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# Electrochemical sensor for multiplex screening of genetically modified DNA: Identification of biotech crops by logic-based biomolecular analysis

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ABSTRACT

Genetically modified (GM) technique, one of the modern biomolecular engineering technologies, has been deemed as profitable strategy to fight against global starvation. Yet rapid and reliable analytical method is deficient to evaluate the quality and potential risk of such resulting GM products. We herein present a biomolecular analytical system constructed with distinct biochemical activities to expedite the computational detection of genetically modified organisms (GMOs). The computational mechanism provides an alternative to the complex procedures commonly involved in the screening of GMOs. Given that the bioanalytical system is capable of processing promoter, coding and species genes, affirmative interpretations succeed to identify specified GM event in terms of both electrochemical and optical fashions. The biomolecular computational assay exhibits detection capability of genetically modified DNA below sub-nanomolar level and is found interference-free by abundant coexistence of non-GM DNA. This bioanalytical system, furthermore, sophisticates in array fashion operating multiplex screening against variable GM events. Such a biomolecular computational assay and biosensor holds great promise for rapid, cost-effective, and high-fidelity screening of GMO.



## 1. Introduction

Genetic modification of crops, fruits, and vegetables has attracted tremendous attention because of its ability to create new genetically altered hybrids offering enhanced productive, ecological, and nutritional value (Hails, 2000; Wolfenbarger and Phifer, 2000), thereby being considered as one of the effective strategies toward alleviating poverty and hunger around the world. Nevertheless, the compatibility of inter-species genes with Nature has not been evaluated systematically (Conner et al., 2003; Dale et al., 2002; Kuiper et al., 2001). The long-term effects of GM foodstuffs on human beings—or, indeed, the global ecology—remain unpredictable. Therefore, reliable assays are urgently required to expedite the quantification of GM materials and to enforce the guidelines for genetically modified organisms (GMOs, so called Biotech crops).

Toward this goal, and in connection with the increasing number and complexity of the overall body of GMOs, event-specific genes—fragments of the unique junction regions between the inserted DNA and the host DNA—have recently been screened for their ability to act as unique identifiers of GMOs (Berdal and Holst-Jensen, 2001; Rønning et al., 2003). Such a technique can also be incorporated with real-time Q-PCR (Permingeat et al., 2002; Querci et al., 2009), microarray (Kim et al., 2010; Leimanis et al., 2006; Xu et al., 2006), and Luminex xMAP (Fantozzi et al., 2008) platform technologies enabling the simultaneous detection of multiple targets/events. Despite significant progress to date, searching an event-specific gene requires cumbersome screening and validation processes, along with complicated procedures (i.e. primer design), consumption of varied substances (i.e. primer, dNTP, and polymerase), and sophisticated instruments (i.e. real-time quantitative PCR).

Logic gates are Boolean functionality-implementing physical devices that perform logic operations from dual or multiple inputs

*Abbreviations:* GMOs, genetically modified organisms; TMB, 3,3′,5,5′-tetramethylbenzidine; Av-GOx, glucose oxidase-labeled avidin; FITC, fluorescein isothiocyanate; α-FITC-HRP, hydrogen peroxidase-labeled anti-FITC antibody.

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to produce a single logic output, thereby allowing the construction of conventional electronic microprocessors. In a similar manner, molecular substrates can be used as inputs for devices that exploit chemical computation (de Silva and Uchiyama, 2007; Elbaz et al., 2009b, 2010; Katz and Privman, 2010). Such molecular logic gates have recently been demonstrated to yield AND, OR, XOR, and NAND functionalities (Andréasson et al., 2011; de Silva and McClenaghan, 2004; Elbaz et al., 2009a, 2009b; Genot et al., 2011; Li et al., 2009; Park et al., 2012; Raymo, 2002; Strack et al., 2008; Willner et al., 2008). Although a substantial body of work has led to the construction of many molecular logic gates, few of these elaborate proof-of-principle concepts have led to the realization of practical systems that can handle the complex requirements of their ultimate applications (Chen et al., 2012; Chuang et al., 2011; Douglas et al., 2012; Halámek et al., 2011; Katz et al., 2012; Konry and Walt, 2009; Margulies and Hamilton, 2009; Melnikov et al., 2010; Xia et al., 2010).

Motivated by need, in this paper, rather than a demonstration of single logic gate configuration that provides single/simple analytical function, we present a biomolecular analytical system with computing functionality mimicking the generation process of an event-specific gene and its feasibility in the analysis of GMOs. This novel, simple, and specific approach provides an alternative means for performing sophisticated multiplex analyses in an array format. For further benefit, this bioanalytical system is endowed with computing functionality that allows decisions to be made rapidly, thereby avoiding potential personal errors and ensuring that analyses of GM events are performed accurately.

