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Robotic spotting of cDNA and oligonucleotide microarrays

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DNA microarrays are a uniquely efficient method for simultaneously assessing the expression levels of thousands of genes. Owing to their flexibility and value, mechanically spotted microarrays remain the most popular platform. Here, we review recent technological advances with a focus on spotted arrays. Robotic spotting still poses numerous technical challenges. To reduce artefacts, many laboratories have recently investigated ways of improving the spotting process. We compare alternative options and discuss implications for next-generation systems. Together with modern approaches to data analysis, such developments bring greatly improved reliability to individual microarray experiments. Advancing towards the ultimate goal of delivering calibrated, truly quantitative gene-expression measurements on a genomic scale, microarray technology remains at the forefront of post-genomic systems biology.

Introduction

Genome sequencing confronts us with the unequivocal fact that we know very little about the function of many genes, even in the most intensely studied genomic regions of key model organisms [1]. Generating loss-of-function or gain-of-function mutations followed by detailed phenotypic analysis has traditionally been used in genetically tractable model organisms. However, this is not always possible, or indeed informative, because many gene mutations have no obvious phenotype. In such instances, gene expression patterns can be used to suggest more appropriate genetic, molecular or biochemical assays. Gene expression patterns can also be used to search for co-regulated groups of genes, having functional implications and giving valuable insights into interactions between genes.

Microarrays exploit the specificity of nucleic acid base-pairing during hybridization to simultaneously assess the expression of tens of thousands of genes [2,3]. Without microarrays, gene expression analysis would be limited to studies of one or a few genes using, for example, Northern blots and real-time quantitative PCR, or to time-consuming and costly approaches like Serial Analysis of Gene Expression (SAGE) [4] and Massively Parallel Signature Sequencing (MPSS) [5]. Microarray experiments are unique in offering cost-effective and efficient analysis of gene expression at the genomic level (Box 1). Although many of the protocols for microarray experiments are not new, some are highly technical and are widely considered to be challenging, most notably the production of the arrays. Many manufacturing considerations similarly apply to all microarray applications (Box 1), therefore, this review will focus on the most popular one, microarrays for gene expression analysis.

Microarrays can be manufactured using robotic spotting of gene-specific cDNAs or long oligonucleotides and by *in situ* synthesis of short or long oligonucleotides. Barrett and Kawasaki have reviewed these established manufacturing processes [12]. More recent approaches include voltage dependent nanopipettes [13], piezoelectric inkjets for non-contact printing [14] and maskless light-directed synthesis of oligonucleotides [15]. However, robotic spotting is still the most popular method because of its wide availability, high flexibility and low cost (Box 2). Although spotted microarrays can provide accurate measurements at the genomic level [16,17], similar to other microarray platforms, their sensitivity is limited by high levels of

Box 1. Microarray experiments

Spotted microarrays were developed for identifying differences in gene expression between samples based on the relative amounts of sample bound to a particular spotted probe DNA on the microarray [2,3]. The full utility of the technique is clearly reflected in the wide variety of its applications [6], ranging from gene expression analyses to studies of genomic DNA. Comparative genomic hybridization (CGH), for example, is used to identify allelic differences between individuals [7]. Chromatin immunopurification (ChIP) microarrays, or 'ChIP-chips', locate the binding-sites of DNA-binding proteins [8]. The samples to be compared are each labelled with a different fluorescent dye and then subjected to competitive hybridization. The aim of any spotted microarray experiment is to generate spot fluorescence measurements that reflect how much sample is bound to a spotted probe DNA. These measurements are derived from images taken by laser scanners or charge-coupled device (CCD) cameras [9]. Software tools locate and then quantify the fluorescence intensity or 'spot signal' from each spotted probe [10,11]. Downstream data processing varies according to application. In gene expression analysis, for example, typical approaches include gene selection by ranking expression ratios, clustering or probabilistic analysis, with the aim of identifying statistically significant differential gene expression or groups of co-regulated genes. This permits inferences to be made about the regulation of the processes being investigated.

