8. Discussion

The objective of the present dissertation was to create an innovative technological foundation for OFP significantly enhancing its efficiency. By overcoming current methodical limitations, such as the high probe number and the restriction to serial hybridizations, it was aimed at developing the conventional procedure towards a high degree of multiplexing, thereby boosting OFP as an efficient tool for DNA characterization.

Within the scope of this dissertation, several characteristics of PNA hybridizations and subsequent detection by MALDI-TOF MS were examined that led to new insight with regard to PNA hybridization conditions in general as well as hybridization probe length and degree of multiplexing in particular.

Moreover, in a pilot study carried out on selected genomic DNA and cDNA clones, the individual performances of different combinations of PNA probes in multiplexed hybridizations were tested. Essentially, the “proof of principle” of the concept of multiplexed OFP was successfully demonstrated.

In addition, in the course of various industrial collaborations promising substrates and DNA attachment chemistries were evaluated. Some of these showed great potential with respect to their suitability as DNA immobilization systems that allow direct hybridization read-out by MALDI-TOF MS. Yet, further development work is still needed.

In the following, all these accomplishments are discussed and a future perspective is given.

8.1 Parameters of multiplexed PNA hybridizations and subsequent MALDI-TOF MS detection

The length of hybridization probes applied in OFP projects is of pivotal importance. It does not only determine the degree of partitioning of a given set of clones under investigation and hence clustering quality to be accomplished but also defines the overall number of probes needed for a meaningful analysis.

For probe to target hybridizations the optimal yield of information is gained if the probability that a certain oligonucleotide probes hybridizes to a given target DNA is 50% (Herwig et al., 1999 and 2000). One probe yields maximal information when it partitions a given clone set of *N* random clones into two subsets of equal size, i.e. it hybridizes to one half of the clones but not to the other. Two probes should ideally partition *N* random clones into four and *n* probes into 2^n subsets of equal size. Assuming a hybridization probability of 50%, about 17 probes would be needed for a successful partitioning of

100,000 clones. In practice, since hybridizations with DNA octamer and shorter probes fail to yield consistent results, degenerated DNA decamer oligonucleotides are used as hybridization probes which possess an octamer core as informational entity. Calculations of average hybridization frequencies, however, showed that for clone libraries with an average insert size of up to 2 kb the partitioning performance of octamers is rather poor (Herwig et al., 1999 and 2000). To compensate for the loss of partitioning quality, a total number of up to 250 hybridization probes is routinely employed to ensure an efficient clustering and hence OFP analysis.

In the scope of this dissertation, PNA octamers, heptamers, and hexamers were tested as hybridization probes. Recent experiments with fluorescence labeled PNA probes suggested that the hybridization of PNA octamers is feasible (Guerasimova et al., 2001). Therefore, it was expected that owing to the greater stability of PNA/DNA heteroduplexes (Tomac et al., 1996, Jensen et al., 1997) hybridization probes even shorter than octamers can be reliably hybridized. A potential reduction of probe length did not only promise to lead to higher information theoretical partitioning and hence better clustering quality but also to a considerable reduced overall number of hybridization probes needed.

Clustering simulations performed for of 8mer, 7mer, and 6mer probes in dependence of maximum target sequence length as well as the influence of varying probe numbers confirmed these expectations and revealed that hexamers are superior and 70-100 probes should suffice for an OFP analysis.

Initial experimental efforts focused on the evaluation of the applicability of PNA 8mer, 7mer and 6mer probes. Obtained results showed that beside octamers also hexamers and heptamers can be employed rendering a more efficient technological foundation for OFP realistic. As for general hybridization behavior, there was no evidence that longer 8mers hybridize better than shorter 7mers and 6mers. Nor were hexamer and heptamer probes more specific as thermodynamic considerations would suggest owing to their respective shorter length. However, 6mers and 7mers showed more favorable desorption and ionization properties. They were more easily detectable than longer 8mers in the process of MALDI giving rise to higher absolute signal intensities.

From a theoretical and practical point of view, it can hence be concluded that PNA hexamer and heptamer hybridization probes are superior to octamers with hexamers being the most suitable candidate for the OFP characterization of genomic and cDNA libraries. Besides, a considerable reduction of the number of probes needed for an OFP analysis appears feasible.

