Improving DNA-Chip Technology

Chemical Aspects

Jörg D. Hoheisel, Frank Diehl, Marcel Scheideler, Nicole Hauser,
Verena Aign, Stefan Matysiak and Markus Beier
Functional Genome Analysis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, D-69120 Heidelberg, Germany; Tel. +49-6221-424680, Fax +49-6221-424682, Email j.hoheisel@dkfz-heidelberg.de

1. Abstract

DNA-microarrays have become a synonym for the type of analyses that aim at the understanding of cellular functioning in a comprehensive manner. Although still in a relatively early phase, the methodology has already proven its worth. Nevertheless, improvements on various aspects are necessary for making DNA-chips a routine tool in research and diagnostics. In this manuscript, some chemical developments toward this end are being reviewed.

2. Introduction

Quality of analyses on DNA-microarrays in the fields of transcriptional profiling[1,2], analysis of single nucleotide polymorphisms (SNPs)[3] and disease-relevant mutations[4], functional studies on deletion mutants[5] or any of the many other applications depends strongly on the effectivity of the chemistry used to create the respective type of chip (Fig. 1). Critical criteria that affect the performance are the solid support as well as the character of the linker molecule to which the biomolecules are actually attached. The type of bonding between the DNA and the chip is another consequential issue for both immobilisation of pre-fabricated molecules and *in situ* synthesis. In the latter process, the stepwise yield per added nucleotide seriously affects the scale of discrimination and dynamic range in

hybridisation. Last, the determination of the actual quality achieved during chip production is prerequisite for many quantitative measurements and indispensable for clinical applications. In our work, we made an effort to improve and if possible standardise all these elements, besides advancing several other factors that are not directly associated with the chemical production procedures.

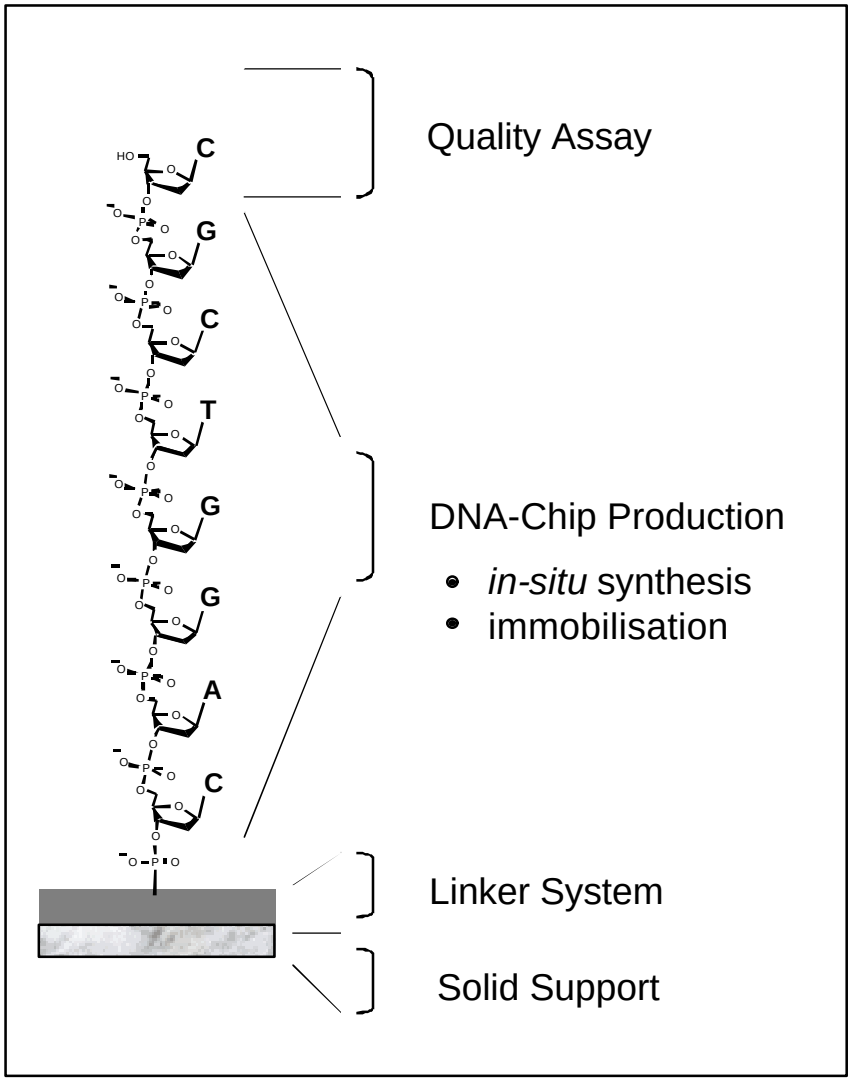

Figure 1. Quality issues of DNA-chip production.