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Box 2. Cost comparison

Microarray technology has relatively high start-up costs, for example, fixed overheads that include the instrumentation, service contracts and staff (Table S1), and consumables, for example, spotting substrates and probe DNA (Table S2). Cost savings can, however, be achieved through increases in throughput (Figure S1), arguing for the pooling of resources and the establishment of core facilities. Core facilities typically charge £50–200 per spotted array. This compares favourably to the commercial alternatives (Table S3); for example, oligonucleotide arrays from Agilent Technologies (http://www.home.agilent.com) cost £300, and Affymetrix GeneChip arrays (http://www.affymetrix.com) cost £500–1000. All prices given exclude the costs of sample processing and hybridization. Gershon [20] extensively discusses the currently available commercial options.

experimental variability [18,19]. Many laboratories have therefore investigated ways to improve the spotting process. Recent progress in dealing with the many inherent challenges is the subject of this review.

Robotic spotting and its variability

Spotted microarrays are printed by dipping pins into probe DNA dissolved in spotting buffer and then depositing each probe on a chemically derivatized glass substrate [9,21]. Most commonly, pins are filled with probe DNA by capillary action and the surface tension between the spotting buffer and substrate then acts to deposit the probe spots [22]. Perturbations in spot structure are common [23] and are a significant source of signal variation [24]. Spot intensity measurements are therefore noisy estimates of how much sample has bound to each probe DNA. This measurement error is in addition to sources of systematic variability or 'bias' [18], such as systematic changes in spot signal during consecutive spot depositions [23,25], variations in hybridization efficiency across the slide [11] and differential dye labelling [26]. Compared to variations from printing, the biases introduced by labelling and hybridization effects are usually stronger and, hence, have received considerable attention elsewhere [19].

In addition to being a challenging aspect of image and data analysis [27–29], spot variations make stringent quality control essential for reliable results [30,31]. However, attention has only recently been focused on the need for a systematic approach to process development [32,33]. It is now recognized that problems of reproducibility and reliability can only be overcome with a combination of more advanced analytical tools and improved experimental techniques [19]. This is complementary, for example, to efforts to calibrate microarray signals from robotically spotted gene-specific cDNA microarrays with RT-PCR measurements [16]. Both developments reflect that, although a replication of measurements reduces the effect of random noise, the measurement bias caused by systematic errors must be addressed specifically.

Spotted probe DNA

Initially, PCR-amplified cDNA clones were used as probes [34]. This is still the method of choice when interrogating samples from organisms with unknown genome sequence [35]. A recently introduced alternative uses probes obtained by PCR from shotgun genomic DNA libraries [36]. However, PCR amplification can suffer frequent failures, variable DNA yields, and brings the danger of cross-contamination [22]. Simply performing PCR on this scale and transferring the PCR products from 96- to 384-well plates for spotting is error-prone [37]. Consequently, resequencing of probe DNA has found that only 66–79% of probes had been correctly annotated and contained no contaminating sequences. Therefore, gel electrophoresis and resequencing of PCR amplicons before printing is highly recommended [38,39].

Because of their length, PCR-amplicon probes are highly sensitive and have an inherent tolerance to small sequence variations. They are thus the method of choice when interrogating samples from one species using probes of another [40]. This feature, however, reduces their ability to discriminate similar sequences within an organism. Many microarray users have started to spot single-stranded long oligonucleotide probes to overcome the limitations of PCR-amplification and to increase target sequence discrimination [41,42]. As a consequence of the higher specificity of oligonucleotide probes, interspecies analyses employing oligo-arrays have to deal with probes explicitly that do not match their target sequences fully [43,44]. The design of oligonucleotide probes in general is a challenge. Poorly designed probes can have a profound impact on target sequence discrimination [42,45,46]. Consequently, modern oligonucleotide design tools use sophisticated algorithms to predict hybridization behaviour [47,48]. Efforts to improve sequence discrimination by improved computational probe design are complemented by recent experimental research into how sequence discrimination varies with oligonucleotide length [46] and hybridization conditions [32]. The design and subsequent experimental validation of a probe set are a considerable investment, which might be best shared through the formation of consortia [49].

