

Direct Acoustic Profiling of DNA Hybridization using HSV Type 1 Viral Sequences



Introduction

This TECHnote describes the detection of specific, conserved DNA sequences of herpes simplex virus (HSV) type 1. HSV causes recurrent mucosal infections of the eye, mouth and genital tract. HSV type 1 establishes a lifelong latent infection within the host which can subsequently reactivate to cause recurrent infections and occasionally life threatening HSV encephalitis. Following infection the virus gains access to sensory nerve terminals and latency is established in corresponding sensory neurons.

A RAPid 4 instrument was used as the biosensor platform for the detection of HSV type 1 viral sequences. One notable advantage of RAP (Resonant Acoustic Profiling) detection over more established optical label-free detection is the relative insensitivity of acoustics to changes in solvent/medium when running samples in complex media such as serum, plasma or whole blood. Optical detection systems suffer from large bulk shifts which need to be minimized by calibration routines and dilution of the sample. In contrast, acoustic systems are not affected by refractive index changes, but are instead sensitive to bulk effects dominated by the viscosity and density of the media. Thus viral detection from clinical samples with minimal sample processing is feasible when acoustic sensors are employed for nucleic acid testing.

Experimental Design

NeutrAvidin™ (NA) prepared in PBS buffer was covalently immobilized to the sensor surface by amine coupling. This was followed by changing the running buffer to Tris buffer, pH 7. The biotinylated complementary surface probe and scrambled surface probe were diluted in Tris buffer to $10\mu\text{g}\cdot\text{ml}^{-1}$ and each injected over separate flow cells for 5 min to create active and control surfaces. A sample of 10mM biotin in Tris buffer was injected for 1min to block the remaining active sites of the NA layer.

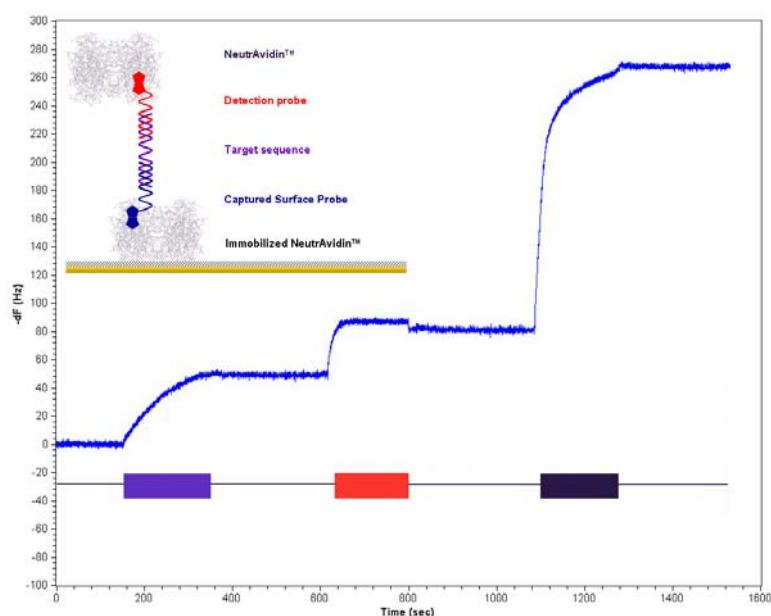


Figure 1: The hybridization assay with signal enhancement is shown. Synthetic target ssDNA oligomers VP16 were injected over the probe-immobilized sensor surface and allowed to hybridize for 3 min. Then the VP16 detection probe at $2.5\mu\text{g}\cdot\text{ml}^{-1}$ was injected to allow hybridization with the VP16 target sequence for 3 min. Finally $5\text{mg}\cdot\text{ml}^{-1}$ NA was injected to the resultant sensor surface to be captured by the VP16 detection probe for 3 min. After a 180 s dissociation period under Tris buffer flow at $25\mu\text{l}\cdot\text{min}^{-1}$, surfaces were regenerated by two successive 30 s injections of 1mM HCl. Frequency change due to oligomer hybridization and NA capture were recorded after the injection finished.

Table 1: ssDNA sequences used in this study. Probes were synthesized by TIB Molbiol, Berlin, Germany.

Name	ssDNA sequence
VP16 surface probe	5'-Biotin-CTC GTT GGC GCG CTG AAG CAG GTT TTT G-3'
VP16 scrambled surface probe	5'-Biotin-ACC TGG GCA TGT ATG GTG TCG TCG CGT T-3'
VP16 target sequence	5'-AAA ACT TCC GTA CCC CTC AAA AAC CTG CTT CA-3'
VP16 detection probe	5'-GGG TAC GGA AGT TTT TCA CTC GAC-Biotin-3'



