

DNA SENSOR BASED ON PIEZOELECTRIC QUARTZ TECHNOLOGY FOR DIAGNOSTIC SCREENING OF GENETIC DISEASES

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The specific sequences detection in human genome is becoming increasingly important in diagnosis of diseases caused by gene mutations. The mutations responsible for numerous inherited human disorders, often associated to the presence of various pathology including tumours, are now known and this knowledge is steadily increasing as the sequencing of human genome is completed. In these last few years, and more so in the future, the development of DNA-sensors as diagnostic instruments represent one of the industrial applications of greater success. DNA sensors, in fact, can detect the presence of genes or mutant genes associated with human pathologies.



Fig.1 Libra 3 Nanogravimetric DNA-sensor



Fig.2 Piezoelectric quartz

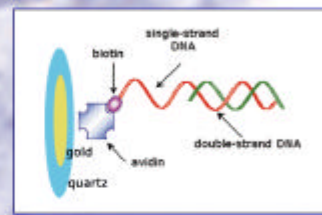


Fig.3 Schematic diagram of DNA immobilization and hybridization on golden quartz

The Technobiochip DNA-sensor, LIBRA 3.0, is based on piezoelectric quartz crystals that allows the direct monitoring of the interactions between the nucleic acids complementary strands (Fig.1). It offers the possibility to monitor in real time the hybridization event by measuring the quartz oscillation frequency variation in response to the mass increase. In this sensor a 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).

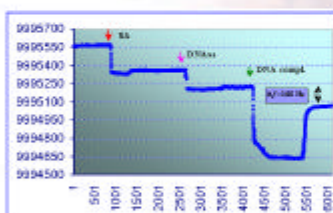


Fig.4 Complementary DNA sequences hybridization example revealed by nanogravimetric sensor

The golden quartz surface is functionalized, as shown in Figure 3, by flowing first a streptavidine solution, then a biotinilated DNA single strand (probe) specific for the genetic alteration related to a particular disease. In the following step total human genomic DNA (target) flows on the quartz surface: hybridization event can be revealed only if the target contains a complementary sequence. An hybridization example between two complementary DNA sequences, as revealed by quartz oscillation frequency variation, is shown in Figure 4.

Our sensor has been used to reveal genetic mutations related to Neuroblastoma, the most common extracranial cancer of pediatric age. Complex patterns of genetic abnormalities interact to determine the clinical phenotype including amplification of the MYCN oncogene and most frequently deletions of chromosome arms 1p and 9p. Chromosome 9p has been observed deleted in about 25-30% of NBs and it is used as a marker to evaluate the patient outcome. We have set up a method for the screening of the cromosomal regions 9p21-9p23 using D9S1810 microsatellite as marker. Human genomic DNA EcoRI cut, extracted from tumoral and normal samples, were flowed on quartzes previously coated with D9S1810 microsatellite. The happened hybridization, as revealed by Δf of 250 Hz, is shown in Figure 5. This data suggest that our sensor is able to reveal this genic deletion related to Neuroblastoma.

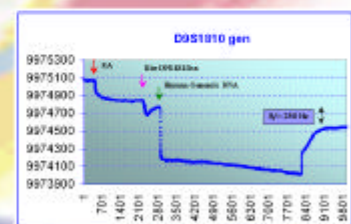


Fig.5 Nanogravimetric revelation of the hybridization between D9S1810 microsatellite and tumoral human DNA

Moreover we tested our device to reveal MYCN genic amplification. At this purpose, DNA extracted from the human Neuroblastoma cell lines GIMEN and SKNBE presenting only one and 60-100 MYCN copies respectively, were used for the hybridization step. The different Δf , as shown in Figure 6A e 6B, reveals the sensor capability to discriminate between amplified cell lines and not.

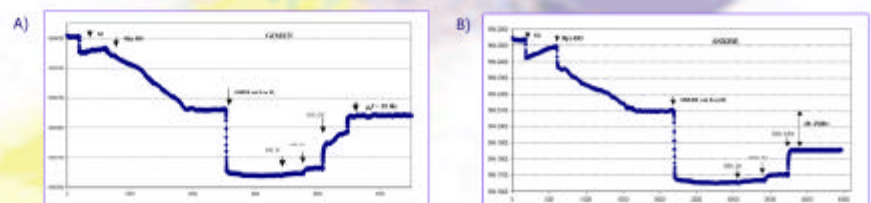


Fig.6 MYCN amplification screening: the graphs reveal the happened hybridization between MYCN and DNA extracted from the human Neuroblastoma cell lines GIMEN (A) and SKNBE (B).

In conclusion we have demonstrated that the Technobiochip DNA Sensor is able to reveal genetic alterations such as deletions and amplification related to human diseases. Therefore it could be used in future for the diagnosis of serious pathologies in which this kind of mutations are present.