Glass microscope slide substrates

In-house coated poly-L-lysine glass microscope slides [2] have been surpassed by commercial aminosilane substrates [22,34], with respect to spot morphology and its consistency [22,50]. For these chemistries, the probe DNA is initially bound to the slide by electrostatic attraction and then cross-linked to the substrate by ultraviolet radiation or heat [41]. A succinic anhydride wash is then performed to prevent non-specific hybridization to the surface of the substrate [24,51] because high levels of background fluorescence adversely affect spot finding and quantification [10,11].

Modified probes and reactive substrates have also been introduced [52] because sequence discrimination is thought to be enhanced when there is a fixed point of attachment [41] and when probe DNA retention on the slide is higher [53]. Substrates that permit covalent attachment without a need for expensive probe modifications have also been developed; these have no appreciable loss in sequence discrimination [54]. In each case, a bovine serum albumen (BSA) or succinic anhydride wash is performed to reduce the background signal [51].

However, some researchers find that covalent attachment of probe DNA to the substrate is not really required because non-covalently bound probes will remain bound to the substrate surface under standard hybridization conditions [55].

Other substrates have been optimized to produce higher spot signals with reduced amounts of probe material. To this end, an increase in the density of reactive groups of the substrate surface is desirable [56–58], which can be achieved by coating glass microscope slides with dendrimers [57], epoxysilane aminosilane composites [56] or self-absorbing polymers [58].

Spacer molecules that reduce the steric hindrance of oligonucleotides immobilized on the substrate can be added to the 5′-end of probe DNAs [46,59,60]. Commercial substrate coatings that have gel-like properties are also available [61]. Covalent attachment of probes to the 'gel' surface gives conditions similar to hybridization in solution and is comparable to binding probes with spacers onto solid substrates [62].

Spotting buffers

Probe DNA was originally dissolved in high-salt spotting buffers [2,34]. Detergent additives have since been introduced to improve the average spot morphology [63]. However, some laboratories found that detergent additives cause higher probe carry-over and increased variability between consecutive spot depositions [23,24]. The introduction of hygroscopic spotting buffers resulted in improved spot structures [22,24,64] and higher spot signal intensities [22]. Hygroscopic additives, however, increase the average spot size [65] but this can be overcome partially by reducing spotting pin speed or using pin microvibrations and microtapping [9,65]. Printing at lower operating temperatures and using more hydrophobic substrates also helps to reduce average spot size [22]. Nevertheless, some microarray facilities continue to use detergent additives, arguing that the alternative hygroscopic buffers have not produced a significant improvement in spot morphology or its consistency [63].

Variations in spot signal caused by spotting effects are present in many recently published microarray datasets [25]. The variety of spotting buffers represented suggests that no spotting buffer type is ideal. For instance, although high-salt buffers evaporate faster, they enable easy desiccation and rehydration of probes, which reduces the variability between print-runs due to changes in probe DNA concentration in the microtitre plates. Although hygroscopic spotting buffers evaporate at a much slower rate, they cannot easily be desiccated and rehydrated; hence, hygroscopic buffers might be better suited for applications in which probe DNA is not limiting or when a smaller overall number of probes needs to be spotted, as for low-density printing or limited issue (or boutique) arrays.

Spotting pins

The traditional robotic spotting pin is made from stainless steel or titanium [2] and a capillary is cut into the tip using electrical discharge machining or laser cutting [66]. Inconsistencies inherent in the manufacturing of these pins mean that subtle pin-to-pin differences exist [66,67] and consequently pin-specific systematic spotting variation is observed [68]. New spotting pin technologies have been developed to address this issue.

