QCM DNA SENSOR FOR GMOs DETECTION

Passamano M*, Pighini M

Technobiochip S.c.a.r.l., via Carrara 12/A 04100 Latina, Italy
* Phone: +39-0773632643; Fax: +39-0773631009; e-mail address: m.passamano@technobiochip.com

Abstract: A Quartz crystal microbalance DNA sensor for the detection of genetically modified organisms (GMOs) is presented. A gene fragment characteristic of GMO phenotypes has been immobilized on the quartz surface and the hybridization between the immobilized probe and total DNA extracted from food samples and from certified reference materials was performed. The data obtained reveal the sensor's ability for sensitive and specific detection of GMOs, so providing a useful tool for screening analysis in food samples.

Keywords: DNA-sensor, GMO.

1. Introduction

DNA sensors based on QCM have been applied with success to real problems from environmental to clinical analysis [1]. These devices have been presented as an effective alternatives to gel electrophoresis and to traditional methods for specific DNA sequences detection, where labeled probes are required. Using QCM sensing, with an appropriate DNA probe immobilized on the sensor surface, it is possible to detect a specific target sequence in solution. So in this last few years, and more so in the future, their development as a diagnostic or analytical instrument represented one of the industrial applications of greater success.

One of the most actual and needful DNA sensor applications is the detection of genetically modified organisms (GMOs) in foods. A genetically modified organism (GMO) is referred to as a living organism whose genome has been modified by the introduction of an exogenous gene able to express an additional protein that confers new characteristics, i.e. herbicide tolerance, resistance to virus or antibiotics or insects. The extensive introduction of GMOs in agriculture and the increasing number of GMO-derived products launched into the food market have led to a strong demand by customers for strict regulations and labeling of such products. EU regulations provides a threshold level of 1% for the GMO content of food. A GMO content above this threshold would require the labeling of the respective product, while a content below this threshold is considered an unpreventable contamination. At present, in order to enforce such regulations, the approaches for GMO identification are still largely based on immunoassay analysis and quantitative PCR. The first one rely on the determination of the protein product of the newly introduced gene. The second one is characterized by the amplification of GMO-specific DNA by polymerase chain reaction followed by an identification of its specific amplification products, for example by agarose gel electrophoresis, restriction fragment length analysis, southern blot hybridization or DNA sequencing [2]. These methods require a well-equipped laboratory and a careful adaptation to the specific equipment by experienced investigators to optimize the results [3]. Furthermore, they are very expensive and laborious [4]. On the basis of the interesting performances of Technobiochip DNA-sensor, we decided to set up a reliable, fast, cost-effective method for GMO screening in food samples. Our DNA-sensor, µLibra, is a nanobalance measuring system which allows the direct monitoring of the interactions between the nucleic acids complementary strands. It offers the possibility to monitor in real time the hybridization event, measuring the quartz oscillation frequency variation in response to the mass increase. The golden quartz surface is functionalized by a biotinylated DNA probe layer (characteristic of GMO phenotype) through the high affinity with streptavidin, previously deposited on the quartz. The hybridization between probe and complementary sequence can be measured by a resonance frequency decrease. As a result, it can discriminate the presence of the exogenous gene in free and not GMO foods. The results of the QCM assay in terms of sensitivity, specificity and reproducibility are discussed.

2. Material and methods

DNA amplification and cloning

Using the Bt-176 DNA sequence (GMOIdent kit; Gene Scan, Germany) as template, we amplified a 200bp fragment of Cry1A(b) gene which confers resistance to insects in maize. The primers used are shown below.

Forward: 5' GGT GGC ACG TTG TTG TTC TGA 3'
Reverse: 5' GTG CTG CTC AGG GTG CGG 3'
The amplification conditions were the following: 10' at 95°C; 30'' at 95°C; 40'' at 58.5°C; 40'' at 72°C; 40 cycles; final extension at 72°C for 15'. The obtained DNA has been analyzed and purified with the QiAquick nucleotide removal Kit (Qiagen). The yield of the PCR fragment wasn’t sufficient to perform µLibra experiments; so it has been cloned using the pGEM®-T Easy Vector System (Promega, USA). The Cry 1A (b) gene fragment DNA, after digestion of expression vector with EcoRI, has been analyzed on agarose gel 0.8% and purified with Nucleospin Extract Kit (Macherey-Nagel). Finally it has labeled with biotin using as reagent the Biotin-16-2'-deoxy-uridine-5'-triphosphate (Roche).

**Nanogravimetric DNA-sensor**

µLibra (produced by Technobiochip S.c.a.r.l.) is a Quartz Crystal Microbalance system based on quartz crystal resonators. The instrument is composed by a Main unit and a Cell Base Unit with two 10 MHz oscillators which can support two measuring chambers. The two channel acquisition system allows single cell operation as well as working/reference measurements. The oscillators either work in air or in solution by two low-volume flow-through cells (Fig. 1). The piezoelectric quartz crystal with gold electrodes on both surfaces (AT-cut of 10 MHz) was purchased from International Crystal Manufacturing Co. Inc. (Oklahoma, U.S.A.). The instrument offers a rapid and easy way to detect and measure mass deposition on quartz crystal. In fact the mass variations are proportional with the quartz crystal resonance frequency variations as described by the Sauerbrey equation [5]. The µLibra mass sensitivity for ICM 10MHz quartzes is 19 ng/Hz (Table 1).

