

1. Summary

Oligonucleotide fingerprinting (OFP) represents a powerful means of cDNA and genomic DNA library characterization and normalization. As a result of OFP and its high degree of normalization non-redundant "unigene" sets are created for an organism or specific tissue thereof. These embody highly valuable resources for subsequent downstream applications and hence represent an important step towards a functional interpretation of the obtained sequence data.

Nevertheless, current technological limitations, such as the high number of oligonucleotide probes required and the restriction to serial hybridizations, impede a higher throughput and thus further boost of OFP as an efficient tool for DNA characterization.

The objective of this dissertation was to overcome these limitations and create an innovative technological foundation for OFP. By applying multiplexed peptide nucleic acid (PNA) hybridizations and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a means of hybridization detection it was aimed at developing the procedure to a high degree of multiplexing.

Within the scope of the dissertation, several characteristics of PNA hybridizations and subsequent analysis by MALDI-TOF MS were investigated that led to new insight. It could be demonstrated that by the use of PNA oligonucleotides hybridization probe length can be reduced to hexamers providing the basis for a significant reduction of the overall number of hybridization probes needed for an OFP analysis. Besides, owing to positive charge-tagging of PNA an unparalleled degree of multiplexing could be accomplished. A simultaneous hybridization of 21 different PNA oligonucleotides was shown to be feasible. Furthermore, in an extensive pilot study carried out on selected genomic DNA and cDNA clones, the applicability of the multiplexed OFP concept was successfully demonstrated. Moreover, several substrates and DNA attachment chemistries were evaluated with respect to their suitability as MALDI-TOF MS compatible DNA immobilization systems of which nylon membrane-based and polyamidoamine (PAMAM) dendrimer-based systems possess great potential.

By a further optimization of PNA hybridization probe sets and complete automation of the entire procedure, multiplexed OFP is expected to be established as routine application with a significantly enhanced throughput.