Ceramic pins adapted from the microelectronics industry have recently been applied to robotic spotting [67] and Reese *et al.* [66] manufacture capillary pins by photolithography of stainless steel foil. As these new pin types are not yet generally available, no direct comparisons have as yet been made. They are, however, cheaper to manufacture and could, in theory, be used to print up to 225 000 spots on a standard microarray slide [66]. In reality, practical limitations such as the number of spots that can be printed per pin loading and instrument speed mean that the current generation of spotters is inappropriate for work at this scale. However, spotting robot performance is catching up fast; the current recorded maximum spot number for the traditional spotting pin is 82 944 spots per microarray [66]. Alternatively, a higher spot density could be used to reduce the overall size of an array with a more moderate number of spots. Such a shrinkage of array size could help to reduce spatial spot signal variation.

Spotting variability and its optimization

Evaporation during printing appears to be the main cause of spotting variability [18,23]. The volume of solvent within the 384-well micotitre plate [18] and within the pin reservoir is reduced [23], which increases the probe concentration and alters the spotting buffer transfer characteristics. Consequently, the fluidic properties of the deposited spot and the distribution of probe DNA within the spot area are altered [23,65]. Moreover, spotting buffer viscosity increases with higher probe concentration, reducing the average spot size [65]. Higher probe concentration also increases the spot signal intensity [69] until the substrate reaches its probe DNA saturation point [53]. An analysis of these effects is complicated by additional sources of variation during spotting and in subsequent steps of the microarray experiment. Spotting buffer pH, for example, can affect spot signal because of its effects on the ionic charge of DNA and its subsequent binding to the slide [10]. Hybridization conditions also affect the structure of the spots [50].

There exist many complex interactions between effects, therefore, it is not possible to predict the performance of a particular combination of spotting buffer and substrate adequately. Consequently, several groups have attempted to optimize the spotting process for improved spot structure and higher spot signals by testing different spotting buffers [22–24,63], slide chemistries [22,50,52,70] and post-printing substrate treatments [24,51] under a range of instrument settings [9,22,65,71]. Several suitable spotting conditions have indeed been identified. Only recently, however, has it become possible to perform a systematic analysis of probe DNA deposition. Moreover, analysis of spot morphology and its variance has relied on comparisons of individual spot images [23,24] and pixel intensity summaries that assumed a flat, uniform spot structure [51,63]. Current developments include assessments of spot structure and variance without presuming a

particular spot structure [28] and a systematic comparison of spot signal intensity for different spotting reagents [33]. These studies have only examined a few aspects of the spotting process in isolation; therefore, further work will be required for a comprehensive systematic evaluation of the entire spotting process. Building on developments like these, it can be anticipated that large-scale systematic and quantitative evaluations will increasingly replace approaches of individual ad hoc process improvements. Such systematic analyses will be resource intensive because of the high number of experiments required to assess multiple effects and their interactions [33], making these studies most amenable to high-throughput facilities. Complementing ongoing quality improvements and developments to reduce levels of systematic variability is the use of per-chip quality indicators [31] and precise records of how the microarrays have been printed [72].

Spotted microarray quality control

Most microarray facilities assess their microarray slides for quality before hybridization to prevent precious and expensive biological material being wasted on sub-standard microarrays. Typical quality control includes screening for printing artifacts, such as missing, oversized or poorly formed spots. Spots can be visualized readily using DNA-binding fluorescent dyes or stains that can be imaged using a standard microarray scanner [73]. Alternative, possibly more accurate methods [10] include hybridization with dye-labelled random 9-mer oligonucleotides or dye-labelled oligonucleotides that hybridize to short marker sequences present in all probe DNAs.

In practice, DNA binding dyes and oligonucleotide hybridizations have been of limited use in assessing the quality of individual microarrays because most laboratories only assess a random selection of slides to verify the quality of a particular batch [73]. The addition of free dyes to the spotting buffer, which can be washed off before hybridization [71,73], or pre-labelling of probe DNA with a fluorescent dye other than Cy5 or Cy3 [74], are alternative approaches with the advantage of being suited for routine use on all arrays of a batch. Probes pre-labelled with an additional dye also provide a means by which spotting artefacts can be corrected with a combination of quality filtering and normalization [74]. Multi-channel designs generally seem to be promising, for example, enabling simultaneous hybridization of three samples to each microarray [75]. Although these approaches are not yet in widespread use, they demonstrate the progress achievable with modern protocols.