## 2. Materials and methods

### 2.1. Reagents and materials

Sodium chloride (NaCl, ≥99%), ethylenediaminetetraacetic acid tetrasodium salt hydrate (EDTA, 99%), hydrochloric acid (HCl, 37%), 3-mercaptopropionic acid (MPA, 99%), casein, bovine serum albumin (BSA), citric acid, and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (St. Louis, MO). Tris (base), potassium phosphate dibasic ($K_2HPO_4$), and potassium phosphate monobasic ($KH_2PO_4$) were obtained from J.T. Baker (Phillipsburg, NJ). HRP-conjugated FITC-antibody (α-FITC-HRP) was acquired from NOVUS Biologicals (Littleton, CO). Avidin-conjugated glucose oxidase (Av-GOx) was obtained from Rockland (Gilbertsville, PA). Synthetic oligonucleotides designed for use in this study are numbered (Table 1) and elaborated in the Supplementary material. All the probes were purchased from either Eurofins-MWG (Ebersberg, Germany) or Protech (Taipei, Taiwan). Deionized water (18 MΩ cm) from a Millipore Direct-Q system (Billerica, MA) was used to prepare all aqueous solutions.

Four buffer solutions were employed in this study: (i) an immobilization buffer (IB, pH 8) containing 10 mM Tris, 1 mM EDTA, and 0.3 M NaCl; (ii) a hybridization buffer (HB, pH 7.2) containing 1 M phosphate buffer and 2.5% BSA; (iii) a binding buffer (BB, pH 7.2) containing 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, 137 mM NaCl, 2.7 mM KCl, and 0.5% casein; and (iv) a washing buffer (WB, pH 7.2), which was the same as BB, but without casein.

### 2.2. Preparation of DNA recognition interfaces on electrode

An aliquot (6 μL) of the capture probe mixture (500 nM thiolated probes **1**, **2**/200 μM DTT in IB) was pipetted over the Au working electrode of the 16-sensor array and left to incubate overnight at 4 °C in humidified atmosphere; it was then washed with deionized water and dried with $N_2$ gas. Subsequently the monolayer-modified Au electrode surface was treated with 1 mM MPA (6 μL; equilibrated in IB) for 1 h at room temperature to yield the DNA recognition interface. Finally, the sensor was washed thoroughly with deionized water and dried under $N_2$.

### 2.3. Assay with DNA hybridization

Desired concentrations of target DNA were incubated with 0.5 μM FITC-conjugated probes (**3**, **4**, and **5**) and 0.5 μM biotinylated probe (**6** and **7**) in HB for 15 min at room temperature to ensure homogeneous hybridization. Aliquots (6 μL) of the hybrid mixture were loaded over the DNA recognition interfaces of Au sensors and incubated for 30 min at room temperature. After a washing with deionized water and drying under $N_2$, the as-prepared sensor was incubated for 30 min with a mixture (6 μL) of α-FITC-HRP (25 μg $mL^{-1}$) and Av-GOx (25 μg $mL^{-1}$) in BB, enabling conjugation of the enzymes required for the enzymatic processing. Finally, the unbound tagged enzymes were washed away with WB, providing a sensor ready for electrochemical examination.

### 2.4. Instrumentation and measurements

A multi-channel CH Instruments (CHI 1030) electrochemical analyzer (Austin, TX) was used for all electrochemical measurements. Analyses of GM materials were performed on a 16-sensor Au electrode array chip obtained from GeneFluidics (Monterey Park, CA); each sensor featured a central Au working electrode (2.5 mm in diameter) surrounded by a quasi-Au reference electrode and an Au auxiliary electrode. To ensure reliable output

