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A bridge-like solution for Universal Microarray applications

Giorgio Ventimiglia *,[1], Enrico Alessi [1], Salvatore Petralia [1]

STMicroelectronics–AMG (Analog, MEMS Group), Stradale Primosole 50, 95121 Catania, Italy

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ABSTRACT

In this work, we provided the evidence of an alternative DNA detection methodology, in the DNA microarray domain, based on a Universal Microarray approach. Despite the classic hybridization of an oligonucleotide immobilized onto a proper surface with a DNA target extracted from a biological sample and amplified by a PCR reaction, here we reported an alternative method based on the usage of generic oligonucleotides microarray sequences, coupled to special probes contained in the hybridization buffer, at end user level. These special probes present one region complementary to the oligonucleotide on the surface (microarray) and the other part recognizing a certain DNA target sequence. These special probes act like a bridge connection and the final hybridization leads to a three-units complex formation. We demonstrated that the hybridization occurs, and therefore the DNA target recognition, although with less efficiency, that is reasonable because of the thermodynamics constrains in the formation of the unstable three-units complex. However, signal detection is still comparable with standard hybridization.

1. Introduction

Microarrays consist of ordered probes in array format, commonly including nucleic acids, proteins, polymers, carbohydrates and other suitable substances, for screening and diagnostic purpose. The probes, covalently attached or physisorbed onto a proper surface of technological interest (glass, quartz, silicon, ITO, plastic, textile, etc.), are used to recognize biological targets by means of a coupling reaction. This recognition process, named hybridization for nucleic acids applications, occurs thanks to the complementarity between some sequences contained in the probes anchored onto the surface and specific regions present in the biological target. The hybridization takes place by hydrogen bond formation between adenine and thymine, from one side, and guanine and cytosine from the other when using DNA (with RNA, thymine is replaced by uracyl). The detection of the hybridization can be optical [1,2] electrochemical [2,3] or using devices sensitive to the mass [4,5].

Depending on the application, the biological target can be amplified by means of the PCR (Polymerase Chain Reaction). The microarray detection module can be integrated with other technological modules (microfluidic, microelectronic, and optical) into modern integrated devices to build up miniaturized labs, usually called Lab-on-Chips [6].

Today, the common strategy for microarray production is to print [7,8] customized microarrays whose content depends on the specific bioassay [9]. However, this approach can be expensive and time consuming because it requires, for any new product or modification of an existing product, a new probe design and production versioning, thus affecting the manufacturing cycle time. To overcome this problem, researchers have focused on Universal Microarray-like approach. It consists on the printing of generic microarray layout suitable for different bio assays. The first real advantage to use the Universal Microarray approach, from a microarrays manufacturer point of view, is to handle a single probes set, not linked to any bio-assay specific content, simplifying the manufacturing line and reducing the cycle time, with costs saving. The second advantage is to offer the microarray technology as an open system, enabling the end user to apply it for various bio assays. The concept of Universal Microarray is present in the state of the art since 1999 and several papers have been written [10–15]. However, the Universal Microarray approach did not get success in the story of microarrays because mainly applied to Gene Expression Microarrays and not to the emerging market of diagnostic microarrays aimed to the detection of few targets per single test. For example, some articles report the application of Universal Microarray in conjunction to PCR (Polimerase Chain Reaction) and LDR (Ligase Detection Reaction), while others cover the concept of species non-specific universal array and refer to the printing of all the DNA sequences in combinations/permutations with a certain

* Corresponding author.
E-mail addresses: giorgio.ventimiglia@st.com (G. Ventimiglia), salvatore.petralia@st.com (S. Petralia).
[1] The authors equally contributed.

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	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	col 8	Col 9	col 10	Col 11	Col 12	Col 13	Col 14	Col 15	Col 16	Col 17	Col 18	Col 19	Col 20	Col 21
row 1	ST1	BG1	ST1	empty	BG2	N-BG1	empty	N-BG2	BG3	BG1	empty	BG1	BG3	N-BG2	empty	N-BG1	BG2	empty	ST1	BG1	ST1
row 2	ST1	empty	BG2	N-BG1	empty	N-BG2	BG3	empty	BG1	ST1	N-BG3	ST1	BG1	empty	BG3	N-BG2	empty	N-BG1	BG2	empty	ST1
row 3	empty	BG2	N-BG1	empty	N-BG2	BG3	empty	BG1	ST1	BG2	empty	BG2	ST1	BG1	empty	BG3	N-BG2	empty	N-BG1	BG2	empty
row 4	N-BG1	empty	N-BG2	BG3	empty	BG1	ST1	empty	BG2	N-BG1	N-BG3	N-BG1	BG2	empty	ST1	BG1	empty	BG3	N-BG2	empty	N-BG1
row 5	N-BG2	BG3	empty	BG1	ST1	empty	BG2	N-BG1	empty	BG3	empty	BG3	empty	N-BG1	BG2	empty	ST1	BG1	empty	BG3	N-BG2
row 6	ST1	N-BG2	BG3	empty	BG1	ST1	empty	BG2	N-BG1	N-BG2	empty	N-BG2	N-BG1	BG2	empty	ST1	BG1	empty	BG3	N-BG2	ST1

