Abstract

After the completion of the Human Genome Sequencing Project, genome research is bound to focus on genome variation studies. Therefore efficient high-throughput genotyping technologies for DNA markers, particularly for single nucleotide polymorphisms (SNPs) are very sought-after. Many methods for SNP analysis, such as DNA microarrays, gel-based and plate-reader based assays have been developed but none of these compete with the rapid detection and resolution of mass spectrometers. Particularly matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) has revolutionised the analysis of proteins and DNA. Nevertheless, it was universally acknowledged that one of the major problems of DNA analysis by MALDI-MS was to achieve sufficient sample purity. This limits significantly high-throughput, as automation of current purification procedures is cumbersome and expensive. The main benefit of the novel approaches described in this thesis consists in the use of MALDI-sensitivity enhancing DNA chemistry termed “charge-tagging”. This DNA modification chemistry was implemented in several different molecular biological procedures for the generation of allele-specific products of SNPs. Therefore purification or concentration procedures as for any other technique using MALDI-MS are not required. Each of the presented approaches starts with a PCR. Assays termed the GOOD assays and the simplified GOOD assay use primer extension for the generation of allele-specific and charge-tagged DNA products. Additionally, procedures using ligases or flap-endonucleases for the generation of such products are shown. Among these methods the simplified GOOD assay is potentially the most powerful as it is executed with the shortest reaction sequence and completely void of chemistry. The simplified GOOD assay is a purification-free, single-tube, three-step procedure consisting of PCR, primer extension and phosphodiesterase II digestion followed by mass spectrometric analysis, while for the GOOD assays an additional alkylation step takes place after phosphodiesterase digestion. The GOOD assays and the simplified GOOD assay are performed with simple liquid-handling at lowest manageable volumes, thermal incubation and thermocycling steps and were successfully implemented in an automated process for high-throughput SNP genotyping.