**Table 1**
DN A probes and oligonucleotides used in this study.

| No. | Oligonucleotide sequence (5′–3′) | Description |
|---|---|---|
| 1 | GACCACTGTCGGCAGAGGCAT-$(CH_2)_3$-SH | Thiolated capture probe specific for *35 S* promoter |
| 2 | TTGTCGTGTTCCACAGTGGTA-$(CH_2)_3$-SH | Thiolated capture probe specific for *Glb1* promoter |
| 3 | FITC-$(CH_2)_6$-ATGCACTAGCCAGAGGATTTGCG | FITC-tagged probe specific for *CP4-EPSPS* gene |
| 4 | FITC-$(CH_2)_6$-ATGCACTAGGTGTTTGTGGCTCT | FITC-tagged probe specific for *pat* gene |
| 5 | FITC-$(CH_2)_6$-ATGCACTATTGCTACTCCAACGG | FITC-tagged probe specific for *cordapA* gene |
| 6 | Biotin-(TEG)-ATGCACTATCAACAGCGACGACT | Biotin-tagged probe specific for endogenous soybean gene *Lectin* |
| 7 | Biotin-(TEG)-ATGCACTATTGCAGCGCTCTGAC | Biotin-tagged probe specific for endogenous maize gene *SSIIb* |
| 8 | TGCCTCTGCCGACAGTGGTCATGCACGCAAATCCTCTGGCAAGCAAGTCGTCGCTGTTGA | Target sequence from GM event GTS 40-3-2 |
| 9 | ATACCTGATCGTACTGGTATATGCACGCAAATCCTCTGGCAAGCAAGTCGTCGCTGTTGA | Partial complementary to *CP4-EPSPS* gene and *Lectin* |
| 10 | TGCCTCTGCCGACAGTGGTCATGCAAGAGCCACAAACACCAAGCAAGTCGTCGCTGTTGA | Target sequence from GM event A5547-127 |
| 11 | TGCCTCTGCCGACAGTGGTCATGCACGCAAATCCTCTGGCAAGCAGTCAGAGCGCTGCAA | Target sequence from GM event NK603 |
| 12 | TGCCTCTGCCGACAGTGGTCATGCAAGAGCCACAAACACCAAGCAGTCAGAGCGCTGCAA | Target sequence from GM event T25 |
| 13 | ATACCTGATCGTACTGGTATATGCACGCAAATCCTCTGGCAAGCAATACCTGATCGTACG | Partial complementary to *CP4-EPSPS* gene |
| 14 | TGCAGGTTATCTTGGTCTTTTCAACGAAAACGAGTCTGGTGATCAAGTCGTCGCTGTTGA | Target sequence from *Lectin* gene |
| 15 | ACCACTGTGGAACACGACAAATGCACCGTTGGAGTAGCAAAAGCAGTCAGAGCGCTGCAA | Target sequence from GM event LY038 |

signals throughout the investigations, the sensory chip was equipped with a tailor-designed adapter system to facilitate measurements (Fig. S1). Prior to measurements, a polyester film coated with adhesive having a 6-mm-diameter opening was applied on the surface of the chip to create a cavity for sample loading. To perform the chronoamperometric detection of GM materials, 45 μL of TMB/glucose mixture [0.1 mg $mL^{-1}$ TMB and 250 mM glucose in 150 mM phosphate/citrate buffer (pH 5, containing 1% DMSO)] was placed on the sensor surface. After incubation for 2 min, the chronoamperometric experiments were conducted at −100 mV and the signaling current was sampled after 60 s.

## 3. Results and discussion

### 3.1. Sensing principle of biomolecular computational assay

Contrary to the conventional GMO analyses, in which sequential examinations on promoter and coding genes led to uneconomical and inefficient assay, we are approaching a simple and rapid format to offer an effective GMO identification. The principle of the GMO detection is illustrated in Fig. 1. The facile processing of this bioanalytical system relies on integration of three hybridization activities, two affinity recognition events, and concatenated biocatalytic reactions that lead to a resulting output, displayed as the electrochemical transformation of oxidized TMB ($TMB_{ox}$) to reduced TMB ($TMB_{red}$). Hybridization of the immobilized capture probes (**1**) and the targeted segment (**I**) forms the backbone of the **Identity** gate. Concurrently, an **AND** gate leveraging two hybridization events of the targeted segments (**II** and **III**), against FITC-conjugated reporter (**3**) and biotin-conjugated reporter (**6**) probes, respectively, operates in parallel with the **Identity** gate. Subsequently, both glucose oxidase-labeled avidin (Av-GOx) and hydrogen peroxidase-labeled anti-FITC (α-FITC-HRP), bound to corresponding antigens, enable an enzymatic cascade reaction to occur (Wilner et al., 2009a, 2009b). That is, once the substrates (glucose, TMB) are present, GOx catalyzes the oxidation of glucose to produce $H_2O_2$, which is further reduced by HRP along with the