Besides the actual experimental verification of array quality, a major concern is the continuous tracking of all steps in array manufacture. Only seamless production logs can provide the assurance that a particular batch of arrays meets stringent quality requirements. When planned carefully, manual logs in spreadsheets can meet this need. Alternatively, data can be maintained by a laboratory information management system (LIMS). Particularly for microarray manufacture, excellent open-source solutions are freely available, such as BASE [76], which has a web-browser front-end for ease of use. For reliable production quality, it is also advisable to develop, document and consistently employ standard operating procedures (SOP; see http://www.flychip.org.uk, for examples). In addition to better day-to-day consistency and reduced operator effects, this also facilitates knowledge transfer between laboratories.

Microarray database standards

The need for precise descriptions of microarray experiments for data archival and subsequent analysis has led to the Minimum Information About a Microarray Experiment (MIAME) standard (http://www.mged.org/Workgroups/MIAME/miame.html) [77], tying in with related efforts of database standardization, like the Gene Ontology (GO) project (http://www.geneontology.org) [78]. Such developments form the basis of public microarray databases, such as ArrayExpress [79]. As with DNA sequences and protein structures, submission of microarray data to a standards compliant repository is increasingly required for the publication of microarray studies [72]. Standard compliance ensures the widest possible dissemination of results, encouraging independent verification of analyses and derivative work by others, including computational method development and meta-analyses that combine multiple datasets. The standard is instrumental not only in making the data available in a common format but also in providing the necessary semantic standardization that makes it possible to compare and integrate results from different sources. The emphasis on an accurate documentation of protocols in the standard is further justified by the observation that, in multi-centre studies, which laboratory generated a set of microarray data is still a dominant factor in the analysis [80].

Concluding remarks

Recent developments show that the experimental variability of spotted microarrays will decrease as new spotting reagents, systems, processes and techniques are implemented and further improved in the coming years. Downstream data analysis will become more accurate with the provision of spot quality control measurements, documented precisely in standardized databases. When combined with robust estimates of spot signals from advanced image analysis tools and state-of-the-art low-level data processing and normalization, this will greatly improve the reliability of individual microarray experiments. Moreover, data with lower bias better support the combined study of experiments from multiple laboratories, modern data analysis and modelling. In summary, microarrays are moving towards the ultimate goal of delivering calibrated, truly quantitative gene expression measurements on a genomic scale. Microarray technology thus remains at the forefront of modern experimental methods that shape the way we can interrogate complex biological systems.

Supplementary data

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References

1 Ashburner, M. *et al*. (1999) An exploration of the sequence of a 2.9-Mb region of the genome of *Drosophila melanogaster*: the Adh region. *Genetics* 153, 179–219

