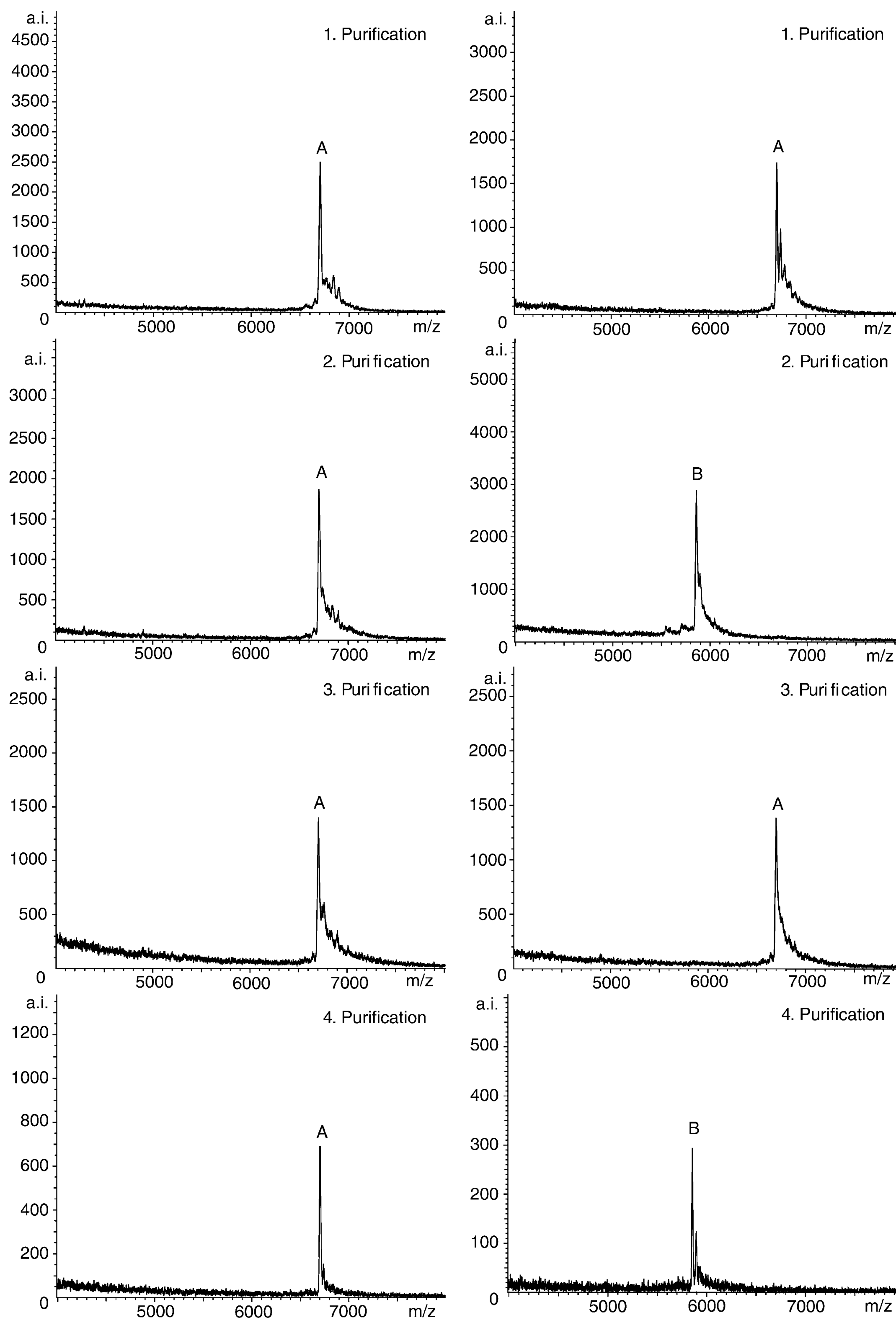

Fig. 5. We used 5 pmol of oligonucleotides A (TCCTTACAAATGCTATTGAGptTT; pt, phosphorothioate linkage) and B (AATTGAATGGCTCTAGGAC), which were diluted in 7 μl reaction solution. For the experiments on the left side oligonucleotide A was purified using the same resin four times (1–4 purifications). On the right side experiments are shown where oligonucleotides A and B were alternately purified using the same resin four times. No contamination was observed. Decreasing recovery could be explained because of residual oligonucleotides partly remaining on the column material and thereby—during several purification cycles—incrementally forming monolayers that after four cycles completely impede interaction of new oligonucleotides with the purification matrix.

oligonucleotide peaks were observed when blank samples were used.

