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Automated MALDI-TOF Mass Spectrometry based SNP Genotyping

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Poster

With the advancement of the human genome project the determination of the general structure of the human genome approaches its completion. A "working draft" covering 90% of the genomic sequence has already been published [a, 1]. Pharmacogenomic research now has to elucidate the molecular polymorphisms underlying individual phenotypic differences. This knowledge will significantly increase the understanding of individual predispositions to certain diseases, as well as the efficacy and potential adverse effects of certain drugs [2].

The most common genetic variations are single nucleotide polymorphisms (SNPs). A SNP is the occurence of a different nucleotide in different individuals at a given chromosomal position [3]. It is estimated that SNPs account for approximately 90% of the individual genotypic variations [3]. Accordingly, in order to fully exploit the available genomic information, there is a growing demand for reliable, fast, cost effective and highly automated analysis methods for SNP genotyping.

SNP genotyping can be addressed by several different methods and technologies. However, many of them do not allow the required sample throughput, or they rely on indirect detection reactions, involving specific nucleic acid labelling or hybridization steps. In contrast, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) measures directly a physical property of the analyte - its molecular mass. Accordingly, MALDI-TOF MS SNP genotyping is not susceptible to background problems resulting from hybridization based detection reactions.

Here we present our MALDI-TOF MS SNP genotyping system that allows fully automated data acquisition and SNP genotyping (Bruker Daltonik, Bremen, Germany).

The sample preparation starts with a PCR amplification of a genomic DNA fragment containing the polymorphic site of interest. After a purification step with the genopure magnetic bead system (Bruker Saxonia, Leipzig, Germany), the PCR products are used as templates to generate allele-specific

products in a primer extension reaction. These are precisely spotted on an AnchorChip target (Bruker Daltonik, Bremen, Germany) after an additional purification step.

The MALDI-TOF MS data acquisition is completely automated by the AutoXecute 5.0 software (Bruker Daltonik, Bremen, Germany). Automated allele calling is performed using the new genotools 1.0 software [4] (Bruker Daltonik, Bremen, Germany).

The MALDI-TOF MS technology enables a throughput of several thousands of DNA samples per day. However, sample throughput may be further increased by the analysis of pooled DNA samples. This is especially useful to screen a high sample number for a DNA containing a rare polymorphism. The DNA pool displaying the desired allele can easily be identified and subsequently splitted into individual DNA samples. This approach will significantly decrease the necessary number of samples to be screened. Moreover, recent advances concerning the calculation of allele-frequencies from the peak intensities of spectra from pooled DNA samples look promising [5].

Literature

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