Detection of genomic DNA by PCR-free piezoelectric sensing

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Supporting Information

Materials

10 MHz AT-cut quartz crystals (14 mm) with gold electrodes (area of 42.6 mm^2) were obtained from Nuova Mistral (Latina, Italy). The experiments were performed using a crystal analyzer (Model QCA 917, Seiko EG &G, Chiba, Japan) and the crystals housed inside a methacrylate cell.

11-mercaptoundecanol, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) and formamide were purchased from Sigma-Aldrich (Milan, Italy); epichlorohydrin and N-hydroxysuccinimide (NHS) from Fluka (Milan, Italy). Ethanol and other reagents for the buffers were purchased from Merck (Darmstadt, Germany), dextran T500 from Amersham Biosciences Europe (Milan, Italy).

Samples

Synthetic oligonucleotides were purchased from MWG Biotech (Ebersberg, Germany),

Biotinylated probe (35S, 25 mer):

5' Biotin-GGC CAT CGT TGA AGA TGC CTC TGC C-3'

Complementary target (35S, 25 mer):

5'-GGC AGA GGC ATC TTC AAC GAT GGC C-3'

Non complementary (Tnos, 25 mer):

5'-GAT TAG AGT CCC GCA ATT AAT CAT T-3'

Blocking oligonucleotide 1 (35S1, 21 mer):

5'-GCT CCT ACA AAT GCC ATC ATT-3'

Blocking oligonucleotide 2 (35S2, 18 mer):

5'-CTC CAA ATG AAA TGA ACT-3'

PCR was conducted on plasmidic DNA (pBI121), while the genomic DNA was extracted from the leaves of the *Nicotiana glauca* (Ng) tobacco plant, modified and grown at the Dipartimento di Biologia Animale e Genetica, Università di Firenze, Italy.

PCR and digestio n blanks were also prepared containing all the PCR (or digestion) reagents except for the template DNA.

Methods

Immobilization of the probe on the sensing surafce

The crystal was firstly cleaned with a boiling solution of H_2O_2 (30%), NH_3 (30%) and milliQ water in a 1:1:5 ratio, then it was modified for the immobilization of streptavidin (200 µg/ml in acetate buffer 10 mM, pH 5.0) on a 11-mercaptoundecanol self-assembled monolayer previously modified with carboxylated dextran.[1]

The biotinylated probe (1 µM in immobilization buffer (NaCl 150 mM, Na_2HPO_4 20 mM, EDTA 0.1 mM, pH 7.4)) was then added, to bind streptavidin.

Hybridization

The hybridization with the target solution was performed adding 100 µl of the oligonucleotide/sample solution, in hybridization buffer (NaCl 300 mM, Na_2HPO_4 20 mM, EDTA 0.1 mM, pH 7.4), to the cell well. The reaction was monitored for 10 minutes (oligonucleotides) or for 20 minutes (PCR products and genomic DNA), then the sample was removed and the sensor surface washed with buffer to remove the unbound sequences.

In all the experiments the single stranded probe was regenerated by two consecutive treatments of 30 seconds with 1 mM HCl allowing a further use of the crystal.

All the experiments were performed at room temperature.

Fragmentation of genomic DNA with restriction enzymes

The genomic DNA was digested using a selected restriction enzyme to obtain DNA fragments, containing the target sequence. By the WebCutter 2.0 software (web available), it was verified that the consensus sequence recognized by the enzyme was not present inside the target DNA sequence. This is important to ensure that the fragmentation does not affect the ability of the target sequence to hybridize to the immobilized probe. The BaMH 1 enzyme was used. After the digestion, the DNA samples consisted of many fragments differing in length; the one containing the target sequence was 872 bp long.