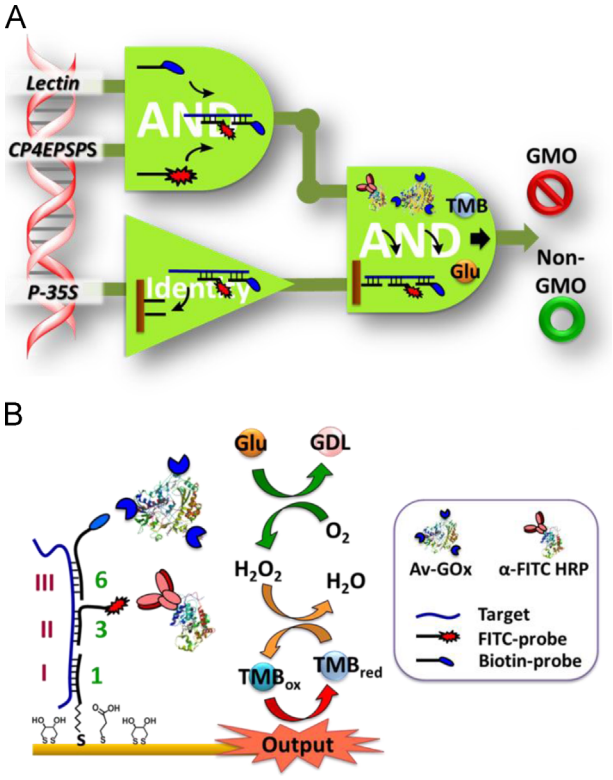
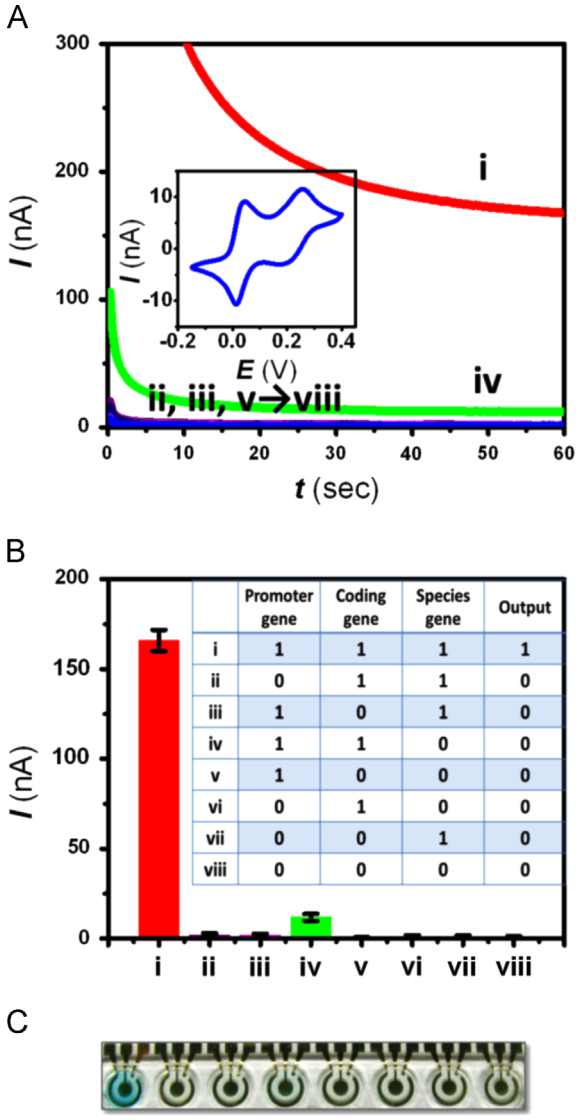



**Fig. 1.** (A) Equivalent circuit computed for GMO analysis. The first AND gate operates in parallel to an identity gate; they are subsequently interplexed to a second AND gate in series to ensure the rapid digital identification of a specific GM event. The cartoons depicted on the gate symbols are the equivalent biochemical activities. (B) Envisioned configuration of biomolecular detection of GMO on the chip, wherein the computing relies on three hybridization reactions, two affinity recognition events, and biocatalytic cascade reactions in connection to a resulting output displayed in terms of the electrochemical transformation of oxidized TMB ($TMB_{ox}$) to reduced TMB ($TMB_{red}$). Right: schematic representations of the various substances involved in the gate.




| | Promoter gene | Coding gene | Species gene | Output |
|---|---|---|---|---|
| i | 1 | 1 | 1 | 1 |
| ii | 0 | 1 | 1 | 0 |
| iii | 1 | 0 | 1 | 0 |
| iv | 1 | 1 | 0 | 0 |
| v | 1 | 0 | 0 | 0 |
| vi | 0 | 1 | 0 | 0 |
| vii | 0 | 0 | 1 | 0 |
| viii | 0 | 0 | 0 | 0 |

