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Quality control of chip manufacture and chip analysis using epoxy-chips as a model

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Abstract

Biochips are miniaturized, highly ordered analysis systems which offer the unique advantage of highly parallel analysis of thousands of analytes at the same time. Although this technique has been enthusiastically developed and has promised to improve and speed up numerous biological assays, the quality control of chip manufacture, chip analysis and data management has received less attention.

The following article compares three epoxy-containing chip surfaces (ARChip Epoxy, 3D-Link™, and EasySpot) with respect to their autofluorescence, immobilization capacity, background fluorescence and hybridization efficiency. Since data collected from biochip experiments are random snapshots with errors, inherently noisy and incomplete, we tried to evaluate technical factors causing variability and to set up quality control procedures for chip manufacture and chip analysis. Variabilities caused by arraying, glass substrate and polymer coating, fluorescent label and experimental conditions are discussed in details.

Keywords: Biochip; Microarray; Quality control; Epoxy

1. Introduction

Biochip technology has been revolutionizing biological and biochemical analysis [1–7]. The major advantage of biochips over conventional biological techniques is the highly parallel, addressable, miniaturized array format which allows simultaneous detection of different targets, virtual automation and functional integration for high throughput screening. Biochips consist of a highly ordered grid of biomolecular probes immobilized on a solid support, such as glass, coated with a reactive polymer. The arrays of biomolecular probes are either formed by in situ light-directed combinatorial synthesis or by spotting pre-synthesized elements.

In low- and medium-density chips pre-synthesized elements are used. Depending on the size of the probe covalent or electrostatic immobilization is preferred. In general, oligonucleotides and DNA fragments of approximately 20–70 bases are amino-modified and bound covalently to the chip surface. Complete or partial complementary DNA of up to 5000 nucleotide bases is bound to the chip by electrostatic adsorption. In order to covalently attach oligonucleotides to the chip pre-activated or surface-modified solid supports, homo- or hetero-bifunctional crosslinkers and modified oligonucleotides are needed [8].

Commercially available glass chips provide reactive aldehyde-, amino-, mercapto- or epoxy-groups for covalent binding of DNA. Chips for non-covalent immobilization use nylon, poly-L-lysine and nitrocellulose as immobilization matrix, interfacial streptavidin–biotin layers or 3D link hydrogels, such as polyacrylamide.

An alternative method is the covalent attachment of disulfide-modified oligonucleotides to 3-mercaptopropyl silane-modified surfaces via a thiol/disulfide exchange reaction, which allows array densities of about 20,000 spots/cm^2. The results show that the hybridization efficiency was directly related to the probe attachment density [9]. The covalent and directed immobilization of DNA on glass type oxidic surfaces is described in [10]. A characteristic feature of the protocol is the deposition of DNA-droplets on a heated surface which results in a more efficient coupling reaction (150–300 fmol/mm^2). Lindroos et al. [11] compared six different commercially available slides with respect to the fluorescence background, the efficiency of the attachment reaction, and the signal-to-noise ratio. Dolan et al. [12] reported on diazotized chip surfaces for the immobilization of unmodified oligonucleotides. The authors showed that the developed *p*-aminophenyltrimethoxysilane (ATMS)/diazotization chemistry was superior over commercial poly-L-lysine

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and silylated slides with regard to probe concentration and fluorescence background. When using polyamidoamine (PAMAM) dendrimers containing 64 primary amino groups as linkers, the signal intensity could be increased significantly compared with amino- and epoxy-silanized surfaces [13]. Zhao et al. [14] described the efficient binding of oligodeoxyribonucleotides modified with multiple phosphorothioate moieties to bromoacetamidosilane-coated slides.

As obvious from the cited literature, the sensitivity and fluorescence background of biochips critically rely on the effective immobilization of biomolecular probes on the chip. As a matter of different surface chemistries probe concentration, probe length and print buffer need to be optimized for a high quality chip performance. Slide autofluorescence, reproducibility of arraying, spot morphology, binding efficiency and probe purification are also crucial for the accuracy and reliability of chip analysis data.

In the present paper, we compare the ARChip Epoxy to two competitors (3D-Link™ and EasySpot) with respect to the slide autofluorescence, immobilization capacity, background fluorescence, spacer length and signal-to-noise ratio. We discuss several technical factors that cause variability disguising actual differences in signal intensities. Because there are no standards available yet in chip technology, the chip surfaces are qualified by hybridization assays performed with the same target, and under the same hybridization and scanning conditions.

