

# ELECTROCHEMICAL DNA SENSOR FOR DETECTION OF GENETIC MUTATIONS RELATED TO *NEUROBLASTOMA*



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DNA sensors can detect the presence of genes or mutant genes associated with human pathologies. Biosensors are devices that combine a biological recognition agent, which gives selectivity, with a transducer, which provides sensitivity and converts the recognition event into a measurable electronic signal. Biosensors for the detection of nucleic acids use single-strand DNA for the recognition of complementary sequences. The Technobiochip Electrochemical DNA Sensor uses the  $\mu$ AUTOLAB II potentiostat and the GPES 4.9 software (Eco Chemie B. V., Utrecht, NL). This system enables the monitoring of adsorbed DNA at graphite electrodes using the Potentiometric Stripping Analysis (PSA) technique for detection of hybridization. Ag/AgCl (3 M KCl) and platinum wires are used as reference and counter electrode respectively. The procedure involves the use of an electroactive indicator, daunomycin hydrochloride, which intercalates the double-stranded DNA. We set up a method in which the single-stranded biotinylated DNA is immobilized on the graphite electrode surface using the avidin-biotin high affinity interaction. Then the electrode is incubated with the sample solution containing the target sequence, and it is immersed in the daunomycin solution. After washes, we performed PSA technique in SSC 2X at constant current (1  $\mu$ A). During the stripping process the potential is recorded and processed. Then the response is recalculated to  $(dt/dE)$  vs E and a peak shaped pattern is obtained, allowing to reveal the happened hybridization directly by the size of daunomycin stripping peak.

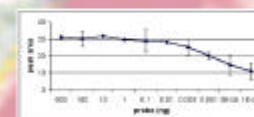


We first tested the sensor functionality using as probe a 18-mer related to cystic fibrosis (FC) and as target FC itself and its complementary sequence (CF). The results indicate a peak area increase when hybridization happens, as shown in Figure 1.

Fig. 1 Sensor response by PSA. The red lines show the peaks after the oligo immobilization; the blue ones show the same measurements after:  
A) interaction with a non-complementary sequence;  
B) hybridization with complementary sequence



In order to define the optimal quantity of probe immobilized on the electrode surface we performed a scale-down experiment ranging from 0.5  $\mu$ g to 0.1  $\mu$ g. The graphic shows that the electrode is saturated with a quantity of probe up to 0.1  $\mu$ g and therefore the next experiments have been performed using 1  $\mu$ g of probe.



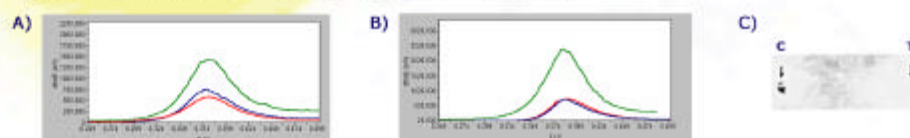
After validation of the sensor using oligonucleotides we tested our device with a whole gene. At this purpose we used *N-Myc*, belonging to *Myc* oncogene family. In Figure 2 the PSA response before and after the hybridization reaction with a non-complementary sequence (Fig. 2A) and with *N-Myc* itself (Fig. 2B) is shown.



Fig. 2 Sensor response by PSA. The red lines show the peaks after *ssMyc* immobilization; the blue ones show the same measurements after:  
A) interaction with a non-complementary sequence;  
B) hybridization with *Myc* itself.

All these results confirm the ability of our sensor to discriminate between complementary and non-complementary sequences. Then we used the sensor to reveal genetic mutations related to *Neuroblastoma*, the most common extracranial cancer of pediatric age. At this purpose we set up a method for the screening of the chromosomal regions 9p21-9p23 and 1p36, whose deletions in *Neuroblastoma* are known, using as markers D9S1810 and D1S244 microsatellites respectively. Data in Figure 3 show that our sensor is able to reveal genic deletions related to *Neuroblastoma*. Then we used the classical technique ECL to verify these results. Control (C) and tumoral (T) digoxigenin labeled total human genomic DNAs were used for the hybridization step on the same electrodes of Fig. 3B. Then they were treated with anti-DIG and exposed to autoradiographic film. The ECL response confirms the Technobiochip sensor results.

Fig. 3 Use of the sensor for deletions screening. The red lines show the peaks after the probe immobilization: A) D9S1810 microsatellite; B) D1S244 microsatellite. The green lines show the hybridization with normal total human genomic DNA Eco RI cut. The blue lines show the missing hybridization due to the use of total human genomic DNA Eco RI cut, extracted from tumoral samples.  
C) ECL performed on the electrodes shown in Fig. 3B



In conclusion we can assert that the Technobiochip Electrochemical DNA Sensor is able to reveal genetic deletions related to human diseases, as in *Neuroblastoma*, and then it can be used for the diagnosis of serious pathologies in which this kind of mutations are present.