**Fig. 2.** (A) Chronoamperometric transients of the bioanalytical system in the presence of eight combinations of inputs [promoter gene, coding gene, and species gene]: (i) (**1**,**1**,**1**), (ii) (**0**,**1**,**1**), (iii) (**1**,**0**,**1**), (iv) (**1**,**1**,**0**), (v) (**1**,**0**,**0**), (vi) (**0**,**1**,**0**), (vii) (**0**,**0**,**1**), (viii) (**0**,**0**,**0**). Inset: cyclic voltammogram of the substrate solution (0.1 mg $mL^{-1}$ TMB and 250 mM glucose) recorded over the bare gold electrode chip. (B) Bar chart of the magnitudes of responses of the combinations (i–viii) generated from the system for GMO analyses at 60 s. Inset: corresponding truth table drawn from the combinations of input signals (i–viii). (C) Visualized output interpretations displayed in an array corresponding to the combinations of input signals (i–viii). Blue color representing the oxidized form of TMB, indicates the simultaneous presence of promoter, coding, and species genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resulting $TMB_{ox}$ to configure the **AND** gate utility. Fig. 1B provides a schematic representation of the envisioned configuration of the biomolecular operation on a gold electrode chip. From examinations of the effectiveness of this platform technology, which involves a promoter, new coding, and species genes fragments from a GM organism as relevant inputs to the system, we ratified its practicability for high-fidelity recognition of GM events. Given that the three inputs are concomitantly present in the specific strain, an affirmative output indicates the existence of the identified GM material.

### 3.2. Validation of the biomolecular computational assay for GMO

Fig. 2 displays the output behavior of the system (sampled from the electrochemical transformation of $TMB_{ox}$ to $TMB_{red}$) for different combinations of inputs. We employed oligonucleotide sequences fragmented from regions of the promoter, new coding, and species genes of the GTS-40-3-2 GM event (one of the most successful GM crops and has become dominant in several countries where its planting is permitted) as segments **I**, **II**, and **III**, respectively, to allow meaningful coding of the specified GM event. As can be seen in the cyclic voltammogram of substrate solution (0.1 mg $mL^{-1}$ TMB and 250 mM glucose in 150 mM phosphate/citrate buffer), recorded at the bare gold electrode of the chip (inset of Fig. 2A), the lower reduction peak of TMB approximately situates in 0 V. Thus −0.1 V was applied to provide a sufficient driving force, overpotential, facilitating the reduction of $TMB_{ox}$. Fig. 2A reveals that the combination (**1**,**1**,**1**) provided a current output (165.9 nA at 60 s) that was substantially greater than those from the other combinations; a histogram representing the corresponding output amplitudes of the eight combinations (Fig. 2B) indicates the largest dynamic range for (**1**,**1**,**1**), thereby agreeing with the truth table shown as inset. Only the concurrent presence of the three genes selected (namely logic **1** at all inputs) resulted in a high current readout, allowing unambiguous identification. This detection event could also be visualized in an array (Fig. 2C), where only the combination (**1**,**1**,**1**) provided a blue indicator, enabling facile and effortless access to an output interpretation modality that could potentially be applied to in-field measurement.

### 3.3. Optimization of assay system

To ensure high-fidelity analyses of the GM materials, we optimized the operating parameters to maximize the dynamic difference of outputted signal obtained from GM-present (**1**,**1**,**1**) and -absent (**0**,**0**,**0**) circumstances. We investigated the effects of the concentrations of glucose, capture probe, reporter probe, and levels of the enzymes involved in the biocatalytic AND gate on the performance of the biomolecular computational system. Glucose is the primary substrate of the GOx/HRP concatenated enzymatic reactions. The elevated quantity of glucose expedites the catalytic reaction to yield apparent outputted signal (Fig. 3A). The preferred concentration of glucose at 250 mM was employed for the following study. In addition, concentrations of capture probe, reporter probes, and enzymes which may govern the signal/noise ratio were also extensively examined. The surface coverage, which is dependent on the applied concentration of capture probe, dominates the steric effect exerted on DNA hybridization and detectable concentration range for the current bioanalytical system. Furthermore, the integrated signal generation is based on the amount of reporter probes hybridized with target analyte, leading

