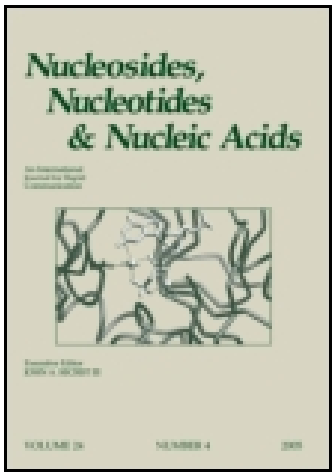

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Genosnip: SNP Genotyping by MALDI-TOF MS Using Photocleavable Oligonucleotides

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

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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 *geno*SNIP, a novel method for SNP analysis using size reduction of primer extension products by incorporation of a non-hydrogen bonding

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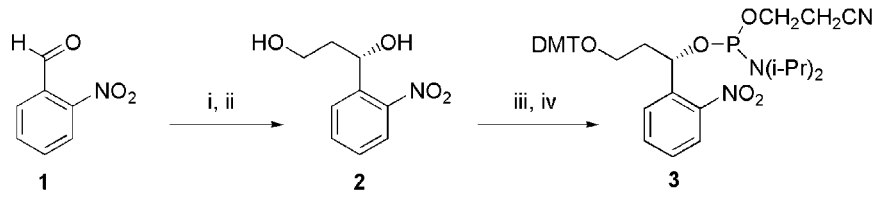
DNA building-block for phototriggering strand breaks near to the 3′-end of the extension primer.

RESULTS AND DISCUSSION

The large-scale synthesis of the photocleavable β-CE phosphoramidite building-block (**3**, [1]) was performed using commercially available o-nitrobenzaldehyde (**1**). **1** was converted into o-nitrophenyl-3-butenol using allyltrimethylsilane in the presence of $TiCl_4$. Next, diol **2** was generated by ozonolysis of the previous compound and reductive workup with $NaBH_4$. 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 $TiCl_4/CH_2Cl_2$, $CH_2=CHCH_2Si(CH_3)_3$, H_2O; (ii) O_3/MeOH, $NaBH_4$, H_2O; (iii) $(MeO)_2TrCl$/pyridine/TEA/DMAP; (iv) $[(CH_3)_2CH]_2NP(Cl)OCH_2CH_2CN$/DIPEA/$CH_2Cl_2$.

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

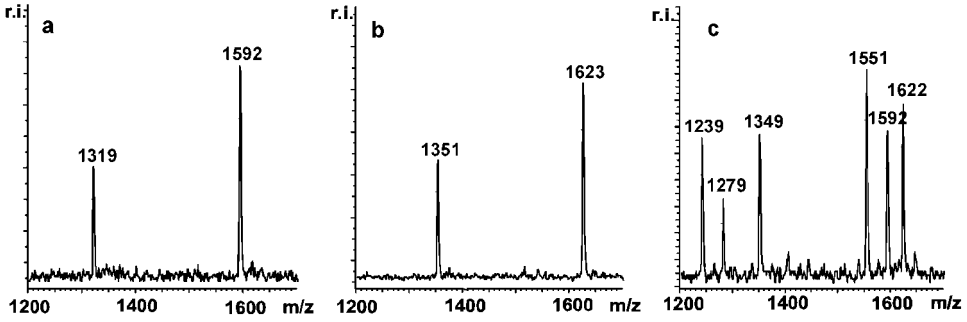


Figure 1. SNP genotyping of three different risk factors (factor V Leiden G 1691A, Prothrombin G20210A, MTHFR C677 T) using the *geno*SNIP assay (a,c); singleplex analysis of MTHFR (a), singleplex analysis of mass increased MTHFR (b), triplex analysis (c).

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Here, we present SNP genotyping by *geno*SNIP/*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′-OMe-guanosine 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 *geno*SNIP/*genostrep* purification empowers rapid and cost-effective genotyping which is easy to automate.

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