Not less crucial than hybridization probe length is the influence of PNA modifications on hybridization and subsequent MALDI-TOF MS detection as well as the determination

of the total number of PNA probes that can be hybridized and detected simultaneously. By this number the degree of multiplexing is ultimately established and the capacity of the concept of multiplexed OFP is defined.

It has been reported that by the introduction of different covalent modifications, such as terminal lysine (Ratilainen et al., 1998) and Fmoc O-linker 8-amino-3,6-dioxaoctanoic acid (Griffin et al., 1997), PNA hybridization and/or MALDI properties are significantly improved. Moreover, quaternary ammonium fixed charge-tags (Gut et al., 1997) were shown to increase the detectability of small DNA oligonucleotides about 100-fold.

Experiments revealed that the incorporation of lysine and/or different numbers of O-linker did neither affect hybridization behavior nor did it enhance MALDI-TOF MS detection. On the contrary, larger numbers of N-terminally attached O-linker mass tags (three or five) consistently yielded weaker signals suggesting that the process of MALDI was increasingly impaired. The presence of one mass-tag, however, did not appear to exert a negative influence on both hybridization and detection but enabled, by means of an improved mass discrimination, the use of more complex PNA pools with sequences of originally identical or too similar molecular masses. Unlike in the case of DNA oligonucleotides, the introduction of quaternary ammonium fixed charge-tags was not found to significantly increase the detectability of PNA probes nor did it positively affect hybridization. Nevertheless, it could be observed that charge-tagging of small PNA oligonucleotides renders them significantly more stable and less prone to alkali adduct formation during MALDI. This phenomenon was in particular observed for PNA hexamers and is probably due to a shielding effect of the fixed positive charge (Bartlet-Jones et al., 1994). Thereby, protonation of the N-terminal amino-group of PNA - a potential source of fragmentation - is impeded and alkali adduct formation is significantly reduced. In summary, it can be concluded that the incorporation of both one O-linker as mass tag and a quaternary ammonium fixed charge-tag is advantageous since it leads to enhanced PNA discrimination and MALDI properties.

Before the investigations performed in the course of this dissertation only little has been known about multiplexing of PNA hybridizations. In their MALDI-TOF MS approach to the analysis of genetic variations Griffin et al. (1997) applied duplex PNA hybridizations and detected up to five PNA probes of unique mass in parallel by combining different hybridization assays. Ross et al. (1997) demonstrated that it is feasible to hybridize four PNA probes simultaneously. However, variations encountered in individual PNA hybridization stability and specificity severely restricted the number of probes simultaneously tested.

First investigations addressed the maximal number of PNA probes that can be hybridized in parallel and resolved in one spectrum. Preliminary experiments carried out

with up to 57 different PNA octamer probes, however, revealed their poor hybridization and MALDI properties and showed that the applied degree of multiplexing was too high for sufficient signal resolution and hence analyzable hybridization results. Further experiments were performed with different, less complex sets of charge-tagged hexamers and heptamers each of which consisted of varying numbers of PNA that fitted in with respect to their individual mass and overall mass resolution and/or possessed comparatively similar desorption and ionization properties. These sets comprise "6mer global" and "7mer global“ consisting of 40 PNA probes, "6mer sub1” (21 probes), “6mer sub2” (17 probes), “6mer sub3” (15 probes), “7mer sub1” (20 probes), and “7mer sub2” (20 probes). Direct MALDI-TOF MS analyses showed an improved overall resolution and significantly higher individual signal intensities compared to octamers, which was attributed to the superior MALDI properties of hexamers and heptamers. In principle, hexamers appeared to be slightly better resolved than heptamers. Hybridization studies comprising all available PNA 6mer and 7mer sets revealed that meaningful hybridization data can only be obtained for charge-tagged PNA sets containing up to 21 different probes. The global PNA hexamer and heptamer sets did not yield distinguishable hybridization data suggesting that the total number of hybridization probes applied in multiplexed PNA hybridizations subsequently analyzed by MALDI-TOF MS is confined to a maximum of about 20 to 30 PNA probes. A more detailed discussion of the results of the comprehensive multiplexed hybridization analysis is represented in chapter 8.2. Nevertheless, the unparalleled degree of multiplexing that was demonstrated to be achievable renders an OFP technology of considerably enhanced efficiency possible.