2 Schena, M. *et al*. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270, 467–470
3 Lockhart, D.J. *et al*. (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.* 14, 1675–1680
4 Velculescu, V.E. *et al*. (2000) Analysing uncharted transcriptomes with SAGE. *Trends Genet.* 16, 423–425
5 Brenner, S. *et al*. (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nat. Biotechnol.* 18, 630–634
6 Heller, M.J. (2002) DNA microarray technology: devices, systems, and applications. *Annu. Rev. Biomed. Eng.* 4, 129–153
7 Pinkel, D. *et al*. (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat. Genet.* 20, 207–211
8 Ren, B. *et al*. (2000) Genome-wide location and function of DNA binding proteins. *Science* 290, 2306–2309
9 Cheung, V.G. *et al*. (1999) Making and reading microarrays. *Nat. Genet.* 21(1 Suppl.), 15–19
10 Brown, C.S. *et al*. (2001) Image metrics in the statistical analysis of DNA microarray data. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8944–8949
11 Yang, Y.H. *et al*. (2002) Comparison of methods for image analysis on cDNA microarray data. *J. Comp. Graph. Stat.* 11, 108–136
12 Barrett, J.C. and Kawasaki, E.S. (2003) Microarrays: the use of oligonucleotides and cDNA for the analysis of gene expression. *Drug Discov. Today* 8, 134–141
13 Bruckbauer, A. *et al*. (2003) Multicomponent submicron features of biomolecules created by voltage controlled deposition from a nanopipet. *J. Am. Chem. Soc.* 125, 9834–9839
14 Laurell, T. *et al*. (1999) Design and development of a silicon microfabricated flow-through dispenser for on-line picolitre sample handling. *J. Micromech. Microeng.* 9, 369–376
15 Nuwaysir, E.F. *et al*. (2002) Gene expression analysis using oligonucleotide arrays produced by maskless photolithography. *Genome Res.* 12, 1749–1755
16 Yuen, T. *et al*. (2002) Accuracy and calibration of commercial oligonucleotide and custom cDNA microarrays. *Nucleic Acids Res.* 30, e48
17 Dudley, A.M. *et al*. (2002) Measuring absolute expression with microarrays with a calibrated reference sample and an extended signal intensity range. *Proc. Natl. Acad. Sci. U. S. A.* 99, 7554–7559
18 Spruill, S.E. *et al*. (2002) Assessing sources of variability in microarray gene expression data. *Biotechniques* 33, 916-920, 922-923
19 Wernisch, L. *et al*. (2003) Analysis of whole-genome microarray replicates using mixed models. *Bioinformatics* 19, 53–61
20 Gershon, D. (2004) Microarrays go mainstream. *Nat Methods* 1, 263–269
21 Holloway, A.J. *et al*. (2002) Options available–from start to finish–for obtaining data from DNA microarrays II. *Nat. Genet.* 32(Suppl.), 481–489
22 Hegde, P. *et al*. (2000) A concise guide to cDNA microarray analysis. *Biotechniques* 29, 548-550, 552-544, 556
23 McQuain, M.K. *et al*. (2003) Effects of relative humidity and buffer additives on the contact printing of microarrays by quill pins. *Anal. Biochem.* 320, 281–291
24 Diehl, F. *et al*. (2001) Manufacturing DNA microarrays of high spot homogeneity and reduced background signal. *Nucleic Acids Res.* 29, E38
25 Mary-Huard, T. *et al*. (2004) Spotting effect in microarray experiments. *BMC Bioinformatics* 5, 63
26 Liang, M. *et al*. (2003) Quantitative assessment of the importance of dye switching and biological replication in cDNA microarray studies. *Physiol. Genomics* 14, 199–207
27 Balagurunathan, Y. *et al*. (2002) Simulation of cDNA microarrays via a parameterized random signal model. *J. Biomed. Opt.* 7, 507–523
28 Kreil, D.P. *et al*. (2003) Quantitative microarray spot profile optimization: a systematic evaluation of buffer/slide combinations. In *German Conference in Bioinformatics*, GCB 2003, (Mewes, H-W, Frishman, D., Heun, V. and Kramer, S. eds.) v.I, 77–81
29 Finkelstein, D. *et al*. (2002) Microarray data quality analysis: lessons from the AFGC project. *Arabidopsis* Functional Genomics Consortium. *Plant Mol. Biol.* 48, 119–131
30 Tseng, G.C. *et al*. (2001) Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. *Nucleic Acids Res.* 29, 2549–2557