The digestion protocol was: 10 µg of genomic DNA have been digested with 2 enzymatic units (10 units/µl), of BaMH 1, (Sigma, Milan, Italy) in restriction enzyme buffer (10 mM Tris –HCl pH8.0, 5mM $MgCl_2$, 100mM KCl, 0.02% Triton X-100, 0.1mg/ml BSA). The reaction was allowed to proceed

at 37°C overnight and then at 75°C for 10 minutes to inactivate the enzyme. Afterwards, the digested DNA was precipitated with 500 µl of absolute ethanol and 20 µl of sodium acetate 3 M (pH 5.2). The precipitating solution was centrifuged at 13000 rpm for 45 minutes at room temperature and the supernatant removed. The DNA pellet was washed with 500 µl of 70% ethanol at –20°C and centrifuged at 1300 rpm for 30 minutes; the supernatant was removed and the pellet was dried in vacuum for 15 minutes and suspended in 100 µl of sterile bi-distilled H_2O.

Blanks consisted of all the digestion mixture (see below) except for the genomic DNA.

The reaction success has been confirmed by electrophoresis analysis, which has been performed using an agarose gel 1% in TAE buffer (40 mM Tris-acetate pH 8.0, 1 mM EDTA) and containing ethidium bromide with a final concentration of 1 µg/ml) (Figure S-1).

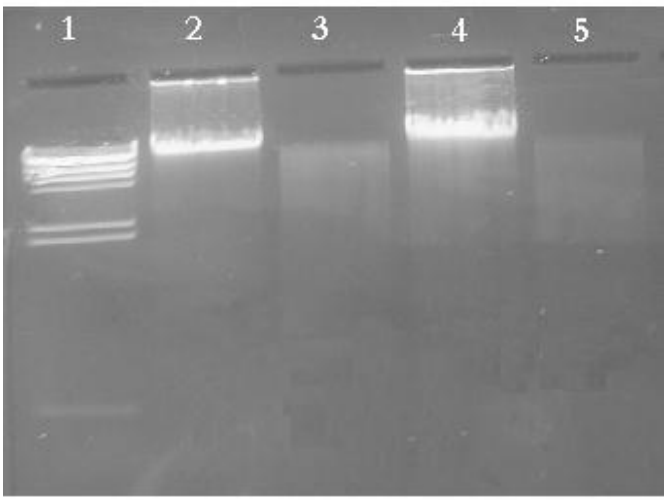

Figure S-1. Fragmentation of genomic DNA with restriction enzymes. Line 1: molecular markers; Line 2: *Nicotiana glauca* wild type not enzymatically fragmented; Line 3 *Nicotiana glauca* wild type enzymatically fragmented; Line 4: *Nicotiana glauca* GR4 transgenic not enzymatically fragmented; Line5: *Nicotiana glauca* GR4 transgenic enzymatically fragmented.

Electrophoresis

The electrophoresis was conducted on agarose gel 2% (1 g in 50 ml of TAE buffer). 1 µg/ml ethidium bromide was employed as fluorescent agent, and a potential of 70 V/cm was applied for at least 60 minutes.

The electrophoresis system "Minipac II P.S. 304" was obtained from Apelex (Massy Cedex, France). Ethidium bromide and agarose were purchased from IBI, Shelton Scientific inc. (Shelton, USA); the marker l-DNA-Hind III Digest from Amersham Biosciences Europe (Milan, Italy), the marker GeneRuler DNA ladder plus from Fermentas (Maryland, USA).

Sample amplification

The samples supplied were PCR amplified fragments of the 35S sequence (243 pb). PCR amplicons (180 bp) from the NOS terminator were also supplied and used as negative control.

The DNA was amplified according to the EU protocol.[2] The concentrations of the amplified sequences were controlled by the spectrophotometer GeneQuant (Amersham, Sweden).

Denaturation

PCR-amplified and genomic DNA are double stranded therefore they need to undergo denaturation to separate the two strands before they can be injected in the cell and hybridize to the immobilized probe.

Three approaches were tested: thermal, chemical and thermal combined with blocking oligonucleotides.

Thermal denaturation

The DNA samples were heated at 95°C for 5 minutes and then cooled in ice for 1 minute and immediately injected in the cell.