Beside hybridization probe length, modifications of PNA probes and degree of multiplexing, other not less relevant characteristics of PNA hybridizations with subsequent MALDI-TOF MS detection were examined. These comprise the impact of individual and global probe concentration, target DNA concentration, temperature as well as different additives on PNA hybridization.

PNA-DNA heteroduplexes generally exhibit a very high thermal stability due to missing interstrand repulsion between charged DNA phosphate groups and the uncharged peptide backbone of the PNA molecules. However, strong variations in stability and specificity of these heteroduplexes occur which appear to be highly dependent on the individual sequence (Griffin et al., 1997) rendering specific, i.e. sequence-dependent hybridizations difficult to achieve. Since hybridization properties of DNA, RNA as well as PNA oligonucleotides are still not predictable (SantaLucia et al., 1996, Sugimoto et al.,

2001) empirical examinations were performed in order to study PNA hybridization properties and optimize hybridization conditions towards better specificity.

The impact of individual probe concentration was investigated in two different ways. Individual PNA concentrations were either adjusted with respect to each other (“equalized sets”) or employed in an equimolar fashion (“equimolar sets”). Experimental results yet revealed that altered individual probe concentrations have virtually no impact on PNA presence and signal intensity strongly suggesting that hybridization results are exclusively target DNA sequence-dependent. As a consequence, only equimolar sets were used for subsequent hybridization analyses. On the other hand, in the course of further studies on the impact of global PNA concentration and target DNA concentration on hybridization, for hexamers it appeared that hybridization profiles, i.e. presence and respective signal intensities of probes, depend very much on global probe concentration and overall target DNA concentration. For all tested concentrations a constant change of profile was observed. Too low concentrations gave rise to hybridization spectra of inferior quality that seemed to be less distinguishable. Too high concentrations in turn unexpectedly altered overall hybridization profiles which might be due to PNA aggregate formation (Tackett et al., 2002). As a compromise, standard concentrations were chosen that ensured hybridization results of sufficient quality but, from an economical point of view, did not cause excessive costs.

Several substances, such as salts, DNA melting agents and detergents, were tested within the scope of this dissertation that have been known to influence the outcome of desoxy- and ribonucleic acid hybridizations towards higher specificity, though rather little has been reported for PNA hybridizations. Furthermore, the impact of temperature on PNA hybridization on its own as well as in combination with some of these substances was investigated.

Although the concentration of salt and the presence of detergents has been demonstrated to significantly influence hybridization specificity on solid supports with regard to full-match and mismatch duplex discrimination (Weiler et al., 1997), no such experience could be gained for aqueous solution hybridizations. Neither sodium chloride, TMACl and TEACl nor sodium N-lauroyl-sarcosine, SDS, and Tween-20 showed an increased discriminatory effect at the concentrations tested but affected hybridization results in a rather unfavorable way, i.e. overall signal intensities were decreased without a concomitant increase in specificity. The presence of betaine caused a similar effect. Of the tested substances only formamide showed a favorable impact on hybridization resulting in an increase of overall PNA signal diversity. This finding was ascribed to an induced melting of duplex DNA rendering target DNA more accessible to hybridization probes – a phenomenon that has been known for decades (Bonner et al., 1967;

McConaughy et al., 1969). However, a higher degree of specificity could not be accomplished. Further experiments on the combined impact of temperature and formamide demonstrated that the elevation of hybridization temperature in the presence of formamide does not lead to greater specificity but to deteriorated hybridization profiles, i.e. presence and respective intensity of PNA signals.

In contrast to hexamers, heptamers yielded ambiguous results. However, this relative ambiguity which was observed in terms of unstable hybridization profiles, cannot be satisfactorily explained.

Despite the evaluation of a wide range of substances in different combinations unspecificity of PNA hybridizations could not be eliminated even under stringent conditions. Several reasons could explain this persisting phenomenon of unspecificity.

First, unlike DNA oligonucleotides, PNA is a achiral molecule which also binds to complementary sequences in a parallel fashion (Egholm et al., 1993, Pfeffer et al., 1993). Although the antiparallel fashion is energetically favored parallel binding does also occur. Therefore, a certain degree of unspecificity has always to be attributed to this particular PNA characteristic rendering unambiguous full match-mismatch discrimination problematic.