3. Solid support

Currently, two support media are being used most for DNA-arrays: nylon filters[6] and glass slides[7,8]. Because of the porous structure, relatively large amounts of DNA can be applied to nylon membranes resulting in strong signal intensities and good dynamic range but also relatively high background, all produced by the very same structural characteristic - the large surface area per spot. Advantageous is that nylon filter arrays can be re-used, because the DNA sticks relatively well to the surface. However, spot sizes cannot be reduced to a level possible with non-porous media.

This ability of miniaturisation in combination with chemical inertness and low intrinsic fluorescence are the main advantages of polypropylene and especially glass permitting high probe concentrations even from small samples. The planar surface structure, however, restricts the loading capacity. Another disadvantage is the limitation in the number of experiments that can be done with an individual microarray. Many systems reported to date permit only one experiment per chip[7,8], preventing proper quality checks on the chip itself and some appropriate controls of the experimental data. In addition, the ability to re-use chips eliminates from experimentation the variance between individual chips, thus increasing the experimental reliability of the analyses.

In an effort to solve these problems, we opted for a very flexible strategy by which a versatile linker structure could be created on glass and polypropylene[9]. Linker attachment to the support is achieved *via* amino-groups. They are added by a derivatisation of glass with aminoalkylsilane or plasma-amination of polypropylene. Subsequently, the linker is generated by an iterative cycle of acylation and amination. By varying the amine, linker characteristics can be modulated to fit the respective use best. Features such as loading capacity, linker length and charge can be adapted. Another factor that can be influenced during linker-synthesis is the hydrophilic or hydrophobic character of the surface by incorporation of the respective form of amines. Most importantly, however, all linker derivatives permit covalent bonding of DNA to the support, hence warrant re-usability of the arrays. Attachment of pre-fabricated oligomers was found to occur *via* their termini only so that their entire sequence is accessible for hybridisation to target molecules. Last, the linker system is not restricted to the attachment of aminated biomolecules but amicable to alternative reactive groups such as thiol, for example.

Apart from providing a support for nucleic acids, also the linkage of peptides was demonstrated. Protein and peptide-arrays could become at least as important a field of microarray analyses as the DNA-chips are today.

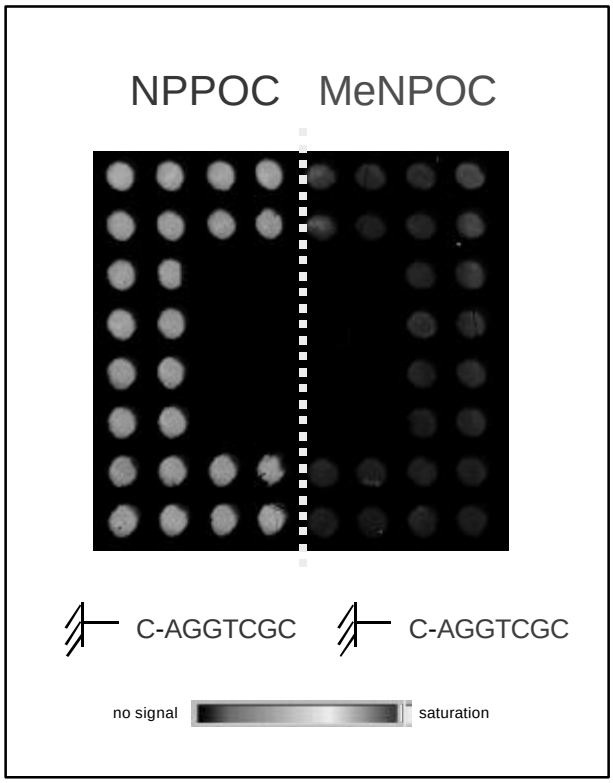

Figure 2. Comparison of oligonucleotide synthesis. d(CGCTGGAC)-sequences were generated using NPPOC and MeNPOC phosphoramidites at the optimal conditions for the respective chemistry. Upon hybridisation of 5'-Cy5-labelled complementary oligonucleotide, signal intensities were scored. The bar at the bottom indicates the false colour values (reproduced with permission from reference 11).

4. *In situ* synthesis

Both for normal phosphoramidite-based synthesis of oligonucleotide as well as photolithographically controlled *in situ* synthesis, procedures were developed based on the photosensitive 2-(2-nitrophenyl)propoxycarbonyl (NPPOC) group that yielded near quantitative amounts of product in each individual condensation reaction[10,11]. This is in contrast to the efficiencies of other established chemistries, including the commercially used ((α-methyl-2-nitropiperonyl)oxy)carbonyl (MeNPOC) chemistry of Affymetrix which results in yields of 85% to 90% per synthesis step[12,13]. Thereby the

accumulative total yield increases by more than threefold in case of octamers (Fig. 2), and this difference widens to a factor of considerably more than one order of magnitude for 20-mer oligonucleotides.