Chemical denaturation

The samples were added to a solution containing 20% formamide and NaOH 0.3 M and left at 42°C for 30 minutes. Then HCl 0.3 M was added to the samples cooled in ice for 1 minute.

Thermal denaturation with blocking oligonucleotides

This denaturation procedure combines two effects. At first the DNA is dissociated by keeping it at a very high temperature, then its re-association is prevented by creating steric hindrance. The steric hindrance is accomplished by the bond of two oligonucleotides, one to the strand containing the target sequence and the other to the other strand. The blocking sequences stick close to the target/probe sequences but do not overlap them so that the target is free to hybridize to the immobilized probe (short) but it cannot bind the digested fragment (long).

1 µM solution of both blocking oligonucleotides was added to the samples that were then heated at 95°C for 5 minutes. The samples were then cooled at 50 °C for 1 minute (Figure S-2).

The blocking oligonucleotides added are the primers used to amplify the 35S DNA, so the binding temperature is chosen according to the annealing temperature of the primers in the PCR EU protocol considered.

After the treatments the sample is injected into the cell for the interaction with the immobilized probe.

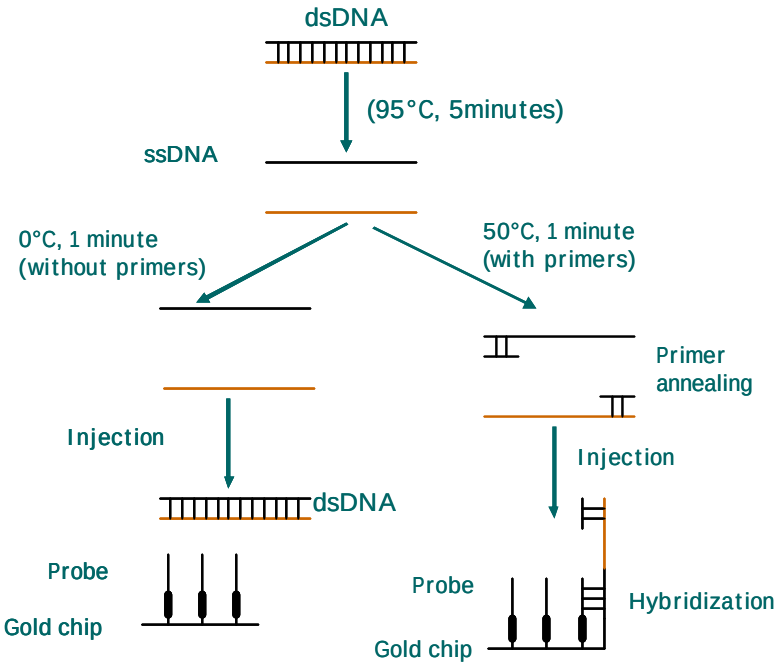

Figure S-2. Treatments to dissociate the dsDNA PCR samples. Left: high temperature denaturation (95°C for 5', 1 minute in ice). Right: high temperature denaturation with oligonucleotides (95°C for 5', 1 minute incubation with oligonucleotides at 50°C).

Results

PCR samples and genomic digested DNA were tested after treatment with the three denaturation procedures. PCR negative controls, PCR blanks, WT digested genomic DNA and "digestion blanks" were also tested after the same treatments. Representative raw sensor signals recorded during the experiments with the PCR samples and the genomic DNA are reported in figures S-3 and S-4.