Second, the sequence dependence of PNA/DNA duplexes is most likely different from DNA/DNA duplexes. Due to the asymmetry of PNA/DNA duplexes, reverse enthalpy-entropy compensation[14] arises allowing the PNA backbone to better accommodate mismatches by means of a less ordered, hence more favorable mismatch surrounding (Ratilainen et al., 1998 and 2000). Unexpected duplex stabilities with up to three consecutive mismatches in the center of PNA oligomers were described (Weiler et al., 1997) suggesting that PNA-DNA mismatch stability is not dependent on the location within the duplex but highly sequence- and context-dependent. The composition of neighboring base pairs was found to be a primary determinant of mismatch stability where flanking G-C base pairs seem in general to stabilize the mismatch relative to flanking A-T base pairs (Ratilainen et al., 2000). The entirely unspecific adhesion of homopurines, especially homoguanines, to DNA forming complexes even persisting boiling buffer was explained by possible PNA precipitation upon self-aggregation (Weiler et al., 1997).

[14] For many different biological systems, compensation between enthalpy (mainly hydrogen bonding energies and van-der-Waals interactions) and entropy (mostly rearrangements of the molecules, solvent water, and counterions) is observed. This is normally expressed in tighter binding (larger $-\Delta H°$) accompanied by a larger loss of degrees of freedom (larger $-\Delta S°$) in a compensatory manner, resulting in relatively small changes in binding free energy ($\Delta G°$).

Third, sequence-unspecific non-Watson-Crick aggregate forming between multiple strands of PNA and complementary as well as non-complementary sequences of DNA was reported (Tackett et al., 2002). Being temperature sensitive, these interactions were shown to be promoted at moderate to high concentrations of PNA oligonucleotides (in the range of 500 nM to 10 µM) due to excess formation of PNA aggregates.

Since PNA probes were applied at moderate concentrations and many PNA probes used in the course of this dissertation were highly purine-rich - according to their selection with regard to entropy maximization – above mentioned reasons may represent an explanation for the encountered hybridization unspecificity.

8.2 Multiplexed oligonucleotide fingerprinting pilot study

Within the scope of this dissertation, using different PNA hexamer and heptamer sets, a comprehensive multiplexed OFP pilot study was carried out on a number of selected genomic DNA and cDNA clones to demonstrate the "proof of principle" for multiplexed OFP.

31 sequence-confirmed genomic clones, that were void of repeat regions and fell into five clusters of different size and two cluster-independent singletons, as well as 29 repeat-free cDNA clones or derived PCR fragments, falling into five clusters of different size, were analyzed by multiplexed PNA hybridizations. Due to the already mentioned unspecificity of PNA hybridizations, results were analyzed on the basis of correlated individual hybridization profiles. As for the global sets containing 40 PNA probes, no convincing hybridization data could be gained with either probe length. Since always nearly the same distinct PNA showed up, hybridization profiles were too similar for a successful discrimination. Clones belonging to the same cluster appeared to be as randomly correlated as unrelated ones rendering a 40-plexed OFP approach unfeasible.

Hybridization performances of the subsets were in general very heterogeneous, i.e. correlation analyses did not reveal the same quality of results. Apart from PNA set “6mer sub1”, correlation data of only modest quality or no consistent data at all could be obtained suggesting that the individual PNA set composition is of crucial importance. Adjusted correlation values gained for both 20 genomic DNA and 17 cDNA clones hybridized with PNA set “6mer sub2” showed that related clones of a given cluster were hardly separable from unrelated ones and numeric correlation differences were negligible. This finding was in accordance with the recorded spectra. However, a general trend towards correct discrimination was still discernible. In the case of a few genomic DNA clones, the achieved discrimination was noticeably suggesting sequence dependence.

PNA sets “6mer sub3” and “7mer sub1” yielded comparable results for genomic clones whereas for cDNA clones only random data seemed to be generated. In either case, PNA set “7mer sub2” failed to generate consistent data at all.

In contrast, adjusted correlation values determined for both 21 genomic DNA and 23 cDNA clones hybridized with PNA set “6mer sub1” demonstrated that clones of a respective cluster were grouped together and were completely separated from unrelated ones. Although the numeric difference to those unrelated clones was mostly rather small, for some clones, significantly higher correlation differences could be accomplished. This finding holds true for either clone species. The high discrimination performance was also confirmed by original spectra which visually supported the successful correlations.

In summary of the multiplexed PNA hybridization analyses, the following conclusions can be drawn.