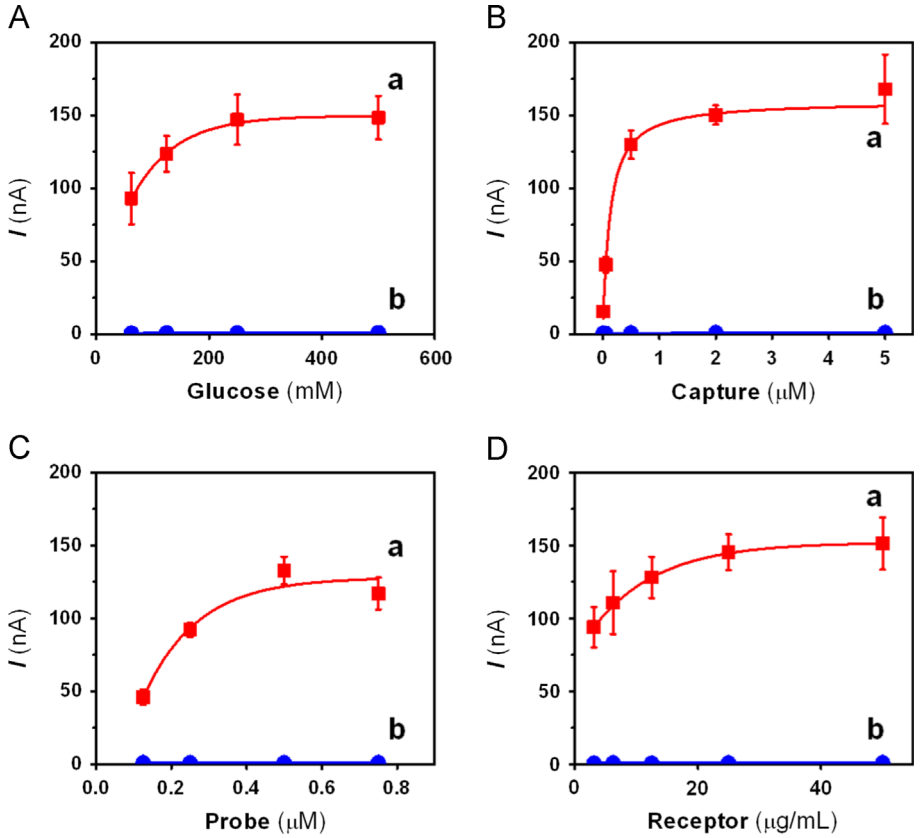



**Fig. 3.** Effects of concentrations of glucose (A), capture probe (B), reporter probe (C), and reporter enzymes (D) involved in the biomolecular circuit upon the output signal in the presence (red squares) and absence (blue circles) of 0.1 μM target. Output current was recorded from the chronoamperograms at 60 s under the operation of the biomolecular computational assay at an applied potential of –0.1 V. See Section 2 for details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the binding of enzyme receptors. Apparently in Figs. 3B, C, and D, the factors under investigation principally drive the output signal rising with elevated concentrations, and achieving the saturated plateaus. Excessive load of enzymes, nevertheless, cause undesired nonspecific binding on electrode surface. Accordingly, the concentrations of capture probe, reporter probe, and enzymes are exploited to be 0.5 μM, 0.5 μM and 25 μg $mL^{-1}$, respectively, for subsequent operations of the assay.

### 3.4. Assay performance of GMO detection

Furthermore, we evaluated the possibility of using current system for on-site testing, where the resulting output signal would be governed by both the concentration of total DNA and the ratio of genetically modified genes. We constructed a contour profile of the outputted current (acquired via chronoamperometry) for the assay system by scanning the concentration of total DNA and the percentage of genetically modified genes from 0 to 20 μM and from 0% to 5%, respectively (Fig. 4A). The relative GMO content (percentage, %) of a sample can be expressed as the amount of genetically modified material to the total material. Over the investigated range of total DNA concentrations, the current response exhibited a maximized gradient with respect to the GM percentage (123.3 nA $\%^{-1}$ at 10 μM, 175.5 nA $\%^{-1}$ at 20 μM). It was observed that a minor increase in the percentage of genetically modified genes yielded a dramatic increase in the current response, revealing the superior sensitivity of this system. For example, at 5% tolerance level for genetically modified material (allowed level of GM foods in Japan and Taiwan), the output at 156.2 nM (equivalent to 7.8 nM GMO) was 25.0 nA, substantially greater than the background readout ($<1$ nA), suggesting the potential feasibility of applying this system to field analysis. To evaluate the influence of quantity of non-genetically modified DNA upon the outputted readout, the current response was investigated against the concentration of GMO in connection to varied percentage of the GMO content (Fig. 4B). Notably, the output current was independent of the percentage of the GMO (%), inferring that a giant body of non-GM DNA would not interfere with the sensing performance of the system, thereby ensuring reliable, and high-fidelity measurements. We also determined the limit of detection (LOD) of the system by iterating the operation until the current level was separated by three standard deviations ($3\sigma$) from the background output, obtaining a value of 25 nM nucleic acid when measuring a sample containing 0.9% GMO (Fig. S2). Accordingly, the LOD corresponded to 225 pM of genetically modified DNA level. Compared with previously reported assay (Orbach et al., 2012) offering detection limit of 10 nM, wherein three nucleotides were utilized as inputs to generate single output, LOD explored from the present biomolecular computational system is substantially more sensitive. To sum up our findings, we may conclude that improved analytical performance provided by such biomolecular devices allows greater precision and consistency between individual assays and better hybridization specificity, accomplished by three separated probes and their corresponding targeted sequences, enables enhanced target discrimination. Our results confirm the utility of this novel assay for identifying GM event; and with the currently achievable LOD, only 558 mg of soybeans are required to implement measurement (see the detailed estimation in the Supplementary material). Moreover, DNA extraction procedures might concomitantly bring in residual substances that interfere with the performance of the system. Accordingly, we systematically evaluated the analytical interference caused by various potential residual substances (Fig. S3). Gratifyingly, the output current was minimally (3–14%) altered by the presence of excessively-loaded potential residues, including 1% of EtOH, isopropanol, $CHCl_3$, and CTAB; as well as the possibility for interference when using commercial DNA extraction kits is negligible.

