Supporting Information

"Off-On" Electrochemical Hairpin-DNA-Based Genosensor for Cancer Diagnostics

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Figures:

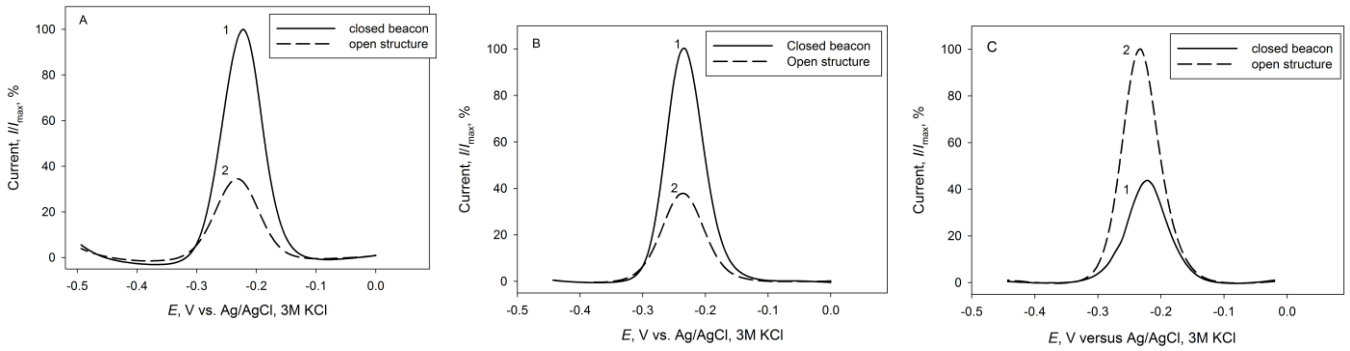


Figure 1S. Representative differential pulse voltammograms recorded for the electrochemical genosensor before and after interaction with a saturated amount (250 nM) of a complementary target DNA. In the genosensor construction, the Au electrode was modified with MB-labeled (A) 33 nts long *TP53*-specific beacon sequence; (B) 27 nts long beacon sequence from refs [1,2], (cathodic currents); and (C) 20 nts long beacon obtained by truncation of the beacon in (B) (anodic current). The studied DNA surface coverage was 4.8±2.1, 5.2±2.3, and 4.1±1.9 pmol cm^{-2}, correspondingly

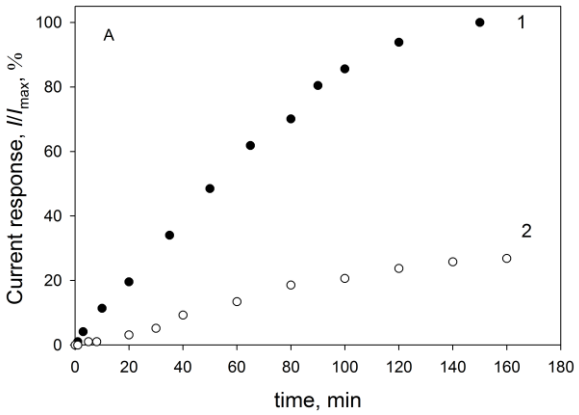


Figure 2S. Time dependence of the *TP53* (20 nts long) genosensor response, (A) normalized for the maximum current signal I_{max} observed at saturation concentration of target DNA, upon reaction with 200 nM of (1) fully complementary target DNA sequence and (2) target DNA sequence with SNP