First, the “proof of principle” for multiplexed OFP could be successfully demonstrated. Hybridization analyses performed with PNA set “6mer sub1” proved that it is feasible to distinguish both genomic and cDNA clones on the basis of their individual hybridization profiles. As for the other sets tested, despite the worse results obtained, a general trend towards correct discrimination was discernible supporting the findings for PNA set “6mer sub1”. Furthermore, by a linked correlation analysis of hybridization results of multiple optimized PNA sets – approaching an overall probe number as suggested by simulations - much higher degrees of discrimination can be achieved.

Second, the different performances of PNA sets are very likely to directly reflect their individual probe composition. Although the sets have been composed roughly according to similar individual PNA MALDI properties (chapter 6.8.1), in the case of PNA sets “6mer sub2” and “6mer sub3” only a very few PNA probes yielded high signal intensities upon hybridization. Possible reasons may be a suppression of weak signals and/or inferior hybridization properties of some PNA probes suggesting that very careful probe selection and PNA set composition are of pivotal importance. With such a set optimization, it is assumed that a total number of more than 21 probes, possibly up to 30, can be simultaneously hybridized in a meaningful fashion.

Finally, compared to hexamers, heptamers generally performed worse. They yielded unsteady hybridization profiles and hence ambiguous results rendering their applicability disadvantageous for multiplexed OFP analyses. This inherent ambiguity cannot be satisfactorily explained. However, purine-rich sequences and a higher tendency towards self-aggregation may represent possible explanations.

8.3 Potential MALDI-TOF MS compatible DNA immobilization systems

Within the course of this dissertation it was aimed at the development of a DNA microarray that allows on-site DNA immobilization, probe hybridization and read-out of hybridization events by MALDI-TOF MS thereby enabling full automation and hence significant acceleration of the OFP process. Since such a system was not available neither in the academic scientific community nor commercially, various surfaces and attachment chemistries were evaluated that promised to feature a stable attachment chemistry with a high DNA immobilization capacity and a high accessibility of immobilized DNA to hybridization probes and laser desorption.

From the very beginning it has been undisputed that the development of a MALDI-TOF MS compatible DNA microarray can only be successful on the basis of industrial collaborations. In order to facilitate industrial cooperations and initiate the development of an MS compatible microarray, a prototype adapter was fabricated that functions as an interface between Bruker MALDI-TOF mass spectrometers and DNA microarrays of universal glass slide format.

Four promising immobilization systems were evaluated based on different three-dimensional matrices for DNA immobilization. These matrices were expected to offer a higher immobilization capacity compared to two-dimensional systems as well as drastically reduced probe-target DNA interferences impairing hybridization as such and its specificity. With regard to such systems, a polyacrylamide-matrix, the streptavidin-based XNA on Gold™ biochip, nylon and nylon-based CAST™ slide, as well as a PAMAM starburst dendrimer-based system were tested - last three in collaboration with industrial partners. Preliminary experiments under high-resolution and optimized conditions demonstrated a promising potential for all systems. However, further investigations revealed inherent drawbacks that, in the case of polyacrylamide- and streptavidin-based system, led to their exclusion at an early stage.

Although polyacrylamide gel pads were employed in MALDI-TOF MS based DNA analysis (Yershof et al., 1996, Stomakhin et al., 2000) experiments showed that dried co-polymerized gel matrices are unstable and disintegrate.

As for XNA on Gold™ biochip, unspecific binding of PNA probes presumably to streptavidin occurred that could not be eliminated by the presence of detergents and rendered a meaningful analysis of hybridization results impossible.

Experiments with metallic CAST™ slides were reproducible and confirmed the preliminary results obtained using small pieces of nylon membrane. However, signal resolution was generally poor. Moreover, empirical observations revealed that hybridization results were detectable at the edges of a membrane piece rather than in the

center, presumably due to membrane thickness and insufficient MALDI matrix crystallization. Besides, the comparative assessment of capacity failed to yield satisfactory results. Both findings were ascribed to the porous nature of nylon membrane. The one-piece nylon membrane probably acts as sponge which renders precise matrix deposition very difficult to achieve and results in immense spread effects and hence inferior, if at all, crystallization.