### 3.5. Multiplex screening of GM events

To demonstrate the versatility of the biomolecular computational assay and its ability to process the increasing number and complexity of GM events, we employed selected GM events—A5547-127, NK603, T25, and LY038—for realized paradigms of genetically modified soybeans and maize (see Fig. S4 in the Supplementary material for details) in a multiplex manner. Desired sequence of a fragment DNA which has been preliminarily blasted to be the representative identity corresponding to the specific promoter, coding, and feature gene was selected and subsequently reconstructed for the targeted species. In accordance with the operational functionality of the equivalent logic shown in Fig. 1, the blue indicator appeared unambiguously in wells a, g, j, and p (Fig. 5A), confirming that output **1** derived from the input combination (**1**,**1**,**1**) was accurately indicative of the presence of specific GM events. Identical conclusion was also achieved by the electrochemical transducer (Fig. 5B). Conversely, the absence of any of the input genes among the promoter, new coding, and species disabled either successful hybridization or facile processing of the enzyme cascade reaction, thereby yielding an OFF output (e.g., wells b–f, h, i, and k–o). These OFF situations were interpreted as non-identified GM events.

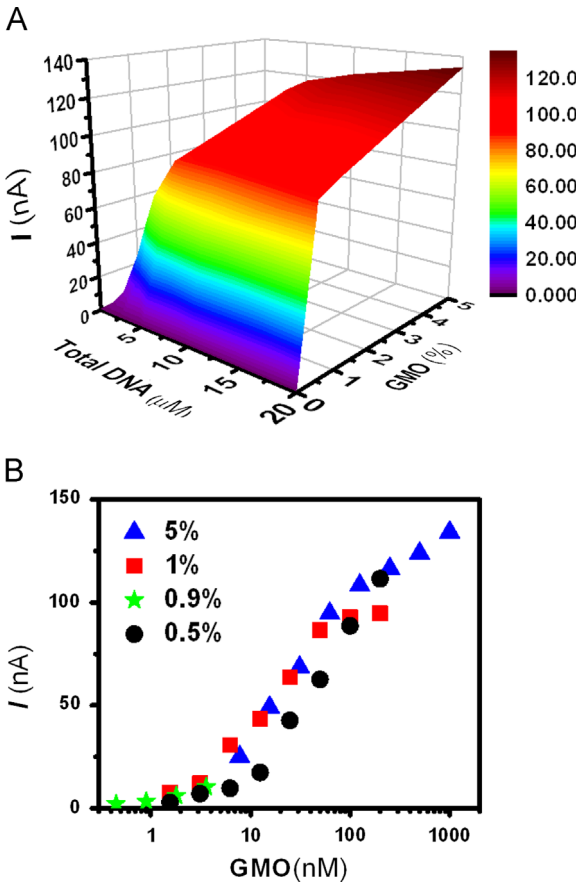



**Fig. 4.** (A) Mapping of the functional dependence of the output current on the total DNA concentration and GMO ratio. Output currents were sampled using chronoamperometry (at 60 s) with input levels of 20, 10, 5, 2.5, 1.25, 0.625, 0.312, and 0.156 μM (total DNA) and 5, 1, 0.5, and 0% (GMO ratio). The linear interpolation was established using the 3D Surface Color Map Surface technique provided by OriginPro 8.5. (B) Relationship between the output current and the concentration of genetically modified genes. The concentration of GMO was calculated by total DNA concentration × GMO (%). Applied potential: −0.1 V and sampled time: 60 s.