Fig. 1. Microarray layout. ST1 is an internal reference for microarray hybridization process verification (fiducial marks): it is a perfect match sequence of a gene of *Arabidopsis Thaliana*. BGs probes are three different perfect-match sequences for the direct recognition of the sequences of HBB gene. N-BGs probes are three different perfect- match sequences complementary to the customized key-probes.

Fig. 2. *In-Check* platform. Composed by a Thermal Control System (TCS) (on the left) and an optical reader (on the right) based on a CCD camera acquisition system. In the inset is reported the LoC device.

length onto a microarray. In particular, zip-code approach is popular but this method needs to change the upstream PCR in order to have DNA sequences suitable for binding the universal probes.

The solution here reported is intended to overcome the above limitations. In particular, the novelty relies in the transferring of the customization content from the microarray level to the reagents kit level. Although the complexity in probe design is still present, from the point of view of microarrays manufacturers the advantage is relevant, because the substrate can be univocally finished with a single set of probes, independently from the final application. Based on this approach, it was conceptualized a universal chip based approach consisting on (i) the use of a generic microarray, (ii) special DNA connectors (named key-probes) added at the end point (end-user level) before the microarray hybridization step, to create a bridge-like solution, between a fluorescent nucleic acid fragment (extracted from a biological sample) to the general sequence of the probe attached to the surface. If compared to the standard two-strands hybridization, data here reported show an acceptable performance for this three-molecules hybridization complex. The experiments were performed using the LoC (Lab-on-Chip) device manufactured by STMicroelectronics, actuated and analyzed using the In-Check platform (developed by STMicroelectronics).

2. Materials and methods

2.1. Chemicals

The following reagents were used for this study. Hydrogen peroxide (29%) ammonium hydroxide (25%) hydrochloridric acid (37%) and methanol for wet processes were purchased by Sigma Aldrich and were used as received. Anhydrous toluene and glycidoxy-propril-trimethoxy-silane (GOPS) for silanization step were purchased by Sigma Aldrich and were used as received. Chemicals for passivation steps: Sodium Dodecyl Sulfate, Sodium Citrate

Table 1
Probe sequences.

Probe Code	Probe sequence 5′–3′ (5′ amino modifier C6)
ST1	AGTGAGGGAGGAGATGGAACCATCT
N-BG1	CACAACACAAGTACCTGACATGGCG
N-BG2	TGGTCTTCTTAAAAGATTAGTAGGT
N-BG3	GGCAAAGGAGCTGCTAAGGGATTTC
BG1	GCAGAGCCATCTATTGCTTAC
BG2	CTAGGGTTGGCCAATCTACTC
BG3	CATCACTTAGACCTCACCCTG
Empty	–

from Sigma Aldrich and were used as received. Bovine serum albumin (BSA) fraction V has been purchased from Euroclone and was used as received. All DNA oligonucleotides were purchased from MWG Biotech, probes for grafting were desalted while Cy5-labeled target (perfect match) were HPLC purified.

2.2. Substrate

The Lab-on-Chip (LoC) device (part of In-Check platform provided by STMicroelectronics), was used as substrate for microarray printing. It consists of a MEMS silicon chip mounted onto a PCB board featured with electrical pads for interfacing with actuating equipment that are part of the platform. The active surface suitable for the printing of the microarray is silicon based and covered by amorphous silicon oxide film, activated and silanized to anchor aminomodified oligonucleotides probes ranging from 15 to 25 mer.

2.3. Surface cleaning and activation

Substrate cleaning and activation processes: silicon oxide surface was cleaned by means of a solution composed by H_2O:NH_4OH:H_2O_2 at 80 °C, for few minutes in a Teflon tank, rinsed by deionized water and dried by nitrogen flow; the activation step was carried out using an acid solution H_2O:HCl at room temperature, rinsed by deionized water and dried by nitrogen flow.

2.4. Substrate silanization

The activated silicon oxide surface was dried by curing and silanized, in nitrogen atmosphere, by immersing the substrates in GOPS in anhydrous toluene solution. Silanized substrates were rinsed in anhydrous toluene and dried in vacuum chamber. Substrates were then cured to stabilize the epoxysilane coating. Silanized substrates were stored in vacuum condition until the oligonucleotides printing.

2.5. Oligonucleotides printing on surface

A high throughput customized piezo spotter has been used to deposit micro drops of about 200 pL, containing amino-modified oligonucleotides at concentration 10 μM, in phosphate buffer. After