2. Experimental

2.1. Materials

Glass slides (25 mm × 75 mm) and Silane-Prep™ slides were purchased from Sigma. 3D-Link™ slides (competitor 1) were obtained from Motorola. SuperClean substrates, EasySpot™ slides (competitor 2), and epoxy resin were received from TeleChem, BioCat and Shell Chemicals, respectively.

Amino-modified Alf1b (5′-CGTTCGYTCTGAGCCAG-3′) modified with C6-, C12- and dT-linkers of various length was ordered from VBC-Genomics.

2.2. Manufacture of ARChip Epoxy

Pre-cleaned glass slides, Silane-Prep™ slides, and SuperClean substrates were coated with 1% epoxy resin in methylethylketone, acetone and toluene under nitrogen using a quadruple film applicator, model 360 from Erichsen (Bickel & Wolf). Amino-modified oligonucleotide probes were spotted in triplicate in 350 pl volumes onto the chip using a piezoelectric biochip arrayer (Packard BioScience).

2.3. Post-arraying and blocking

The ARChip Epoxy, competitors 1 and 2 were placed in a humid chamber at 37 °C overnight. The surface blocking was performed with 50 mM ethanolamine, 0.2 M Tris, pH 9 and 0.1% lauryl sulfate (SDS) for 10 min. After alternating wash in distilled water and in 4 × SSC (0.6 M sodium chloride, 0.06 M sodium citrate)/0.1% SDS, the slides were blow-dried and ready for hybridization.

2.4. Hybridization

Total 16S rDNA sequences were amplified using an initial denaturing step of 5 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 1 min annealing at 52 °C and 1 min extension at 74 °C. The PCR-reaction mixtures (50 μl) contained 5 μl reaction buffer (Gibco, BRL), 200 μM dNTPs, 0.15 μM primers 1520r and Cy5-labelled fD1 (MWG-Biotech, Germany)/or Dy630-labelled fD1 (kindly provided by Dyomics), 3 mM $MgCl_2$ and 2.5 U Taq DNA polymerase (Gibco, BRL) and 1 μl extracted DNA. The amplification products were confirmed by gel electrophoresis on 1% agarose gels. A 2 ng/μl Cy5- or Dy630-labeled Rhizobium fredii in 20 mM Tris, pH 7.4, 0.9 M NaCl, 0.01% SDS and 20% (v/v) formamide was denatured at 95 °C for 5 min, snap cooled on ice and hybridized to the chip at 50 °C for 3 h. At positions on the array where the immobilized probe recognized the fluorescent labeled complementary target, hybridization occurred which was detected with a Genepix™ 4000B from Axon Instruments.

3. Results and discussion

3.1. Autofluorescence and fluorescence background

The ARChip Epoxy was coated with 1% epoxy resin in methyethylketone (MEK), acetone and toluene. Because the autofluorescence and the reproducibility of autofluorescence were best for epoxy resin dissolved in methylethylketone—the mean autofluorescence measured at 100% laser power and 100% photomultiplier tube (PMT) was 749 ± 61 for MEK, 1294 ± 198 for acetone, and 1452 ± 225 for toluene, respectively—MEK was used as solvent for chip manufacture. For the printing of high quality arrays with improved sensitivity the slide coating needs to be uniform and the autofluorescence as low as possible at $\lambda_{ex} = 635$ nm (Cy5 channel) and $\lambda_{ex} = 532$ nm (Cy3 channel). Fig. 1 shows the autofluorescence of ARChip Epoxy 1–3, manufactured on pre-cleaned glass (ARChip 1), Silane-Prep™ slides (ARChip 2), and SuperClean substrates (ARChip 3), furthermore, competitors 1 and 2, both containing a reactive epoxy-group for immobilization of biomolecular probes. For each type of slide, three slides containing an array of 144 virtual spots were scanned, both before arraying (Fig. 1(a)) and after blocking (Fig. 1(b)). As is obvious from Fig. 1(a) the choice of glass substrate is critical for a low and reproducible autofluorescence. The autofluorescence of the tested slides was significantly increased at $\lambda_{ex} = 532$ nm except for ARChip 3 and competitor 1. The autofluorescence of