Use of the NPPOC-group has also been extended successfully to the four ribonucleotides. For many applications - from approaches to highly parallel DNA-sequencing[14] to the creation of double-strand DNA-microarrays[15] - the availability of a free 3'-terminus, hence, a reverse direction of oligonucleotide synthesis, would be advantageous. NPPOC-chemistry should be applicable to this end, also. With these additional features, photolithographically produced oligomer-chips would become an even more versatile tool for many chip-based analysis procedures.

5. Quality control

A routine quality assessment is prerequisite for quantitative measurements, for which the exact amount of probe present at each position is essential to know. But also for purely qualitative assays in a clinical environment such control is necessary. Hybridisation is inadequate for this purpose, since only few sequences can be checked. Covalently adding a fluorescence marker assays all oligonucleotides[13] but influences the subsequent hybridisation because of steric and other effects of the dye molecule. Therefore, a removable fluorescent tag had to be used[11]. It can be added by standard phosphoramidite chemistry as the last step of oligonucleotide synthesis, in our specific case consisting of either a dansyl- or a Cy5-dye attached to a phosphoramidite building block by a base-labile linker. After condensation of the tag monomer to all full-length molecules, a signal from each individual spot can be recorded. Treatment with ammonia then removes the tag (and concomitantly the base- and phosphate-protecting groups) leaving behind a phosphate at the 5'-end of each oligomer, which does not interfere with hybridisation.

6. Alternative probe molecules

Ever since the elucidation of the DNA-structure, chemical derivatives have been synthesised with the aim of modifying various aspects of the biochemical and biophysical properties of nucleic acids. Peptide nucleic acid (PNA; Fig. 3) is one such molecule combining several advantageous features[16]. Substitution of PNA for DNA molecules on the arrays[17] takes advantage of the molecules' superior selectivity in double-strand formation with nucleic acid targets and the significantly higher stability of the duplex

once formed. Since PNA is an uncharged molecule, hybridisation can be carried out in the absence of ions under conditions which disfavour double-strand formation between the DNA target molecules. PNA is not being degraded by either proteases or nucleases. Therefore, even crude isolates could be used for hybridisation. Last, detection methods other than optical measurement are feasible by taking advantage of the structural difference between PNA and DNA for detection purposes[18].

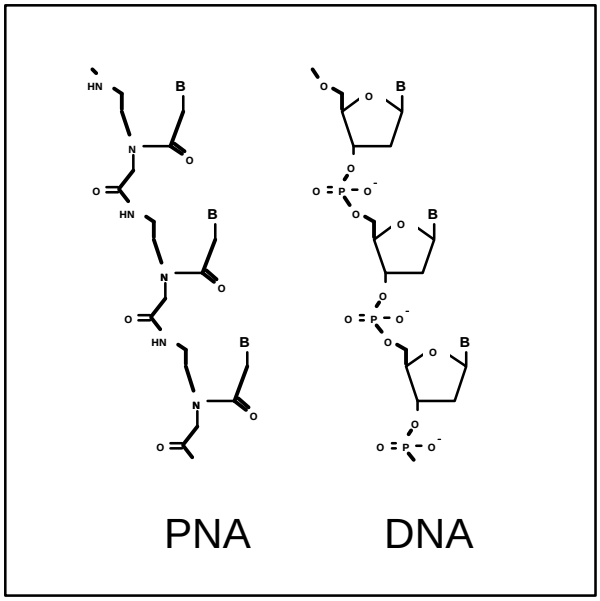

Figure 3. Comparison of DNA and PNA structure. PNA consists of repeating units of N-(2-aminoethyl)-glycine linked by amino bonds. Bases (B) are attached by methylene carbonyl linkages.

7. Conclusions

DNA-chips have come a long way from their conception[19-22] and the initial experiments[23]. While during many years emphasis was on technical developments, now the time of real utilisation has come. Nevertheless, technology needs further improvement in order to make microarrays as versatile and robust a tool as for instance the polymerase chain reaction has become. In combination with technical advances that are still in the making, such as the electric field control of hybridisation - described in more detail elsewhere in this issue - or alternative detection modes like mass

spectroscopy or direct electric measurement, developments on biochemical and biophysical aspects will soon advance the performance of chip-based analyses to a level which will enable the technology to be used as a routine tool in clinical diagnoses, besides its very many other fields of application.

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