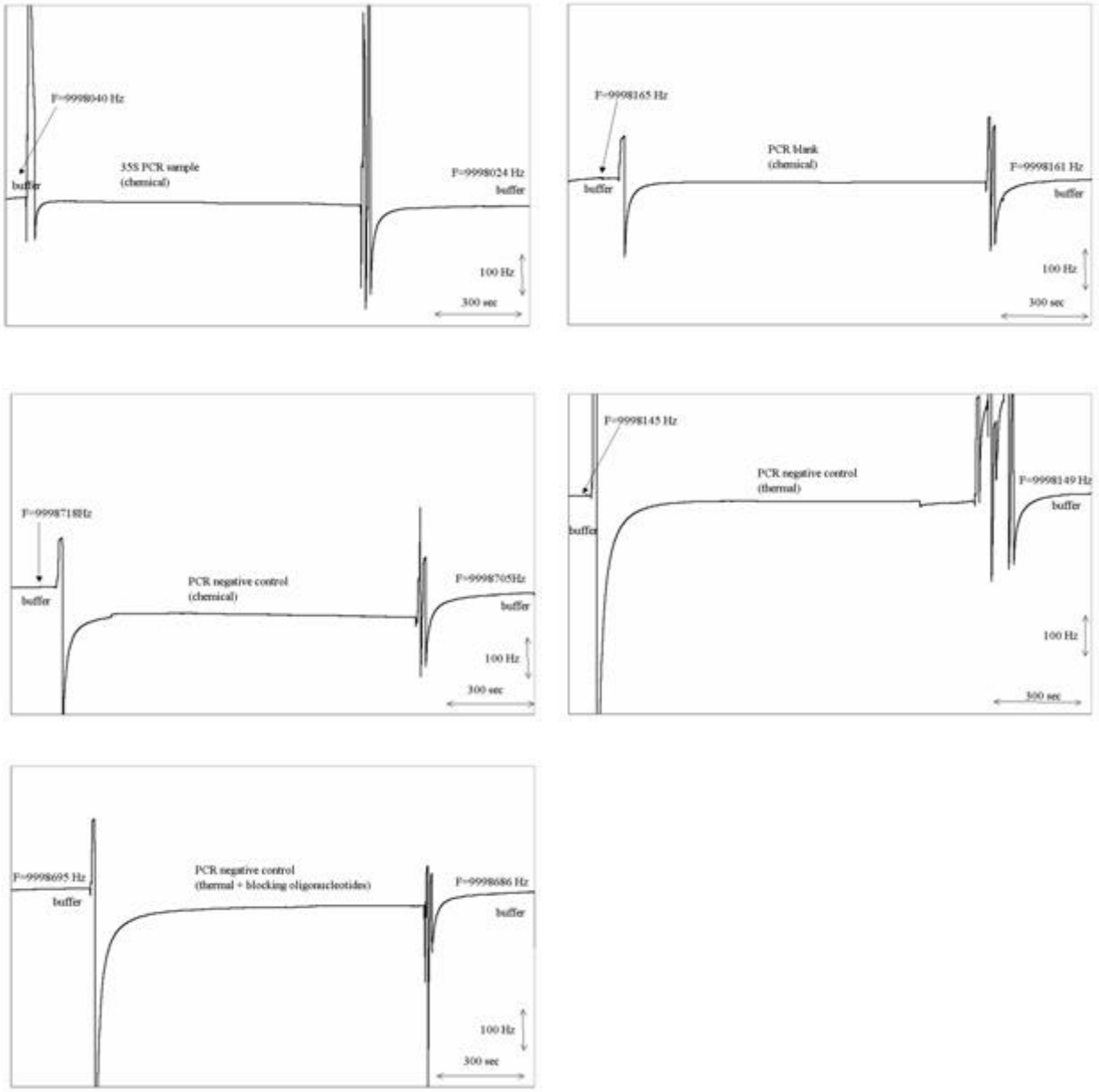

Figure S3. Representative raw sensor signals (Frequency (Hz) vs time (sec)) recorded during the experiments with PCR samples.

