



# Development of a Monitoring System for Microorganisms in the Environment Using DNA Array Technology

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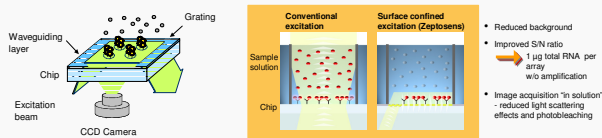
Besides the enforcement of the Swiss ordinance regulating the contained use of organisms, our laboratory is engaged in bio-emergency and disaster response. For these purposes, we establish methods for the identification and quantification of bacteria and viruses in laboratories and in the environment utilizing the following technologies: Real Time PCR, viral infectivity test, in-culture quantitative PCR, sequencing and fragment analysis (RISA). In the event of a major accident where microorganisms escaped from biotech production units or research facilities or in the case of a bioterror event it is not always clear which organisms have been released into the environment and whether or not they have spread and survived. To answer these questions a project has been initiated to develop a DNA microarray-based screening method that allows the fast, sensitive, and reliable detection and identification of a relevant set of microorganisms.

The chosen approach takes advantage of the high-sensitivity planar waveguide (PWG) microarray technology commercialized by Zeptosens. Assay performance is determined by comparing microarray data with those obtained using Real Time PCR. In the first phase of array development we defined and tested the optimal physical parameters for the detection of 16S rRNA genes from *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In the second phase the array was extended to discriminate about 40 bacterial species. We started to optimize the array using multiplex sequence specific end-labeling (SSEL\*). We were able to show that PWG based technology in combination with SSEL is able to detect microorganisms in the range of 50 to a few hundred genome copies compared to 10-20 genome copies using TaqMan PCR. For validation of the detection system spiked environmental samples will be used.

## 1. Phase: Identification of 10-12 Bacteria

### A. Test of Array Platform and Physical Parameters

#### Zeptosens Platform: Planar Waveguide (PWG) based Read-out Technology



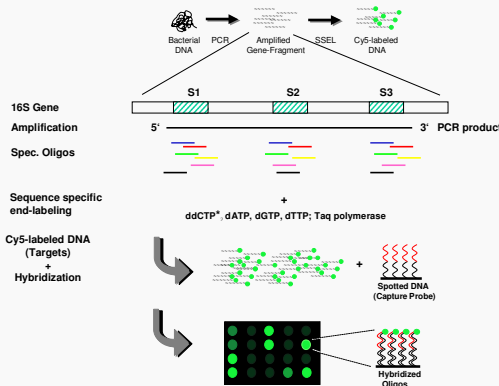
The analytes of interest are immobilized on the waveguide surface. The presence of the analytes in a sample applied to a PWG chip is detected. Upon fluorescence excitation by the evanescent field, excitation and detection of fluorophores is restricted to the sensing surface, whilst signals from unbound molecules in the bulk solution are not detected.

### Pilot-Array: Test of Physical Parameters

In a pilot array we tested a) the length of spotted DNA, and b) the influence of mismatches on hybridization with the labeled oligo. Two different sets of capture probes were used: The first set had a 24-mer variable region, resembling part of the 16S rRNA gene from *Pseudomonas aeruginosa*, the second set had a 33-mer variable region, resembling part of the 16S rRNA gene from *Staphylococcus aureus*. The length of the constant region of each set varied with the total length of the probe.

## B. Labeling

### Sequence Specific End-labeling\* of DNA (SSEL) and Hybridization of Labeled Oligos



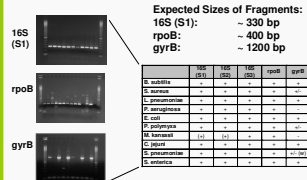
Total DNA from selected bacteria is PCR amplified with universal primers for 16S rDNA (whole 16S region or regions S1-S3). Specific oligos are added and labeled sequence specifically with Cy5. Hybridization follows on Zeptosens platform.

## C. Test of Primersets

### Microorganisms

- Bacillus subtilis*
- Staphylococcus aureus*
- Legionella pneumophila*
- Pseudomonas aeruginosa*
- Escherichia coli*
- Paenibacillus polymyxa*
- Mycobacterium kansasii*
- Campylobacter jejuni*
- Streptococcus pneumoniae*
- Salmonella enterica*

### Test of Universal Primersets



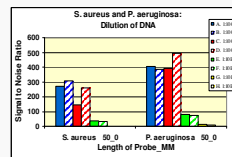
Test of universal primersets for 16S rRNA, rpoB and gyrB genes with 10 different bacterial strains.

## 2. Phase: Array for up to 40 Bacteria

- 170 loci on array → ca. 4-5 probes/MO (1020 different genes per chip)
- Development and testing of PCR followed by sequence-specific end-labeling
- Validation of the system using spiked environmental samples
- Comparing the sensitivity of SSEL combined with Microarray system (Zeptosens platform) to Real Time PCR based system

## A. Comparison of DNA Microarray and TaqMan® PCR

### Detection limit of bacterial DNA in Microarray and TaqMan® PCR



**DNA Microarray:** Genomic DNA of 9 different bacteria is mixed with genomic DNA of *S. aureus* or *P. aeruginosa* (1:10<sup>2</sup>, 1:10<sup>3</sup>, 1:10<sup>4</sup>, 1:10<sup>5</sup>, 1:10<sup>6</sup>). A region of the 16S rDNA gene is amplified and after purification and sequence-specific end-labeling (SSEL) hybridized on a chip.

*S. aureus*: Detection limit at a dilution of 1:10<sup>4</sup>  
*P. aeruginosa*: Detection limit at a dilution of 1:10<sup>5</sup>

The figure shows one out of four experiments. MM: Mismatch

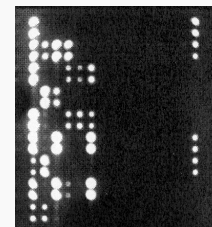
### Detection limit of TaqMan® PCR and DNA Microarray

	Genome Copies	
	Microarray	TaqMan PCR
<i>Staphylococcus aureus</i>	50-800*	10-20
<i>Pseudomonas aeruginosa</i>	80-400*	10-20

\* Range of results from four different experiments

## E. Preliminary Results

### Hybridization Results with Complex Bacterial Mixtures



A region of the 16S rDNA gene from 10 different bacteria was amplified by PCR. After purification, sequence-specific end-labeling (SSEL) was carried out simultaneously with 28 different oligos for this region, followed by the hybridization onto the chip.

- All 10 bacterial species could be detected
- Simultaneous labeling is possible, but some cross-reactivity occurs

## Conclusions

- Capture probes shorter than 70-mers can be used on Zeptosens platform
- Sequences with identities of less than 90% can be discriminated on the array (tested for a 24-mer and 33-mer)
- Detection limits for *S. aureus* and *P. aeruginosa* in DNA Microarray is only up to 40x higher compared to TaqMan PCR
- PCR amplification (16S + rpoB) is possible for up to 10 different bacterial species
- Multiplex labeling is possible. The number of oligos that can be used for SSEL simultaneously needs to be validated
- Oligos with cross-reactivity need to be optimized

## Literature

\* Rudi, K., Treimo, J., Nissen, H., Vegarud, G. (2003), Scientific World Journal 3: 578-584

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