Selected Data

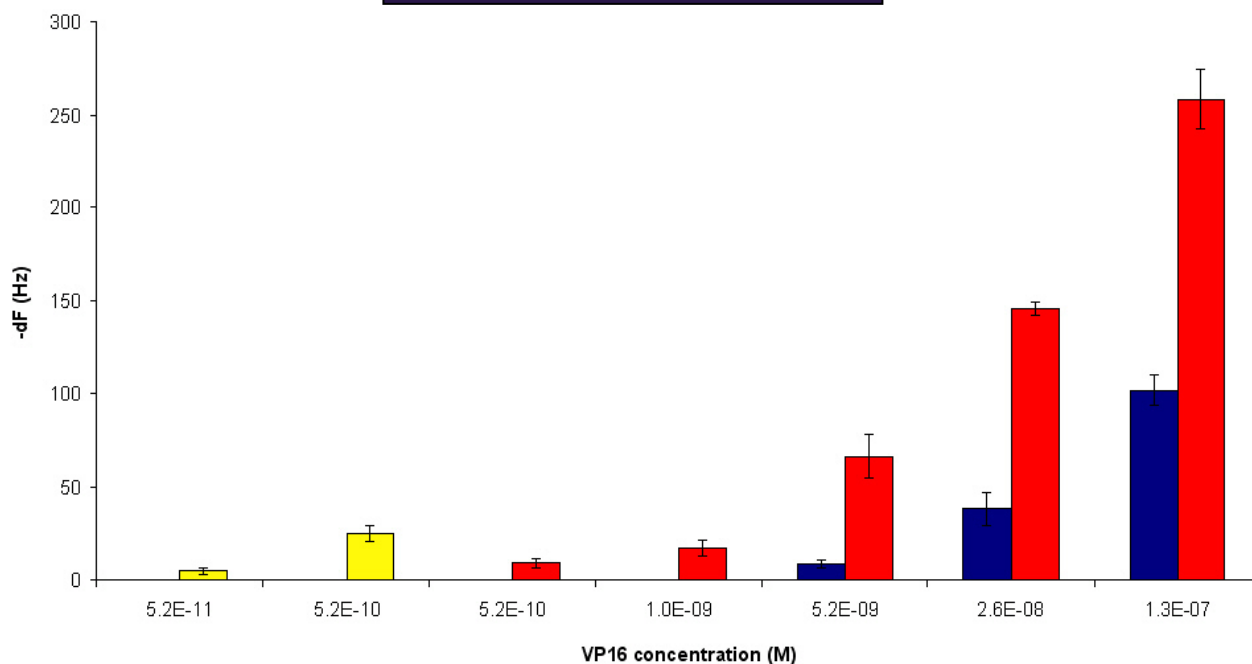


Figure 2: Blue columns: VP16 target sequence hybridization onto the surface probe. Red columns: NeutrAvidin™ capture onto the VP16 detection probe. Yellow columns: NA capture after the injection of solution-hybridized VP16 target sequence and detection probe ($n = 4$ for all results).

The hybridization between the VP16 surface probe and the complementary oligonucleotide VP16 target sequence was tested in a concentration range from $5.2 \cdot 10^{-11}$ to $1.3 \cdot 10^{-7}$ M. The resultant theoretical detection limit of DNA hybridization for this assay with NA signal amplification was $1 \text{ ng} \cdot \text{ml}^{-1}$ ($1 \cdot 10^{-10}$ M).

Notably, it was found that DNA hybridization efficiency can be higher when hybridization is performed at the annealing temperature in free solution rather than via *in situ* hybridization to a probe on the biosensor surface. Hence, the target sequence and detection probe were first hybridized in free solution at 36°C and then injected over the biosensor surface. Subsequent addition of NA resulted in a 5 ± 2 Hz frequency change for $5.2 \cdot 10^{-11}$ M target concentration.

Conclusion

Nucleic acid-based testing has been increasingly used for the detection of viral pathogens. The implementation of biosensors to nucleic acid-based recognition can provide real-time, label-free and quick testing of samples. Piezoelectric biosensors are able to operate in un-diluted, un-processed complex media and, being entirely electronic, are suitable for miniaturization.

The sensitivity of oligonucleotide hybridization achieved by quartz crystal microbalance (QCM) based sensors has been previously reported to be ca. 10^{-8} M. Nanoparticles are commonly used to enhance the sensitivity of hybridization to the region of 10^{-15} - 10^{-16} M. We have shown that without the use of nanoparticles but utilizing capture protein, a hybridization sensitivity of 10^{-11} M can be achieved.

This level of sensitivity necessitates amplification of DNA by means of PCR or PCR-like amplification methods; however, by reducing the detection limit of the DNA to the region of 10^{-18} M or lower, it may be possible to avoid PCR amplification. Further studies will continue to improve the limit of detection for the recognition of HSV type 1 and other viral nucleic acids.

The complete study was published:

Yıldız Uludağ, Xin Li, Heather Coleman, Stacey Efstathiou and Matthew A. Cooper, 2008, Direct acoustic profiling of DNA hybridization using HSV type 1 viral sequences. *Analyst* **133**: 52 – 57.



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