4. Objective of this dissertation

Oligonucleotide fingerprinting has been proven to be a powerful means of cDNA and genomic DNA library characterization and normalization (Craig et al., 1990, Hoheisel et al., 1991, Meier-Ewert et al., 1993, Maier et al., 1994, Meier-Ewert et al., 1998, Radelof et al., 1998, Poustka et al., 1999, Clark et al., 2001, Herwig et al., 2002). 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 for a meaningful analysis and the restriction to serial hybridizations, have to be overcome to further increase the throughput and therefore boost OFP as an efficient tool for DNA characterization.

The objective of the present dissertation is to significantly enhance the efficiency of OFP by developing the conventional procedure to a high degree of multiplexing. This is to be achieved by hybridizing many different PNA probes simultaneously, in multiplexed pools, to target DNA immobilized on a solid surface. Only those PNA probes that hybridize with target DNA will eventually be recorded by MALDI-TOF MS with its parallel detection capacity. Thereby, a mass-specific oligonucleotide fingerprint can be generated that uniquely identifies a respective target DNA (fig. 4.1). Such a system is anticipated to

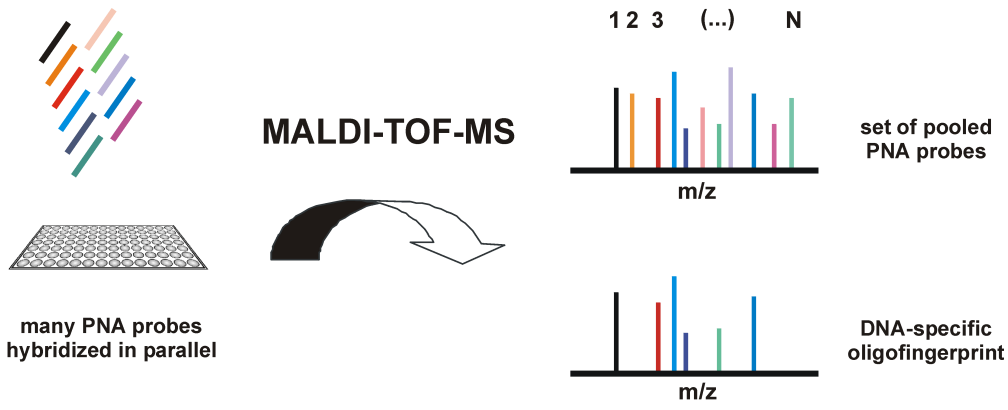

Figure 4.1 Concept of multiplexed OFP

In multiplexed pools many different PNA probes are simultaneously hybridized to immobilized target DNA. Only those probes that hybridize are recorded by MALDI-TOF MS. Thereby, a mass-specific oligonucleotide fingerprint is generated that uniquely identifies a respective target DNA.

increase current data production rates by at least one order of magnitude.

To accomplish this objective, several pivotal questions have to be addressed, such as the number of PNA probes that can be hybridized and detected simultaneously in a meaningful fashion. Moreover, it has to be elucidated whether it is possible to apply oligonucleotides shorter than octamers to reduce the overall number of probes needed for a complete OFP analysis. In an "aqueous solution" model system PNA hybridization conditions are to be optimized for a successful discrimination of different DNA targets. For full automation of the OFP process it would be desirable to have a DNA immobilization system at disposal that allows direct read-out of hybridization events by MALDI-TOF MS. To pursue this approach promising substrates (i.e. surface materials and DNA attachment chemistries) are to be evaluated in terms of their suitability. As a conclusion to this dissertation, the "proof of principle" of the innovate concept of multiplexed OFP is to be demonstrated by the reproducible generation of unique fingerprints for a number of selected target DNAs of known sequence.