We observed that single-strand nucleic acids (DNA and RNA) in a mass range from 1000–10,000 Da could be efficiently purified by the procedure shown here, which is consistent with previous results [10]. The purification efficiency rapidly decreases with molecules heavier than 10,000 Da. Thus, the purification procedure used is suitable for all mentioned procedures for SNP genotyping by mass spectrometry including the GenoSNIP assay that generates rather small DNA products [8]. In addition to SNP genotyping, the purification method shown might also be useful for recent methods to discover SNPs by MALDI detection of RNA fragmentation patterns [14,15]. Furthermore, it can be applied for the MALDI analysis of molecular haplotypes of SNPs using for example the CSH methodology [16].

For users with medium throughput (1000–10,000 SNP genotypes per day) applications, the approach shown including purification could be an alternative as rapid and easy application. In the procedure shown here, only three steps (loading, washing, eluting) are required to obtain purified samples. This makes our procedure easy to apply and allows robust automation. Potential problems associated with purification procedures such as ZipTips™ (plugged tips) or magnetic bead technology (difficulties to separate efficiently the beads from solution) are circumvented by our facile protocol. Here we used 96-formats of purification plates that can be used for medium-throughput applications. Future 384-formats involving lower elution volumes and therefore less time for the lyophilisation step will significantly enhance throughput capability of the system. However, using one of our simple "speed vac" systems and 96 plate-formats allows a throughput of up to 2304 samples per hour, which is beyond the performance of current MALDI mass spectrometers that realistically would allow up to 1000 measurements per hour and instrument. The present cost for purification using our approach is about 0.57€ per sample, while comparable products such as micro-ZipTips™ currently cost about 1.39€ or 1.03€ when respective Zip-plate formats are used. However, all these prices are only momentary and can decrease with a higher production level. As was shown in many mass spectrometry-based SNP assays, by multiplexing (parallel generation of products of several SNPs and subsequent parallel detection) the purification costs can be proportionally reduced as well. For example by "duplexing" reactions the purification costs would be halved [4,5,7,9,20]. Admittedly, the quality of the enzymatic reactions in a multiplex assay depends on the SNPs that have to be combined and in general multiplexed assays are less stable than single-plex reactions. The use of our purification system is reasonable in medium throughput applications, particularly when a simple protocol and high flexibility are required, for example in clinical diagnostics.

By using chemical DNA modification termed charge-tagging, MALDI detection of nucleic acids can be improved thus avoiding purification [17]. This principle has been employed for the GOOD assays, which were efficiently applied for high-throughput candidate-gene association studies (http://www.cng.fr). The GOOD assays fluently connect the reaction steps of PCR, shrimp alkaline phosphatase digestion, primer extension with positive or negative "charge tag" carrying primers containing phosphorothioate linkages, 5′-phosphodiesterase digestion to remove unmodified DNA compounds of the extension primers and alkylation for the charge neutralisation of the phosphorothioate backbone of the products, which are then diluted and efficiently detected by MALDI-MS without prior purification. However, charge-tagging can be associated with several problems such as high costs of primers containing aminopropagyl-modified purines, hazardous chemistry (alkylation), and varying primer extension efficiency due to the introduction of stereocenters by DNA backbone modification [18–20]. Novices in the fields of nucleic acid modification chemistry and mass spectrometry might be swamped with the implementation of the GOOD assays, although once successfully implemented in a high-throughput platform these are very powerful and cost-efficient methods. In contrast to this, the procedure presented here provides a simple protocol that can be easily applied for medium throughput applications, where only a limited number of SNPs is analysed, for example in (clinical) diagnostics. In this case, the raw genotype price is not as important as in a scientific environment, where generally large numbers of different SNPs have to be typed.