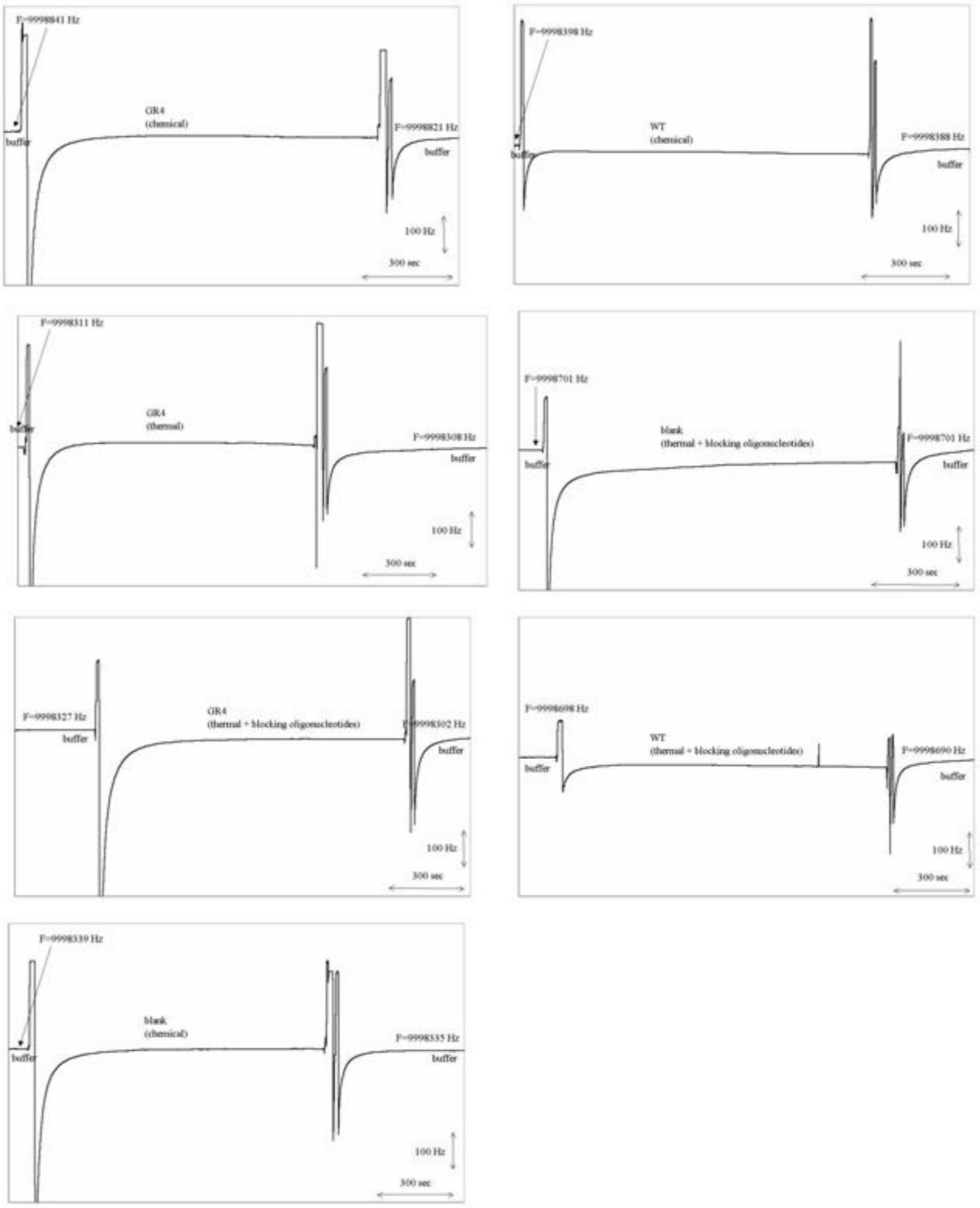

Figure S4. Representative raw sensor signals (Frequency (Hz) vs time (sec)) recorded during the experiments with digested genomic DNA samples.

References

[1]Mannelli, I.; Minunni, M.; Tombelli, S.; Mascini M. *Biosens. Bioelectron.* **2003**, *18*, 129.

[2]Pietsch, K.; Waiblinger, U.; Brodmann, P.; Wurzl, A. *Deutsche Lebensmittel Rundschau Helf* **1997**, *2*, 35.