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Magnetic scanometric DNA microarray detection of methyl tertiary butyl ether degrading bacteria for environmental monitoring

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ABSTRACT

A magnetoresistive biosensing platform based on a single magnetic tunnel junction (MTJ) scanning probe and DNA microarrays labeled with magnetic particles has been developed to provide an inexpensive, sensitive and reliable detection of DNA. The biosensing platform was demonstrated on a DNA microarray assay for quantifying bacteria capable of degrading methyl tertiary butyl ether (MTBE), where concentrations as low as 10 pM were detectable. Synthetic probe bacterial DNA was immobilized on a microarray glass slide surface, hybridized with the 48 base pair long biotinylated target DNA and subsequently incubated with streptavidin-coated 2.8 μm diameter magnetic particles. The biosensing platform then makes use of a micron-sized MTJ sensor that was raster scanned across a 3 mm by 5 mm glass slide area to capture the stray magnetic field from the tagged DNA and extract two dimensional magnetic field images of the microarray. The magnetic field output is then averaged over each 100 μm diameter DNA array spot to extract the magnetic spot intensity, analogous to the fluorescence spot intensity used in conventional optical scanners. The magnetic scanning result is compared with results from a commercial laser scanner and particle coverage optical counting to demonstrate the dynamic range and linear sensitivity of the biosensing platform as a potentially inexpensive, sensitive and portable alternative for DNA microarray detection for field applications.

1. Introduction

Sequence-selective DNA detection has become an increasingly important tool used in understanding molecular biology and unraveling the genetic basis of disease. By employing DNA microarrays in a highly multiplex and parallel format, the arrays and its accompanying imaging platform enable the high throughput biological detection required in areas such as medical diagnostics (Clarke et al., 2001; Heller, 2002), drug discovery (Chin and Kong, 2002) and environmental monitoring (Loy et al., 2002). DNA and protein microarrays represent two of the best examples of how microfabrication technology enables hybridization and detection to be carried out in microminiaturized, highly parallel formats.

The gold standard in DNA microarray technology is the fluorescence based solid-phase assay format. Although hampered by the need for sophisticated fluorescence microscopes/scanners as well as strongly environment-dependent quantum yields of the fluorescent tags, no other scheme for readout is likely to supersede fluorescence detection for standard use in centralized bulk laboratory facilities. However, the current instrumentation has limitations in both flexibility and portability, two important factors for the assay and sensing platform to be deployed in field applications.

Other assay formats have been developed based on either label-free methodologies (Anderson et al., 2008; Piscevic et al., 1995) or using other types of labels such as gold nanoparticles (Reichert et al., 2000; Taton et al., 2000), quantum dots (Gerion et al., 2003) and magnetic particles (Baselt et al., 1998). Although the label-free approach is attractive for its simple operating protocol that eliminates undesirable effects such as steric impediments and instabilities of the labels, the signal detection mechanism is more complicated. Since both target and probe are of the same nature, and often both contribute to the signal, incremental changes due to binding or hybridization events are extremely difficult to sense. On the other hand, magnetic labels have many advantageous characteristics such as robustness, non-toxicity and stable properties over time. The ability to manipulate these particles with on-chip or external magnetic fields (Graham et al., 2005; Wirix-Speetjens et al., 2007), together with the absence of magnetic background in most biological materials, make magnetic particles labeling an extremely promising approach.

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Biosensors using highly sensitive magnetic sensor technology are among the most sensitive and amenable to miniaturization. Biosensor chips based on magnetic sensor arrays have been proposed to create easy-to-use portable lab-on-a-chip devices that are sensitive, versatile and easily integrated with standard silicon integrated-circuit technology. In a typical magnetic array chip, underneath each magnetically labeled DNA spot sits a magnetoresistive (MR) sensor, using either giant magnetoresistive (GMR), spin valves or tunnel junction sensor designs (Ferreira et al., 2003; Megens and Prins, 2005; Rife et al., 2003; Shen et al., 2008). Here, the number of sensors and DNA spots are equal; an array format containing 10^3 DNA spots will thus require 10^3 sensors for a complete analysis. This increases the cost and complexity of the biochip and introduces many technical challenges in designing the biochip for efficient multiplexing. In addition, a good passivation layer between the sensor and the biological solutions is required to ensure sensor integrity and prevent spurious signals due to contamination of the sensor surface, while stringent washing processes are needed in order to reuse the expensive sensor substrate (Schotter et al., 2004). Moreover, errors due to sensor offset drift occurring during the hybridization or washing process complicates the discrimination of true signals even when reference sensors are present on the array (Graham et al., 2004; Xu et al., 2008).

In this paper we describe a different biosensing platform that combines the advantages of stable magnetic labels and highly sensitive MR sensor in a scanning probe format similar to that of a hard disk drive. The biosensing platform is comprised of reusable magnetic "reader" unit and low-cost disposable assay substrates printed with DNA probes and labeled with magnetic tags. The reader consists of a single mechanically scanned MR sensor and associated readout electronics, while the passive, disposable substrate retains the use of the standard glass microscope slide used in conventional fluorescence based assays. By using a single micron-sized sensor to scan across the whole glass slide, large assay areas can be imaged with high spatial resolution. Moreover, the same sensor can be applied to different assays by changing just the disposable substrate without the need to expose the sensor to any biochemical or washing solutions. This platform aims to demonstrate the potential of using small sensitive sensors in a scanning format resembling a hard disk drive to develop a portable biosensing platform for on site environmental monitoring.