The comparison of two batches of PAMAM functionalized silicon with conventional PAMAM glass slides showed that on silicon consistently higher signals and better resolutions are gained, although specificity was not improved. However, a lack of pre-structuring causing an intricate on-slide sample localization and problematic MALDI matrix crystallization led to impaired signal detection. Nevertheless, as confirmed by the comparative assessment of capacity PAMAM based dendrimers represent powerful DNA immobilization systems with PAMAM functionalized silicon being superior to glass. This is possibly due to the aligned layer of thermally oxidized silicon which provides for an optimized substrate functionalization and hence an improved DNA binding capacity.

In the course of this work it has been concluded that an ideal MALDI-TOF MS compatible DNA microarray should comprise following features:

- uniform dimensions
- a stable homogeneously functionalized surface optimized for MALDI matrices
- a high DNA immobilization capacity
- a high accessibility to hybridization probes and laser desorption
- a prestructured coordinate system of separate positions

A uniform microarray format offers the advantage of high flexibility in the form of platform independence, i.e. all kinds of instruments of different companies can be employed.

MALDI matrices are applied as solutions containing harsh organic solvents, such as acetonitrile and acetone, requiring substrates of high stability. Experiments with all immobilization systems showed that for reproducible mass spectrometric results a high quality of matrix/sample crystallization is essential. Even an excellent hybridization performance could be superimposed by poor matrix crystallization resulting in very bad or no signals at all. Furthermore, a study of PNA detection sensitivity on different conductive substrates as well as glass revealed that the threshold of detection is substrate-independent but highly affected by crystallization quality (personal observations). Hence, for enhanced signal intensity and resolution a homogeneously functionalized surface is

necessary that ensures an optimized and highly reproducible crystallization of MALDI matrix.

High DNA immobilization capacity as well as high accessibility to hybridization probes and laser desorption should improve overall signal intensities and signal-to-noise ratios thereby enhancing detection via MALDI-TOF MS. This was in particular reflected in the detection problems encountered with nylon membranes where hybridized PNA probes were hardly accessible to laser desorption.

A prestructured coordinate system of separate positions is a prerequisite for precise laser guidance during MALDI-TOF MS measurements. In combination with substrate functionalization it even offers the potential of creation of separate microenvironments that can be treated individually.

Although no functional MALDI-TOF MS compatible DNA immobilization system could be created in the course of this dissertation, two of the tested systems, metallic CAST™ nylon membrane slides as well as PAMAM functionalized silicon and glass slides, showed a promising potential for further development.

Regarding the CAST™ system, the existing drawbacks could be overcome by the construction of a well-defined prestructured array of separate nylon "spots" that are of optimized pore size and membrane thickness. According to Schleicher & Schuell Bioscience such a system would be conceivable. For protein arraying, an analogous system on the basis of nitrocellulose has already been launched[15].

The same holds true for PAMAM functionalized substrates, where persisting problems, such as the intricate on-slide sample localization and inferior MALDI matrix crystallization, could also be removed by a prestructured coordinate system of separate functionalized positions. Moreover, Chimera Biotec has expressed its general interest in the further development of their functionalization technology towards that direction.

8.4 Outlook

Within the scope of this dissertation, the applicability of the concept of multiplexed OFP was successfully demonstrated.

Furthermore, it could be shown that by the use of PNA oligonucleotides hybridization probe length can be reduced to hexamers. From a theoretical and experimental point of view, hexamers perform clearly better than octamers and heptamers leading to a

[15] FAST™PAK protein arraying kit (http://www.s.-und-s.de)

considerable reduction of the number of hybridization probes needed for an OFP analysis. Moreover, the achieved degree of hybridization probe multiplexing significantly increases throughput and hence efficiency of OFP.

However, the inconsistent performance of some PNA probe sets as well as the lack of automation have to be overcome in order to establish multiplexed OFP as routine application.

Through a careful selection of probe sequences with respect to in particular experimental considerations, it should be possible to establish and further optimized PNA probe sets of equalized performance. On that basis, not only a further increase of the degree of multiplexing can be envisaged (possibly up to 30 probes) but also improved correlation values can be achieved. In combination with an overall probe number of 70-100, as suggested by "in silico" simulations, a much higher degree of discrimination should be accomplished.

Complete automation of the multiplexed OFP procedure could be achieved using a MALDI-TOF MS compatible DNA immobilization system as well as an automated bead-based hybridization system. Whereas the former still requires further development, the latter alternative can be implemented in relatively short term adopting in-house available magnetic bead-based technology for DNA purification.