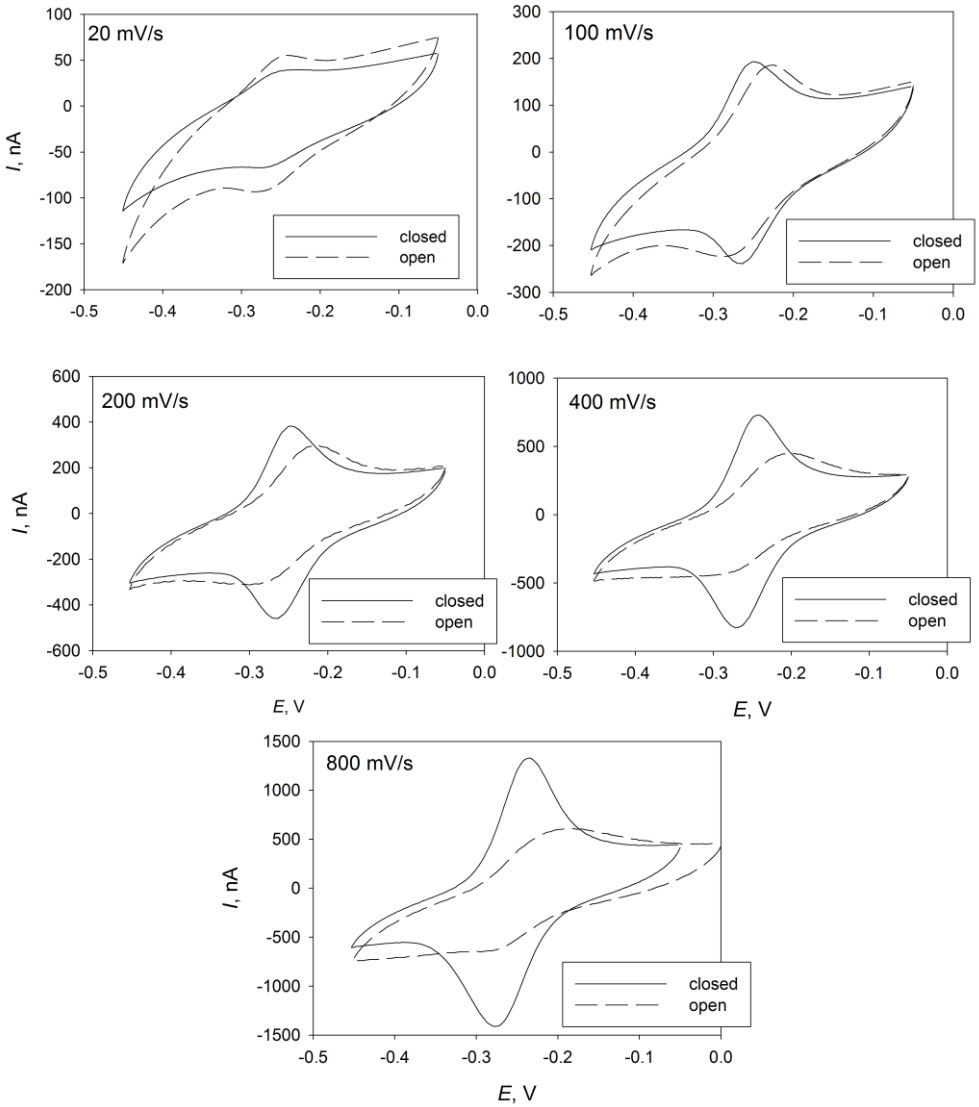
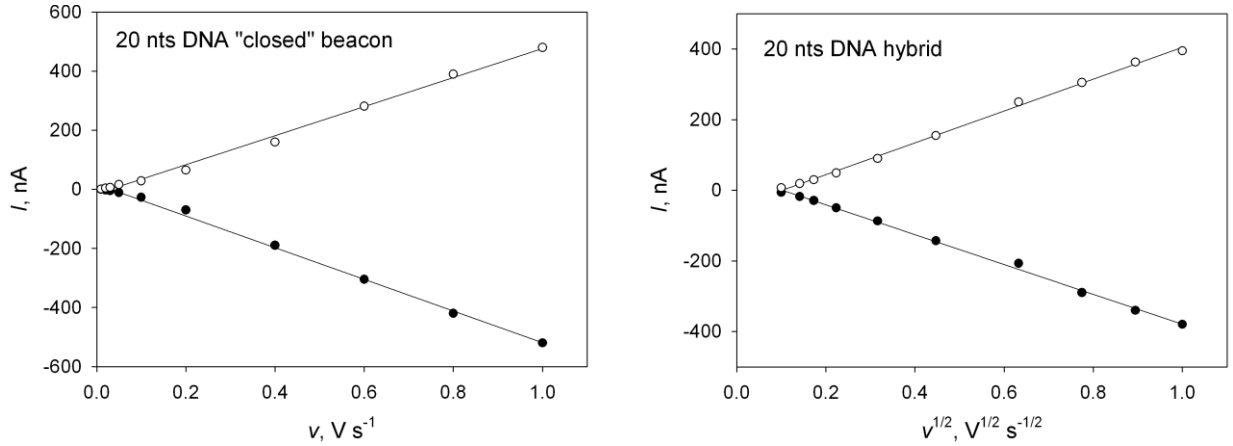


