

high-throughput SNP genotyping on a MALDI-TOF MS system, using ABgene® Thermo-start® DNA polymerase

As part of the German National Genome Research Net (NGFN) we have set up a high-throughput SNP-genotyping facility to analyse candidate genes for complex diseases including environmental (e.g. asthma, atopic dermatitis), neurologic (e.g. Alzheimer's disease, bipolar diseases), cardiovascular and infectious diseases as well as cancer. The facility is funded by the GSF Research Center in Neuherberg and the German Research ministry.

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Complex diseases like cardiac infarction or asthma show a high prevalence in many populations, especially in industrialized nations. They show a complicated mode of inheritance with several genes and environmental factors involved.

Overview

To detect disease genes or disease causing DNA polymorphisms, we established a high-throughput single nucleotide polymorphism (SNP) genotyping facility with a MALDI-TOF MS (matrix assisted laser desorption/ionisation time of flight mass spectrometer, Sequenom Inc. San Diego USA) unit. This instrument gives the capability to perform 20,000 SNP analyses per day.

To find complex disease causing SNPs, we genotype special candidate genes or chromosomal regions from several hundred to thousands of affected patients.

Polymerase Chain Reaction

The isolated DNA is removed from the 96-well 1.2ml Storage Plates (ABgene®), and the fragment of interest is PCR amplified in a heat sealed Thermo-Fast® 384 PCR Plate (ABgene®).

The miniaturised PCR reactions are performed in a final volume of 5µl using 0.1 units of Thermo-Start® DNA Polymerase (ABgene®). The amplicons are then further processed in a primer extension reaction.

Primer Extension Reaction

In this reaction an oligonucleotide is designed to bind directly to the 5' end of the identified SNP. Dideoxy nucleotides (ddNTPs), which cannot be elongated, are substituted for one of the 4 deoxy nucleotides (dNTPs) in the reaction mix. Therefore, if a SNP is present

the complementary ddNTP will be incorporated and different allele specific fragments will be created.

In the example in figure 2, a T/A SNP is analysed. The reaction mix contains one ddNTP (ddATP), which will terminate the DNA chain reaction, and three dNTPs (C, G and T), which can be elongated.

In the case of the first allele ddATP will be incorporated straight away, which results in a product of 24 nucleotides. In the case of the

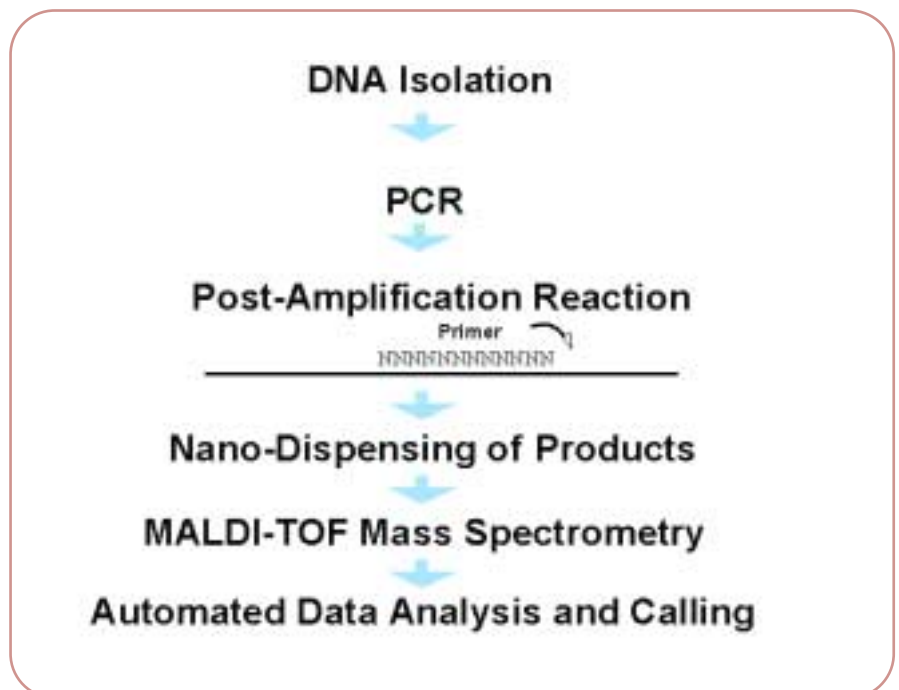


Figure 1: The principle of the MALDI-TOF analysis.

2nd allele, the first two nucleotides (dTTP and dGTP) are incorporated as normal and the extension proceeds up to the T where a ddA is incorporated resulting in a 26 nucleotide long extension product. The two extension products, a 24-mer and a 26-mer, have different masses.

MALDI-TOF MS

The extension products can be spotted on microchips (figure 3) and analysed in the MALDI-TOF Mass Spectrometer.

