

DNA SENSOR BASED ON PIEZOELECTRIC QUARTZ TECHNOLOGY FOR GENETICALLY MODIFIED ORGANISMS DETECTION



PASSAMANO M.; PIGHINI M.

Technobiochip S. c. a r. l., Via della Marina n. 39, 57030 Marciana (LI) - Italy. e-mail: Bio-mol@technobiochip.com

The extensive introduction of genetically modified organisms (GMOs) in agriculture and the increasing number of GMO products launched into the food market have led to a strong demand by customers for strict regulations and labeling of such products. The surveillance of food labeling concerning GMOs requires sophisticated analytical techniques. Present assay systems use essentially quantitative PCR and immunoassay methods: they are very expensive and laborious. 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.



Fig.1 Libra 3 Nanogravimetric DNA-sensor



Fig.2 Piezoelectric quartz

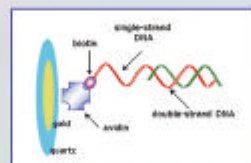


Fig.3 Scheme of DNA immobilization and hybridization on golden quartz

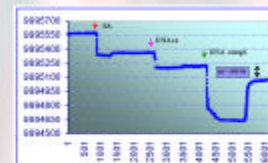


Fig.4 Complementary DNA sequences hybridization example revealed by nanogravimetric sensor

Libra 3 Nanogravimetric DNA-sensor is based on piezoelectric quartz crystals, which allows the direct monitoring of nucleic acids interactions by measuring the quartz frequency variation as result of the superficial mass increase (Fig.1). The mass change, occurring during the DNA hybridization process, is converted into a resonant frequency change that can be easily measured. This DNA-sensor works with two quartz: working and reference quartz (Fig.2). The golden quartz surface is functionalized, as shown in Figure 3, by a biotinylated DNA probe layer 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. In Figure 4 an hybridization example between two complementary DNA sequences.

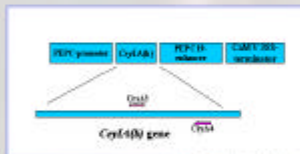


Fig.5 PCR amplification of *CryIA(b)* fragment using as target the BT-176 DNA sequence (Gene Scan).

We are studying some DNA sensor applications for GMOs detection in real samples, using some gene fragments characteristic of GMO phenotypes. In particular we chose as probe the *CryIA(b)* gene, a synthetic DNA sequence (ID I41419) derived from *B. thuringiensis*. It's the most common gene introduced into maize for the protection against insect damage (GM-maize MON810, Bt11, Bt176). A *CryIA(b)* fragment of about 200bp, obtained by PCR amplification (Fig.5) and then cloned in pGEM-T easy Vector, has been used as biotinylated probe in order to functionalize the quartz surface.



Fig.7 Transgenic biscuit

In order to apply our system in the GMOs gene detection, we have firstly verified the hybridization between biotinylated *CryIA(b)* fragment and the same fragment used as target (Fig. 6A). The happened hybridization is revealed by a resonance frequency variation (Δf) of 18 Hz.

Total DNA extracted from a food set (Corn flakes, Pop corn, Muesli etc.) and from certified reference materials have been analyzed by DNA-sensor. In Fig.6B a Libra experiment is reported using as target a genomic DNA from a transgenic cookie made using certified 5% genetically modified MON 810 maize flour (Fig.7). The happened hybridization is revealed by Δf of 15 Hz. The data obtained have been confirmed by classical Southern analysis (Fig. 6C), so providing a classical validation for the nanogravimetric experiments and confirming the sensor ability in GMOs gene detection.

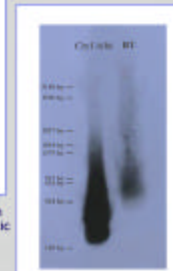
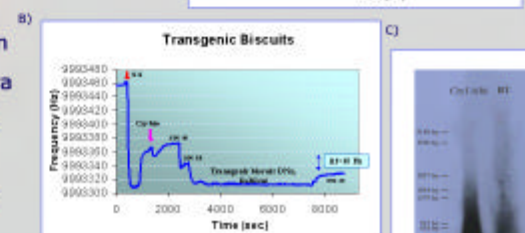
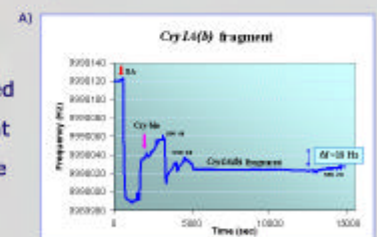


Fig. 6 *CryIA(b)* detection: the happened hybridization on Libra using as target *CryIA(b)* fragment (A) and Transgenic Biscuits DNA (B). Southern Blotting analysis (C) between *CryIA(b)*-bio and the positive control or Transgenic Biscuit DNA (BT).