Figure 3S. Representative CVs recorded with the MB-labeled 27 nts long DNA hairpin probes from refs. [1, 2], immobilized through the alkanethiol linker at the Au electrodes. Potential scan rates are 20, 100, 200, 400 and 800 mV s^{-1}. Solid lines: folded closed state of the beacon; dashed lines: unfolded open DNA state, produced by 2 h hybridization to the complementary target DNA. The DNA surface coverage is 13.8 pmol cm^{-2}. CVs recorded for this surface coverage demonstrate the same pattern as was shown with 33 nts long *TP53*-specific DNA probe at low and high potential scan rates, also at the lower surface coverage (main text, Figure 5). The average surface coverage for this beacon was 5.2±2.3 pmol cm^{-2}, and 13.8 pmol cm^{-2} was the extreme, highest value specially studied to find out if the observed effects were still present.



A B

Figure 4S. Representative dependences of the cyclic voltammetric peak currents *I* on scan potential rate *v* and square root of scan rate $v^{-1/2}$ for (A) a folded "closed" MB-labeled DNA beacon and for (B) an "open" DNA probe, hybridized to the complementary target DNA. The DNA surface coverage is 5.2 pmol cm^{-2}.

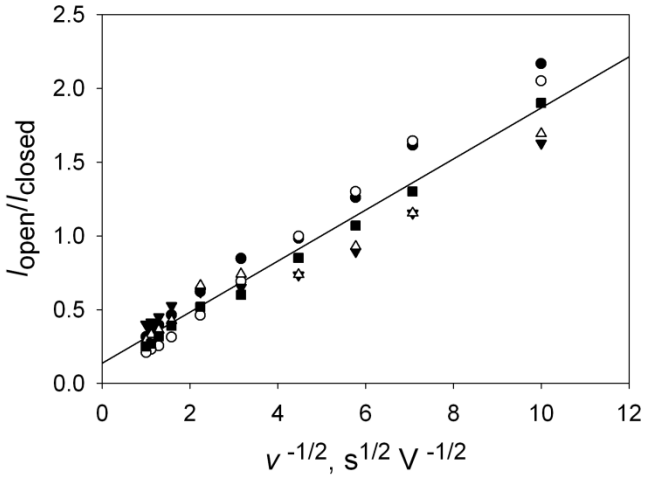


Figure 5S. Dependences of the CV peak current ratio I_{open}/I_{closed} for open and folded closed DNA beacons on the inverse square root of the potential scan rate $v^{-1/2}$ for 27 nts long DNA hairpin probe.

Expression for the relation between peak currents in CV for diffusionless (closed beacon) and limited by the diffusion of redox species (open beacon) electrochemistry, derived for the case of the redox-labeled DNA hairpin beacons.

In linear potential sweep voltammetry the dependence of the voltammetric peak current on potential seep rate for a diffusion limited process is expressed as:

$$I_p^{\,dif} = (2.69 \times 10^5) n^{3/2} A D_{\text{redox}}^{\,1/2} v^{1/2} C^* \qquad (1)$$

where n is the number of electrons transferred, A is the electrode area, D_{redox} is the diffusion coefficient of e.g. the oxidized form, if one consider cathodic process, v is the scan rate and C is the bulk concentration in moles per cubic cm.

Taking into account that all redox species, namely the MB-redox probes, are localized in the near-to-the electrode layer of a maximal thickness l, where l is the length of the DNA, in cm, the concentration of the MB species can be expressed as:

$$C^*_{\text{MB}} = m / A\, l \qquad (2)$$

where m is the number of moles of MB species at the electrode surface.