**Table 1. µLibra specifications.**

<table>
<thead>
<tr>
<th>SPECIFIC FEATURES</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Base frequency range</td>
<td>1-20 MHz</td>
</tr>
<tr>
<td>Data acquisition channels</td>
<td>2</td>
</tr>
<tr>
<td>Acquisition rate</td>
<td>0.5-2 Acq/sec.</td>
</tr>
<tr>
<td>Precision</td>
<td>± 1 Hz</td>
</tr>
<tr>
<td>Mass sensitivity</td>
<td>19 ng/Hz</td>
</tr>
<tr>
<td>Flow-cell volume</td>
<td>25 μl</td>
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The µLibra experiments for GMO detection have been performed using two quartzes, one as working sensor, the other as reference, in a static flow cells. A streptavide solution (0.2mg/ml) was used to prepare the first layer on the golden quartz surface. The high affinity between biotin and streptavide was needful to bind a single strand biotinylated probe (1,5μg) in order to allow the quartz functionalization (Fig. 2). A complementary non-biotinylated sequence has added in the solution (10μg/ml in SSC2X for 1 hour) and the happened hybridization between probe and target can be measured by a resonance frequency decrease as result of the superficial mass increase.

![Fig. 2 Scheme of DNA immobilization and hybridization on golden quartz.](image)

**3. Results and Discussion**

Biosensors for their characteristics (i.e. fast time response, low costs) are very attractive for new applications in different emerging fields like genetically modified organisms detection.

We are currently studying some DNA sensor applications for GMOs detection in real food samples, using some gene fragments characteristic of GMO phenotypes. In particular we chose as probe the Cry1A(b) gene, a synthetic DNA sequence (1947bp long) derived from *Bacillus thuringiensis*, having enhanced insecticide activity in maize. It is the most common gene introduced into maize for the protection against insect damages and allows a constitutive expression of the "Bt endotoxin" in all plant tissues and at all growth stages [6]. A Cry1A(b) fragment of about 200bp,
obtained by PCR amplification and then cloned, has been used as biotinylated probe to functionalize the quartz surface. In order to apply our system in the GMOs gene detection, we have firstly verified the hybridization between biotinylated Cry1A(b) fragment and the same fragment used as target. They have been thermally denatured in order to allow the hybridization. After a period necessary to achieve the equilibrium conditions, the recorded signal related to probe-target interaction is in a resonance frequency variation (\(\Delta f\)) of 18 Hz. The \(\mu\)Libra experiment, above mentioned, is shown in Figure 3.

![Fig. 3. Nanogravimetric revelation of the hybridization between biotinylated Cry1A(b) fragment and the same fragment used as target. SA: streptavidine; Cry-bio: probe.](image)

In order to test the analytical performances of the sensor developed, the sensitivity, specificity, reproducibility and stability have been evaluated using IRMM-413 certified reference materials. Total DNA extracted from IRMM products of dried maize powder with different mass fractions (from 0.1% to 5%) of genetically modified MON 810 maize flour, have been analyzed by DNA-sensor. The resonance frequency shift (expressed in Hz) versus the different % of GMO maize flour DNA are shown in the graph (Fig.4).

![Fig. 4. Calibration curve for the sensor surface with immobilized biotinylated Cry1A(b) fragment using different % of GMO maize flour DNA.](image)

A calibration curve has been derived by interpolating the data points. A resonance frequency variation of 4 Hz is recorded with DNA from 0% GMO maize flour. This value could be due to the electronic noise of the instrument. The analyzed data are in agreement with a linear dependence, so demonstrating a quantitative GMOs detection of the sensor.

Total DNA extracted from food samples has been examined by the DNA-sensor to test its capacity for GMO detection in real samples. DNA from a transgenic cookie, made using certified genetically modified MON 810 maize flour, has been analyzed. The \(\mu\)Libra experiment, above mentioned, is shown in Figure 5. A resonance frequency variation of 15 Hz is revealed.

![Fig. 5. Cry1A(b) detection: the hybridization between biotinylated Cry1A(b) fragment and Transgenic Biscuit DNA.](image)

The data obtained, as confirmed by classical Southern and Dot Blotting analysis (data non shown), reveals the sensor’s ability for GMOs detection. Furthermore, the % GMO content in transgenic biscuit, related to the measured resonance frequency variation, is in agreement with the same value obtained by graphic interpolation in the calibration curve.

4. Conclusions

The surveillance of food labelling concerning GMOs requires sophisticated analytical techniques. A reliable, fast, cost-effective method for GMO screening is presented. In particular we tested our device for the detection of Cry1A(b) gene fragment characteristic of GMO phenotypes. This study has demonstrated the utility and the applicability of the DNA-sensor for GMO detection both in environmental and food analysis. The advantages of this strategy versus the classical detection methods are the label free DNA hybridization reaction (no toxic compounds are required) and shorter hybridization times. Furthermore the DNA-sensor can discriminate, in qualitative and quantitative manner, the presence of the exogenous gene in free and not GMO samples.
Future investigations will allow to verify the DNA-sensor ability to reveal the threshold value of 1% for the GMO content in different food samples, and other experiments will be carried out to improve our QCM assay in terms of sensitivity, specificity and reproducibility.

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References