31 Raffelsberger, W. *et al*. (2002) Quality indicators increase the reliability of microarray data. *Genomics* 80, 385–394
32 Franssen-van Hal, N.L. *et al*. (2002) Factors influencing cDNA microarray hybridization on silylated glass slides. *Anal. Biochem.* 308, 5–17
33 Wrobel, G. *et al*. (2003) Optimization of high-density cDNA-microarray protocols by 'design of experiments'. *Nucleic Acids Res.* 31, e67
34 Schena, M. *et al*. (1996) Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc. Natl. Acad. Sci. U. S. A.* 93, 10614–10619
35 Suchyta, S.P. *et al*. (2003) Bovine mammary gene expression profiling using a cDNA microarray enhanced for mammary-specific transcripts. *Physiol. Genomics* 16, 8–18
36 Zaigler, A. *et al*. (2003) Construction and usage of a onefold-coverage shotgun DNA microarray to characterize the metabolism of the archaeon *Haloferax volcanii*. *Mol. Microbiol.* 48, 1089–1105
37 Knight, J. (2001) When the chips are down. *Nature* 410, 860–861
38 Halgren, R.G. *et al*. (2001) Assessment of clone identity and sequence fidelity for 1189 IMAGE cDNA clones. *Nucleic Acids Res.* 29, 582–588
39 Taylor, E. *et al*. (2001) Sequence verification as quality-control step for production of cDNA microarrays. *Biotechniques* 31, 62–65
40 Renn, S.C. *et al*. (2004) Biologically meaningful expression profiling across species using heterologous hybridization to a cDNA microarray. *BMC Genomics* 5, 42
41 Duggan, D.J. *et al*. (1999) Expression profiling using cDNA microarrays. *Nat. Genet.* 21(1 Suppl.), 10–14
42 Relogio, A. *et al*. (2002) Optimization of oligonucleotide-based DNA microarrays. *Nucleic Acids Res.* 30, e51
43 Wang, Z. *et al*. (2004) Identification and utilization of inter-species conserved (ISC) probesets on Affymetrix human GeneChip platforms for the optimization of the assessment of expression patterns in non human primate (NHP) samples. *BMC Bioinformatics* 5, 165
44 Ji, W. *et al*. (2004) A method for cross-species gene expression analysis with high-density oligonucleotide arrays. *Nucleic Acids Res.* 32, e93
45 Kane, M.D. *et al*. (2000) Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. *Nucleic Acids Res.* 28, 4552–4557
46 Chou, C.C. *et al*. (2004) Optimization of probe length and the number of probes per gene for optimal microarray analysis of gene expression. *Nucleic Acids Res.* 32, e99
47 Rouillard, J.M. *et al*. (2003) OligoArray 2.0: design of oligonucleotide probes for DNA microarrays using a thermodynamic approach. *Nucleic Acids Res.* 31, 3057–3062
48 Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31, 3406–3415
49 Wright, M.A. and Church, G.M. (2002) An open-source oligomicroarray standard for human and mouse. *Nat. Biotechnol.* 20, 1082–1083
50 Carre, A. *et al*. (2003) Molecular interactions between DNA and an aminated glass substrate. *J. Colloid Interface Sci.* 260, 49–55
51 Taylor, S. *et al*. (2003) Impact of surface chemistry and blocking strategies on DNA microarrays. *Nucleic Acids Res.* 31, e87
52 Zammatteo, N. *et al*. (2000) Comparison between different strategies of covalent attachment of DNA to glass surfaces to build DNA microarrays. *Anal. Biochem.* 280, 143–150
53 Peterson, A.W. *et al*. (2001) The effect of surface probe density on DNA hybridization. *Nucleic Acids Res.* 29, 5163–5168
54 Kimura, N. *et al*. (2004) Attachment of oligonucleotide probes to poly carbodiimide-coated glass for microarray applications. *Nucleic Acids Res.* 32, e68
55 Call, D.R. *et al*. (2001) Fabrication of DNA microarrays using unmodified oligonucleotide probes. *Biotechniques* 30, 368-372, 374, 376
56 Chiu, S.K. *et al*. (2003) Synergistic effects of epoxy- and amine-silanes on microarray DNA immobilization and hybridization. *Biochem. J.* 374, 625–632
57 Le Berre, V. *et al*. (2003) Dendrimeric coating of glass slides for sensitive DNA microarrays analysis. *Nucleic Acids Res.* 31, e88
58 Pirri, G. *et al*. (2004) Characterization of a polymeric adsorbed coating for DNA microarray glass slides. *Anal. Chem.* 76, 1352–1358

