This article was downloaded by: [Fondren Library, Rice University] On: 22 November 2014, At: 03:03 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: <u>http://www.tandfonline.com/loi/lncn20</u>

Genosnip: SNP Genotyping by MALDI-TOF MS Using Photocleavable Oligonucleotides

T. Wenzel $^{a\ b}$, T. Elssner a , K. Fahr a , J. Bimmler a , S. Richter a , I. Thomas a & M. Kostrzewa a

^a Bruker Daltonik GmbH , Leipzig, Germany

^b Bruker Saxonia Analytik Gmblt, Permoserstrasse 15, Leipzig, D-04318, Germany Published online: 31 Aug 2006.

To cite this article: T. Wenzel, T. Elssner, K. Fahr, J. Bimmler, S. Richter, I. Thomas & M. Kostrzewa (2003) Genosnip: SNP Genotyping by MALDI-TOF MS Using Photocleavable Oligonucleotides, Nucleosides, Nucleotides and Nucleic Acids, 22:5-8, 1579-1581, DOI: <u>10.1081/NCN-120023038</u>

To link to this article: <u>http://dx.doi.org/10.1081/NCN-120023038</u>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Genosnip: SNP Genotyping by MALDI-TOF MS Using Photocleavable Oligonucleotides

T. Wenzel,* T. Elssner, K. Fahr, J. Bimmler, S. Richter, I. Thomas, and M. Kostrzewa

Bruker Daltonik GmbH, Leipzig, Germany

ABSTRACT

A photocleavable o-nitrobenzyl CE phosphoramidite building-block was synthesised and incorporated within oligonucleotides. After allele-specific primer extension, desalting was performed using *genostrep* purification plates. Release of the SNP information containing part through photocleavage created shortened molecules that are easily accessible for MALDI-TOF analysis. Additionally, incorporation of mass modified nucleosides enables flexible design of multiplex genotyping.

INTRODUCTION

Genotyping SNPs by MALDI-TOF MS is a highly sophisticated method because of its accuracy, cost-effectiveness, speed and automation capability. Major drawback of current methods for MALDI SNP analysis is complex sample preparation. This is obligate because of the strong tendency of larger DNA molecules to form adducts with positively charged ions.

Here, we present *genoSNIP*, a novel method for SNP analysis using size reduction of primer extension products by incorporation of a non-hydrogen bonding

1579

DOI: 10.1081/NCN-120023038 Copyright © 2003 by Marcel Dekker, Inc.

Downloaded by [Fondren Library, Rice University] at 03:03 22 November 2014

1525-7770 (Print); 1532-2335 (Online) www.dekker.com

^{*}Correspondence: T. Wenzel, Bruker Saxonia Analytik Gmblt, Permoserstrasse 15, D-04318 Leipzig, Germany; Fax: + 49 341 243 1313; E-mail: tw@bsax.de.

DNA building-block for phototriggering strand breaks near to the 3'-end of the extension primer.

RESULTS AND DISCUSSION

The large-scale synthesis of the photocleavable β -CE phosphoramidite buildingblock (3, ^[1]) was performed using commercially available o-nitrobenzaldehyde (1). 1 was converted into o-nitrophenyl-3-butenol using allyltrimethylsilane in the presence of TiCl₄. Next, diol **2** was generated by ozonolysis of the previous compound and reductive workup with NaBH₄. Dimethoxytritylation of **2** followed by phosphitylation yielded target compound **3** in excellent yields (overall yield: 60%).

To genotype SNPs by *geno*SNIP genomic regions containing polymorphic sites are amplified by PCR. The PCR products are used as templates for primer extension reactions which generate allele specific products. The primers used in the extension reaction were biotinylated at the 5'-end and they did contain the photocleavable building-block **3** close to the 3'-end. After extension reaction, purification was done by *genostrep*, a purification system based on streptavidin coated microtiterplates.



(i) 1M TiCl₄/CH₂Cl₂, CH₂ = CHCH₂Si(CH₃)₃, H₂O; (ii) O₃/MeOH, NaBH₄, H₂O; (iii) (MeO)₂TrCl/pyridine/TEA/DMAP; (iv) [(CH₃)₂CH]₂NP(Cl)OCH₂CH₂CN/DIPEA/CH₂Cl₂.

Purified extension products were photochemically cleaved releasing shortened DNA molecules as 5'-monophosphates.



Figure 1. SNP genotyping of three different risk factors (factor V Leiden G 1691A, Prothrombin G20210A, MTHFR C677 T) using the *geno*SNIP assay (a,c); singleplex analysis of MTHFR (a), singleplex analysis of mass increased MTHFR (b), triplex analysis (c).

SNP Genotyping by MALDI-TOF MS

Here, we present SNP genotyping by genoSNIP/genostrep using assays for several common genetic risk factors (Prothrombin, Factor V Leiden, MTHFR).

As the primer extension products of MTHFR (M = 1592 Da) and factor V Leiden (M = 1592 Da) show the same molecular weight, we introduced 2'-OMeguanosine close to the photocleavable building-block of MTHFR increasing the weight by 30 Da.

The incorporation of mass modified nucleoside within DNA enables multiplexing. Combination of genoSNIP/genostrep purification empowers rapid and costeffective genotyping which is easy to automate.

REFERENCE

1. Ordoukhanian, P.; Taylor, J.S. J. Am. Chem. Soc. 1995, 117 (37), 9570–9571.