The final expression for the dependence of the voltammetric peak current I_{open}, in A, on the potential sweep rate v can be presented as follows:

$$I_{\text{open}}^{\,\text{dif}} = (2.69 \times 10^5) n^{3/2} D_{\text{open}}^{\,1/2} v^{1/2} m/l \qquad (3)$$

where D_{open} is the diffusion coefficient of the MB species conjugated to the DNA (and actually related to the diffusion of the dsDNA strand), in $cm^2\ s^{-1}$. On the other hand, for surface adsorbed species, with no diffusion limitations of the redox process, the dependence of the voltammetric peak current I_{closed}, in A, on potential sweep rate is expressed as:

$$I_{\text{p}}^{\,\text{surf}} = (n^2F^2/4\text{RT}) v A \Gamma = (9.39 \times 10^5)\, n^2\, v A \Gamma \qquad (4)$$

Where Γ is the surface coverage with MB-modified DNA (adsorbed redox species) expressed as m / A.

Thus, the final relation between the peak currents I_{open}/ I_{closed} can be presented in the form:

$$I_{\text{open}} / I_{\text{closed}} = I_{\text{p}}^{\,\text{dif}} / I_{\text{p}}^{\,\text{surf}} = 0.286\, D_{\text{open}}^{\,1/2} / n^{1/2} v^{1/2}\, l \qquad (5)$$

For the MB redox reaction n equals 2, so expression (5) simplifies to

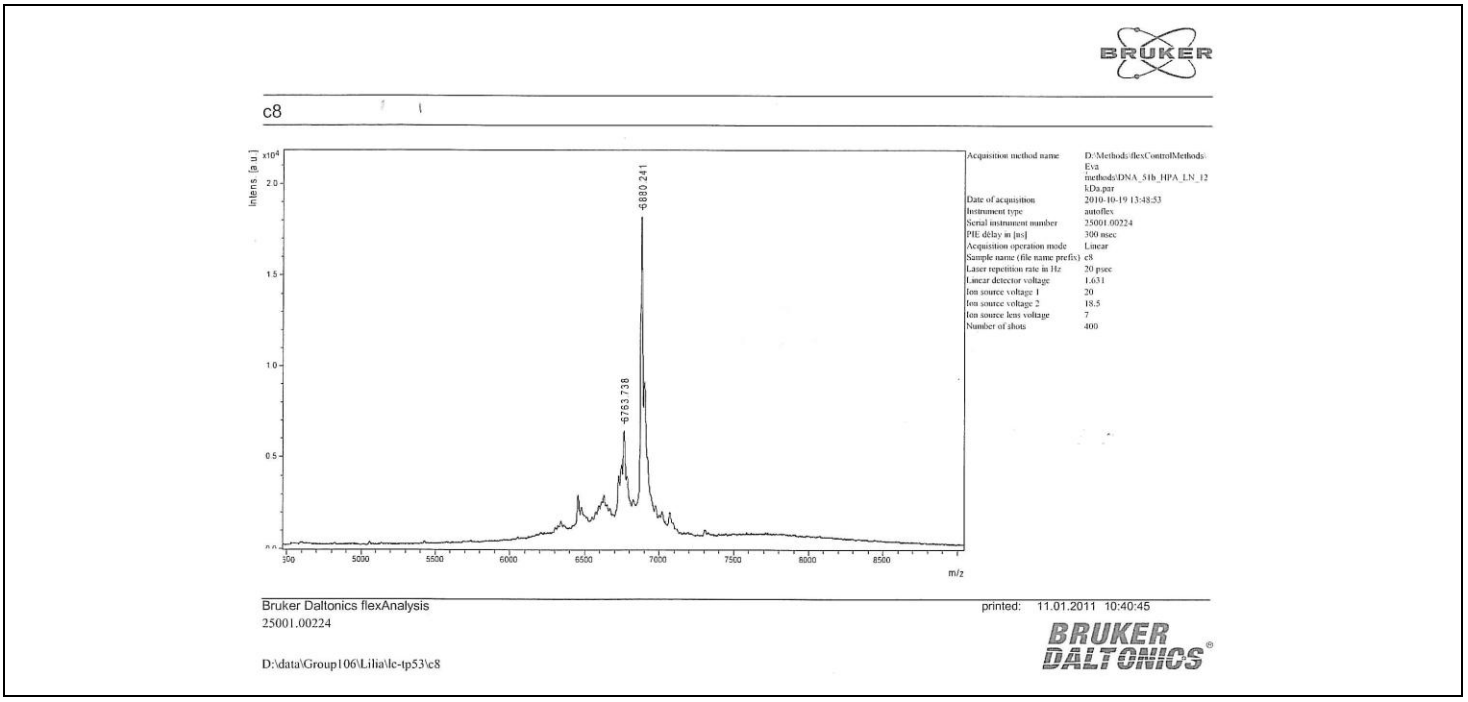
$$I_{\text{open}} / I_{\text{closed}} = 0.2\, D_{\text{open}}^{\,1/2} / v^{1/2}\, l \qquad (6)$$