59 Guo, Z. *et al*. (1994) Direct fluorescence analysis of genetic polymorphisms by hybridization with oligonucleotide arrays on glass supports. *Nucleic Acids Res.* 22, 5456–5465
60 Shchepinov, M.S. *et al*. (1997) Steric factors influencing hybridisation of nucleic acids to oligonucleotide arrays. *Nucleic Acids Res.* 25, 1155–1161
61 Ramakrishnan, R. *et al*. (2002) An assessment of Motorola CodeLink microarray performance for gene expression profiling applications. *Nucleic Acids Res.* 30, e30
62 Dorris, D.R. *et al*. (2003) Oligodeoxyribonucleotide probe accessibility on a three-dimensional DNA microarray surface and the effect of hybridization time on the accuracy of expression ratios. *BMC Biotechnol.* 3, 6
63 Rickman, D.S. *et al*. (2003) Optimizing spotting solutions for increased reproducibility of cDNA microarrays. *Nucleic Acids Res.* 31, e109
64 Calevro, F. *et al*. (2004) Assessment of 35mer amino-modified oligonucleotide based microarray with bacterial samples. *J. Microbiol. Methods* 57, 207–218
65 Tran, P.H. *et al*. (2002) Microarray optimizations: increasing spot accuracy and automated identification of true microarray signals. *Nucleic Acids Res.* 30, e54
66 Reese, M.O. *et al*. (2003) Microfabricated fountain pens for high-density DNA arrays. *Genome Res.* 13, 2348–2352
67 George, R.A. *et al*. (2001) Ceramic capillaries for use in microarray fabrication. *Genome Res.* 11, 1780–1783
68 Fan, J. *et al*. (2004) Normalization and analysis of cDNA microarrays using within-array replications applied to neuroblastoma cell response to a cytokine. *Proc. Natl. Acad. Sci. U. S. A.* 101, 1135–1140
69 Yue, H. *et al*. (2001) An evaluation of the performance of cDNA microarrays for detecting changes in global mRNA expression. *Nucleic Acids Res.* 29, E41–41
70 Guo, Z. *et al*. (2002) Oligonucleotide arrays for high-throughput SNPs detection in the MHC class I genes: HLA-B as a model system. *Genome Res.* 12, 447–457
71 Khitrov, G. (2001) Use of inexpensive dyes to calibrate and adjust your microarray printer. *Biotechniques* 30, 748
72 Stoeckert, C.J., Jr. *et al*. (2002) Microarray databases: standards and ontologies. *Nat. Genet.* 32(Suppl.), 469–473
73 Shearstone, J.R. *et al*. (2002) Nondestructive quality control for microarray production. *Biotechniques* 32, 1051–1052, 1054, 1056-1057
74 Hessner, M.J. *et al*. (2004) Utilization of a labeled tracking oligonucleotide for visualization and quality control of spotted 70-mer arrays. *BMC Genomics* 5, 12
75 Forster, T. *et al*. (2004) Triple-target microarray experiments: a novel experimental strategy. *BMC Genomics* 5, 13
76 Saal, L.H. *et al*. (2002) BioArray Software Environment (BASE): a platform for comprehensive management and analysis of microarray data. *Genome Biol* 3, SOFTWARE0003
77 Brazma, A. *et al*. (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat. Genet.* 29, 365–371
78 Harris, M.A. *et al*. (2004) The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Res.* 32, D258–D261
79 Rocca-Serra, P. *et al*. (2003) ArrayExpress: a public database of gene expression data at EBI. *C. R. Biol.* 326, 1075–1078
80 Lin, X. *et al*. (2003) Making sense of human lung carcinomas gene expression data: integration and analysis of two Affymetrix platform experiments. In *Methods of Microarray Data Analysis* IV (Shoemaker, J.S. and Lin, S.L. eds.) Springer