4. Conclusion

In this communication, we show the application of automated molecular biological reactions used for generating products of SNPs and automated application of micro elution SPE technology (OASIS®) for high-throughput purification of DNA products prior to MALDI mass spectrometry and data analysis. A MALDI mass spectrometer and liquid handling robots, in best case with an integrated vacuum station, are required to efficiently use the technology shown. The whole procedure including automated sample purification is straightforward and easy to apply. Furthermore, due to the repeated use of the purification columns costs are significantly decreased. Although MALDI-TOF-MS is not yet an integral component of molecular biological laboratories, it has the potential to become a 'Gold Standard' tool for the analysis of nucleic acids. With the increased usage of mass spectrometry as a standard instrument for life science applications, future MALDI mass spectrometers tailored to the needs of geneticists, and facile and robust protocols for sample preparation, mass spectrometry technology could become a common tool for biologists who look for easy and accurate procedures to answer their scientific questions.

Acknowledgements

This work was supported by the German National Genome Research Network (NGFN grant 01GR0155) and by the Max-Planck-Society. We would like to thank Stephen Gelling and Carola Burgtorf for critical reading of this manuscript.

References

[1] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[2] T. Bonk, A. Humeny, Neuroscientist 7 (2001) 6.
[3] A.J. Brookes, Gene 234 (1999) 177.
[4] S. Sauer, I.G. Gut, J. Chromatogr. B 782 (2002) 73.
[5] W. Pusch, J.H. Wurmbach, H. Thiele, M. Kostrzewa, Pharmacogenomics 3 (2002) 537.
[6] D.P. Little, A. Braun, B. Darnhofer-Demar, H. Koster, Eur. J. Clin. Chem. Clin. Biochem. 35 (1997) 545.
[7] L.A. Haff, I.P. Smirnov, Nucleic Acids Res. 25 (1997) 3749.
[8] T. Wenzel, T. Elssner, K. Fahr, J. Bimmler, S. Richter, I. Thomas, M. Kostrzewa, Nucleosides Nucleotides Nucleic Acids 22 (2003) 1579.
[9] S. Sauer, D. Lechner, K. Berlin, H. Lehrach, J.L. Escary, N. Fox, I.G. Gut, Nucleic Acids Res. 28 (2000) e13.
[10] M. Gilar, A. Belenky, B.H. Wang, J. Chromatogr. A 921 (2001) 3.
[11] S. Sauer, H. Lehrach, R. Reinhardt, Nucleic Acids Res. 31 (2003) e63.
[12] W. Pusch, K.O. Kraeuter, T. Froehlich, Y. Stalgies, M. Kostrzewa, Biotechniques 30 (2001) 210.
[13] G. Ruano, K.K. Kidd, J.C. Stephens, Proc. Natl. Acad. Sci. USA 87 (1990) 6296.
[14] C.P. Rodi, B. Darnhofer-Patel, P. Stanssens, M. Zabeau, D. van den Boom, Biotechniques Suppl (2002) 62.
[15] S. Krebs, I. Medugorac, D. Seichter, M. Forster, Nucleic Acids Res. 31 (2003) e37.
[16] C. Burgtorf, P. Kepper, M. Hoehe, C. Schmitt, R. Reinhardt, H. Lehrach, S. Sauer, Genome Res. 13 (2003) 2717.
[17] I.G. Gut, W.A. Jeffery, D.J.C. Pappin, S. Beck, Rapid Commun. Mass Spectrom. 11 (1997) 43.
[18] S. Sauer, D. Lechner, K. Berlin, C. Plancon, A. Heuermann, H. Lehrach, I.G. Gut, Nucleic Acids Res. 28 (2000) e100.
[19] S. Sauer, D.H. Gelfand, F. Boussicault, K. Bauer, F. Reichert, I.G. Gut, Nucleic Acids Res. 30 (2002) e22.
[20] S. Sauer, I.G. Gut, Rapid Commun. Mass Spectrom. 17 (2003) 1265.