References:

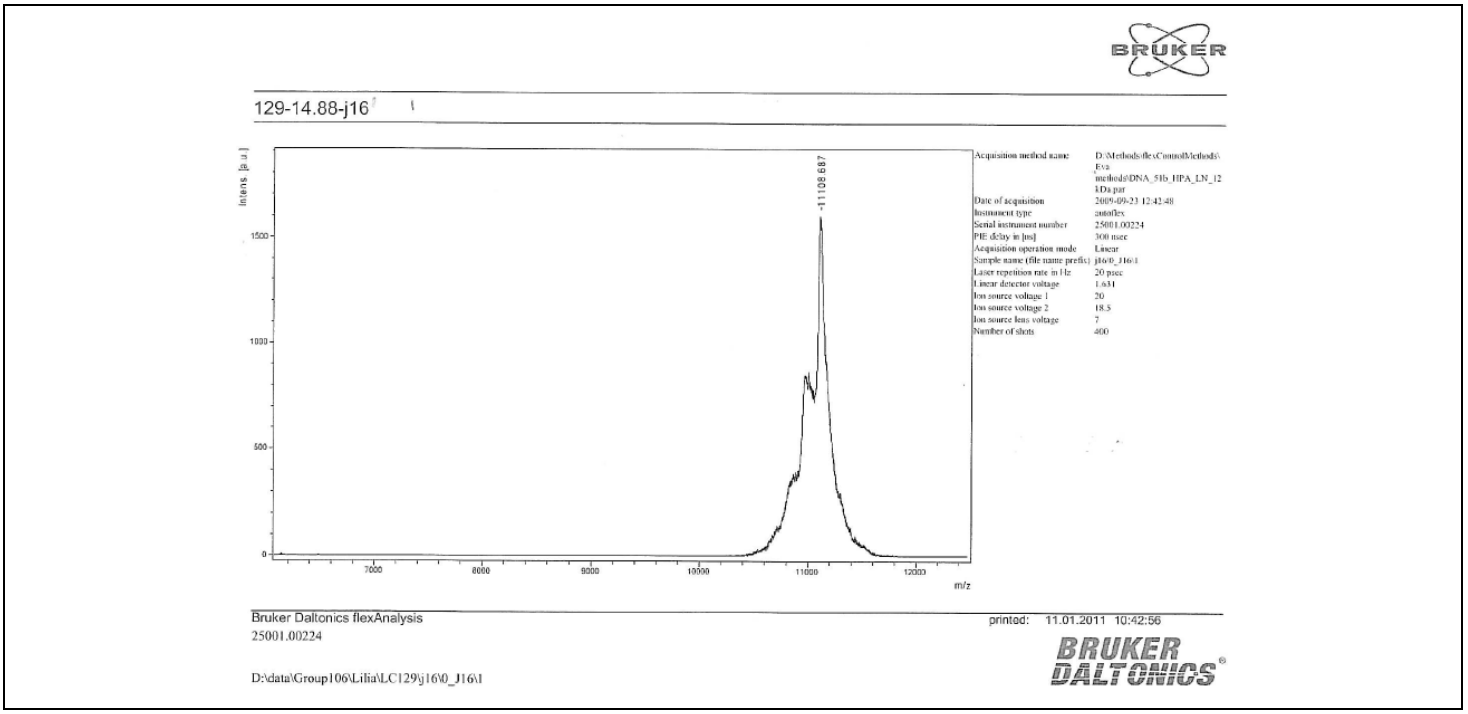
(1) Fan, C.;Plaxco, K. W.;Heeger, A. J. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9134-9137.