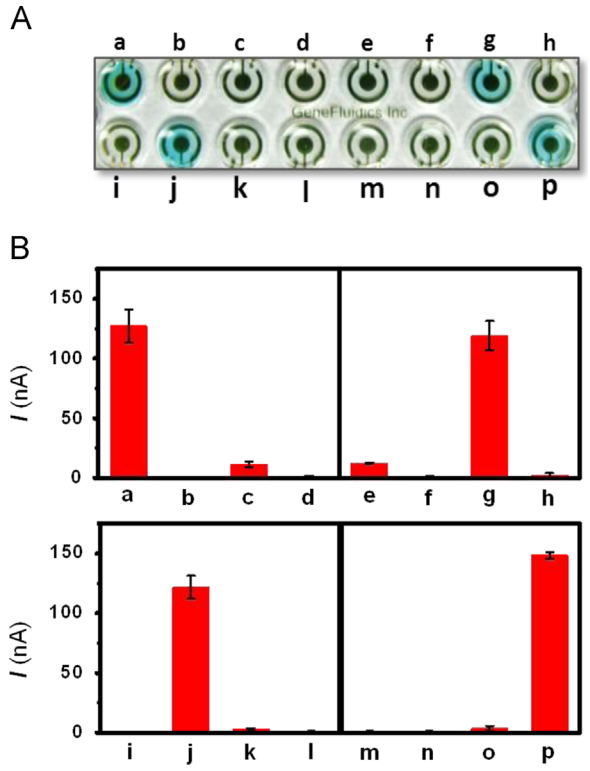



**Fig. 5.** (A) Visualized output with a blue indication revealing the formation of $TMB_{ox}$. Respective probes complementary against events A5547-127 (wells a, e, i, and m), NK603 (wells b, f, j, and n), T25 (wells c, g, k, and o), and LY038 (wells d, h, l, and p) were positioned in desired wells and subsequently examined with the targeted genes of the desired GM events in wells a–d (A5547-127), e–h (T25), i–l (NK603), and m–p (LY038). (B) Histograms of the resulting electrochemical outputs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The concept of biomolecule-processed computational screening of multiple fragments of single-stranded DNA serves as the core function of a GMO analytical platform that circumvents the need for stepwise screen analysis of promoter and coding fragments commonly involved in the conventional strategy. Additionally the cumbersome and complicated processes in screening and utilization of the event-specific gene could also be avoided. The approach using the logic concept to analyze GMO is capable of offering a decisive answer, on basis of molecular computations, to exclude potential personal errors. Simplicity and robustness are inherently desirable properties of the electrochemical devices. While extending the concept to practical applications, such electrochemical devices, provided in current array fashion, can be promisingly employed to mid-sized screening, that is differentiated from the mass screening techniques such as microarray (Kim et al., 2010; Leimanis et al., 2006; Xu et al., 2006), and Luminex xMAP (Fantozzi et al., 2008). The present approach circumvents the need for stepwise screening commonly required in the conventional assay and may lead to the increase in analytical throughput (comparison of various GMO detection methods is summarized in the Supplementary material, Table S3). It also should be noted that an accurate logic interpretation relies on reliable source of targeted sequence (i.e., single-stranded DNA); therefore a pretreatment protocol required for the generation of the specific single-stranded DNA from the duplex structure is indispensable. Moreover, the number of bases in the oligonucleotide separating new coding from species genes may play a role in regulating the efficiency of concerted enzymatic reactions (cascaded with GOx and HRP) which requires further attention to meet sequence diversity in various GMOs.

## 4. Conclusion

In summary, a novel approach for multiplex screening of genetically modified DNA is demonstrated using a biomolecular computational assay that processes multiple genes with resulting digital interpretation. In a more general perspective, our study validates the notion that logic-based biomolecular activities can offer the capability of processing information and, accordingly, the analysis of diverse nucleic acids can, in principle, be implemented within a chip device. The present bioanalytical system allows discrimination of various concentration (0–20 μM) and percentage (0–5%) of genetically modified genes of GM-material, in connection to independent correlation with non-GM DNA and detection limit as low as 225 pM. The influence of potential disturbing factors brought through DNA extraction procedures can even be neglected. This assay of principle is advantageously sophisticated as screening platform for variable GM events configured in array chip. In virtue of the innate properties of this proof-of-concept utility and its resulting elegant performance, we anticipate a multiplex screening to facilitate the analysis of GMOs and other versatile applications.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2013.06.044.

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