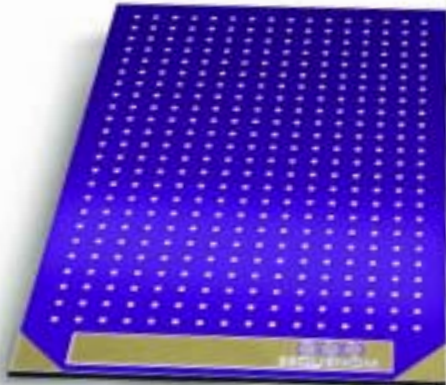


Figure 3: DNA microchip used in the MALDI-TOF Mass Spectrometer.

A laser fires on the spotted products on the microchip and the DNA is accelerated in a vacuum to a detector. Smaller molecules (e.g. a 24-mer) are faster than larger molecules (e.g. a 26-mer) and are detected earlier (figure 4). The mass of every extended product is determined and can be "translated" into one allele of the SNP, in our case T or A.

Discussion

Several hundred samples and at least as many controls for each specific SNP are analysed. If significant differences in allele frequencies between cases and controls are detected, we

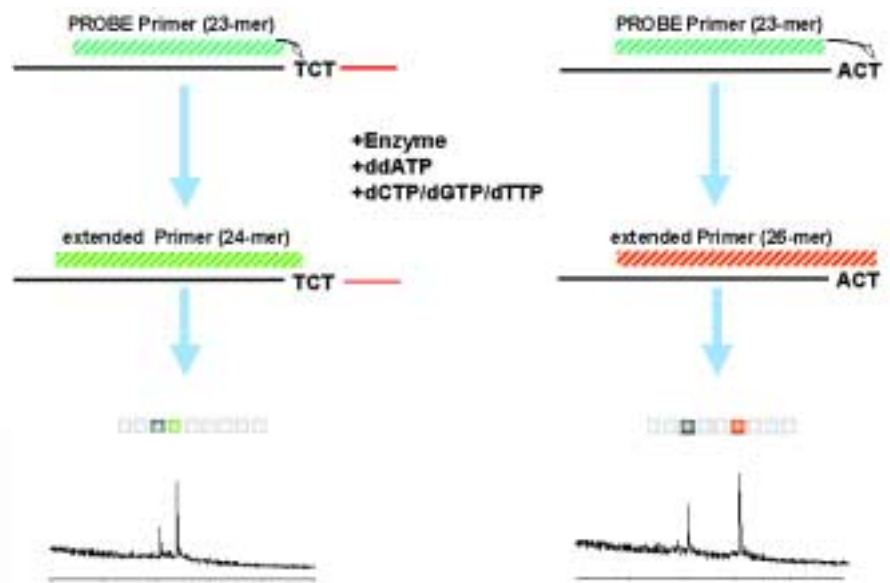


Figure 2: Primer extension reactions to identify SNPs.

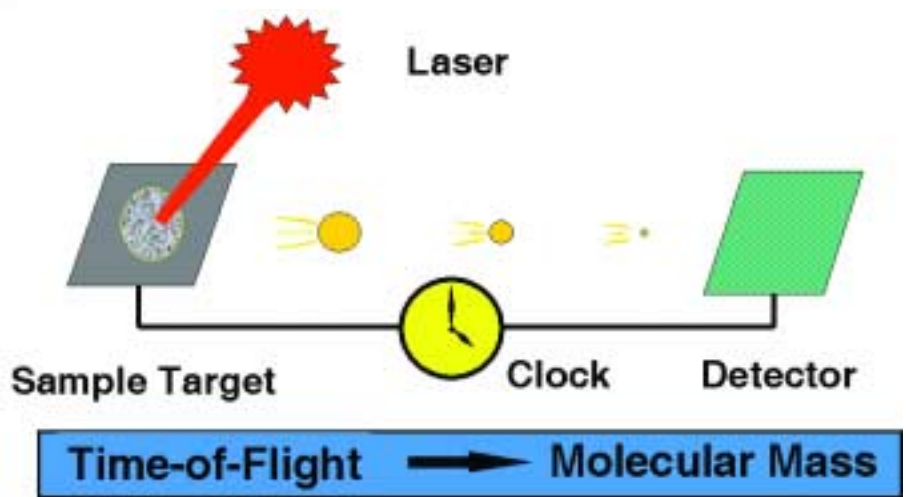


Figure 4: Diagrammatic representation of the MALDI-TOF Mass Spectrometer principle.

have a good indication that this gene or gene variant (SNP) might be co-responsible for disease aetiology. We have recently detected new SNP variants that are likely to play an important role in Alzheimer's disease and

asthma, which will soon be published in high impact journals. We are interested in new co-operative SNP genotyping projects, which should be addressed to the author of this manuscript.

Cat. No.	Description	Quantity
AB-0908	Thermo-Start® DNA Polymerase	250 units
AB-0908/b	Thermo-Start® DNA Polymerase	2,500 units
TF-0384	Thermo-Fast® 384 PCR Plate	50 plates
TF-0384/k	Thermo-Fast® 384 PCR Plate in black	50 plates
TF-0384/w	Thermo-Fast® 384 PCR Plate in white	50 plates
AB-0564	1.2ml Storage Plate	50 plates



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