(2) Lai, R. Y.;Lagally, E. T.;Lee, S.-H.;Soh, H. T.;Plaxco, K. W.;Heeger, A. J. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4017-4021.

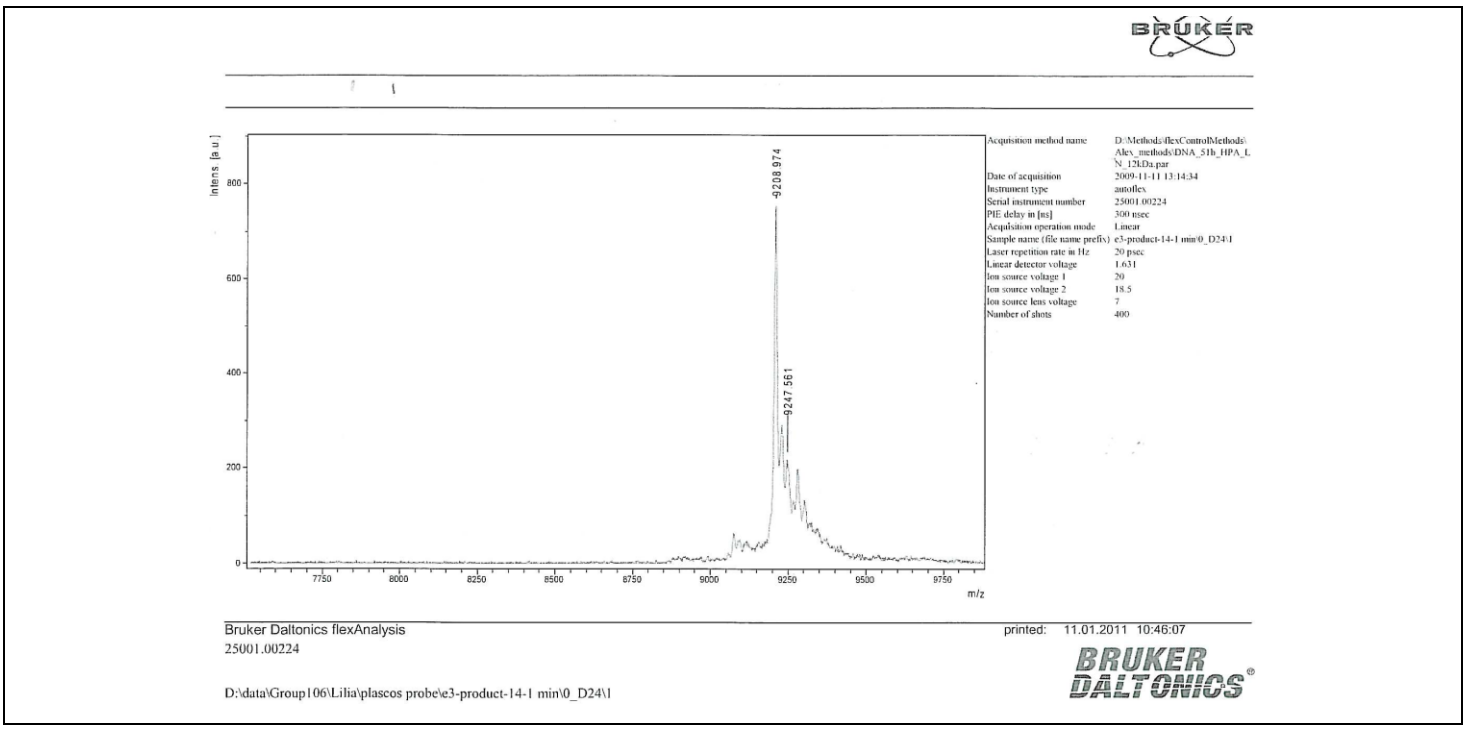
Representative MALDI spectra of the produced MB-DNA conjugates:



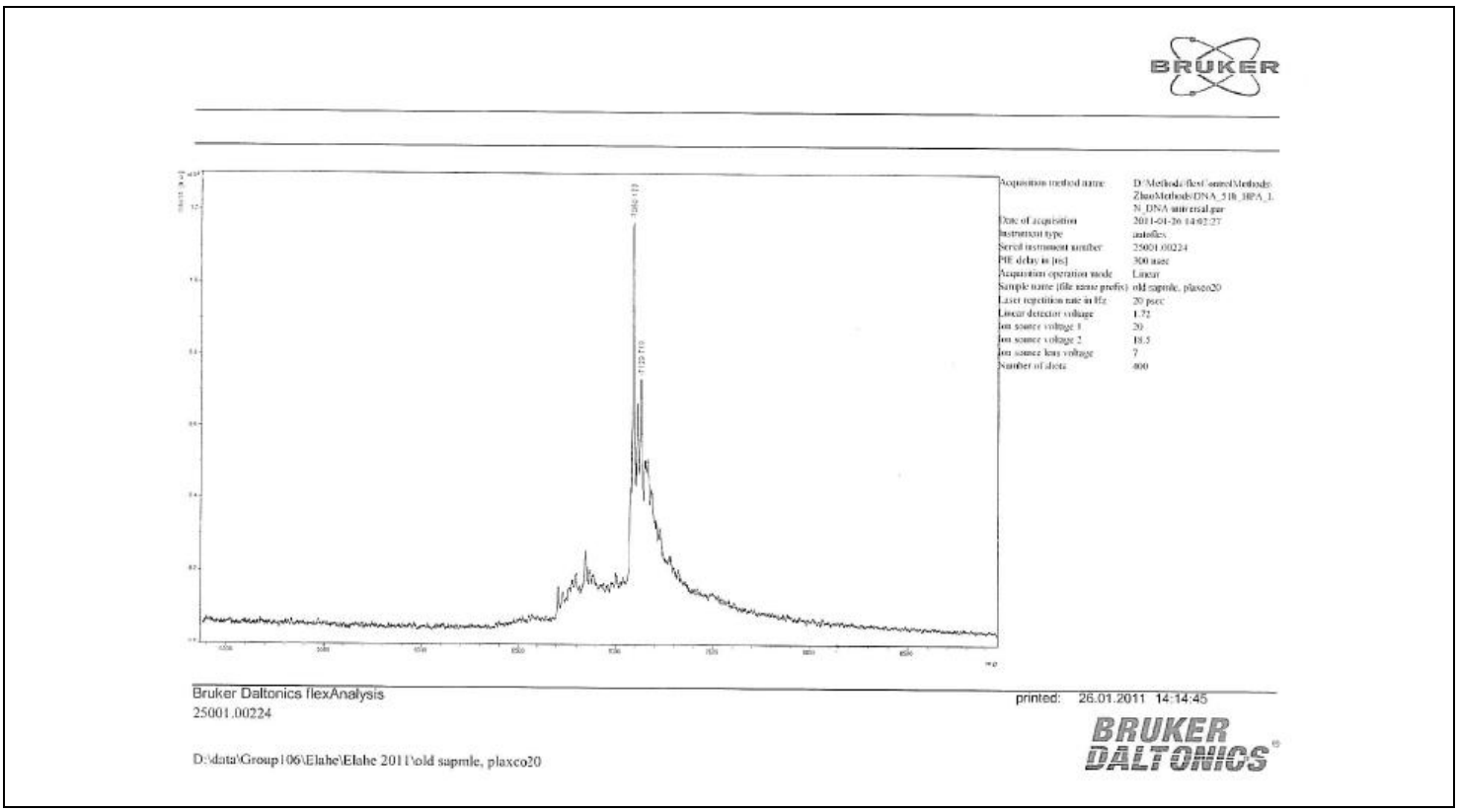
Truncated TP53



33 nts-long TP53



27 nts-long hairpin DNA



20 nts-long hairpin DNA