In this approach, a true magnetic measurement of the DNA array is captured, free from sensor offset errors since the same sensor images both the magnetically labeled spot and the label-free background. This sensing platform is used to quantify *Methylibium petroleiphilum PM1* bacteria, an organism that is naturally present at aquifers contaminated with *methyl tert-butyl ether* (MTBE) and have been linked to the biodegradation of MTBE (Hristova et al., 2003).

Table 1
PM1 bacterial 16S rDNA sequences used in microarray experiments. The sequence in the target that is complementary to the probe is in boldface. Mismatched base pairs are underlined, while the 5′ end of targets used for magnetic labeling and fluorescent labeling is in italic.

Oligonucleotide	Sequence
Probe	5′-NH2-**ACA CGA GCT GAC GAC GGC CATG**-3′
Perfect match target (Magnetic)	3′-TTGTAGAGTGC **TGT GCT CGA CTG CTG CCG GTA C** GTCGTGGACACAAGA-*biotin*-5′
Perfect match target (Fluorescent)	3′-TTGTAGAGTGC **TGT GCT CGA CTG CTG CCG GTA C** GTCGTGGACACAAGA-*Cy3*-5′
2 Basepairs mismatch target	3′-TTGTAGAGTGC **TGT GCT CCA CTG CTC CCG GTA C** GTCGTGCACACAAGA-*biotin*-5′

2. Materials and methods

2.1. Oligonucleotide probe design

Linear DNA oligoprobes were designed based on the *M. petroleiphilum* PM1 16S rDNA gene sequence. Table 1 shows the single stranded DNA sequences for both the commercially synthesized 22-mer oligonucleotide probe and 48-mer complementary target. All DNA sequences were purchased from Integrated DNA Technologies (IDT, IL). The probes were amino modified at the 5′ end to enable covalent immobilization of probes onto a solid support. The targets used for fluorescent labeling were tagged with a cyanine 3 (Cy3) fluorophore, while targets for magnetic labeling had a biotinylated end which serves as the interaction point with the streptavidin coated magnetic particles. A C–C mismatch was inserted into the middle of the sequence at two locations to create a 2 base pair mismatch target.

2.2. Surface functionalization and spotting

Epoxysilane glass slides, Nexterion® E (Schott, NY) were used as the base substrate for DNA microarrays. The epoxysilane coating serves as a uniform surface for biomolecule immobilization via the covalent interaction between epoxide end groups in the coating and nucleophilic groups on the DNA probe, as illustrated in Fig. 1a.

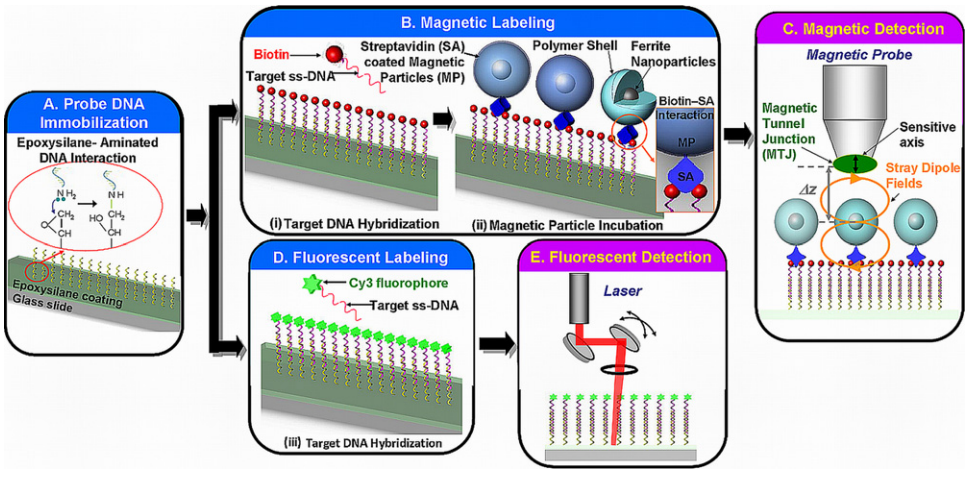

Fig. 1. Bioassay protocol for magnetic and fluorescent labeled DNA microarrays. (A) The oligonucleotide probe was immobilized on epoxysilane glass slides. The microarrays were subjected to labeling with either (B) magnetic particles that involved a two-step process of: (i) hybridization with biotinylated target DNA and (ii) incubation with streptavidin coated magnetic particles, or (D) Cy3 fluorophore conjugated target DNA. The DNA duplex structure was then scanned using (C) a magnetic tunnel junction (MTJ) probe close to the magnetic particles for the magnetically labeled microarray or (E) the commercial laser scanner for the fluorescently labeled microarrays.