



Monitoring for Contaminating Microorganisms in the Environment Using DNA Array Technology

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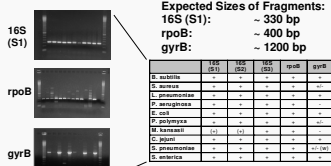
Monitoring for contaminating pathogenic and genetically modified microorganisms requires specific and sensitive detection methods. In our laboratory we establish methods for the identification and quantification of bacteria and viruses on laboratory surfaces and in the environment by applying the following technologies: Real Time PCR (TaqMan) for quantitative detection of specific DNA, infectivity test and in-culture quantitative PCR for the detection of live microorganisms, sequencing for identification of unknown organisms and fragment analysis (RISA) for the discrimination between bacterial strains. In the event of a major accident where microorganisms escaped from biotech enterprises or research facilities or in case of a bioterror event it is not always clear which organisms have been released into the environment. The aim of this project is to develop a DNA array that allows the fast and reliable detection and identification of all (for Switzerland) biotechnologically and bioterror relevant microorganisms.

1. Phase: Identification of 10-12 Bacteria

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

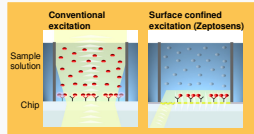
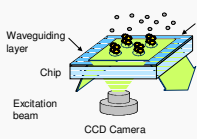


Test of universal primersets for 16S rRNA, rpoB and gyrB genes with 10 different bacterial strains.

A. Test of Array Platform and Physical Parameters

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

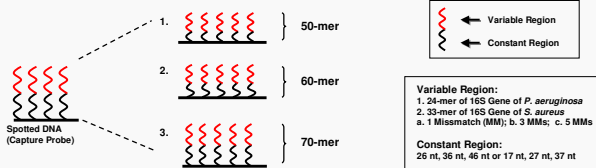
zeptosens
www.zeptosens.com
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- Reduced background
- Improved S/N ratio
- → 1 µg total RNA per array w/o amplification
- Image acquisition "in solution" - reduced light scattering effects and photobleaching

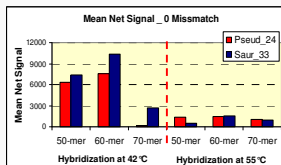
The analytes of interest are immobilized on the waveguide surface. The presence of the analytes in a sample applied to a PWG chip is detected. Upon fluorescence excitation by the evanescent field, excitation and detection of fluorophores is restricted to the sensing surface, whilst signals from unbound 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.

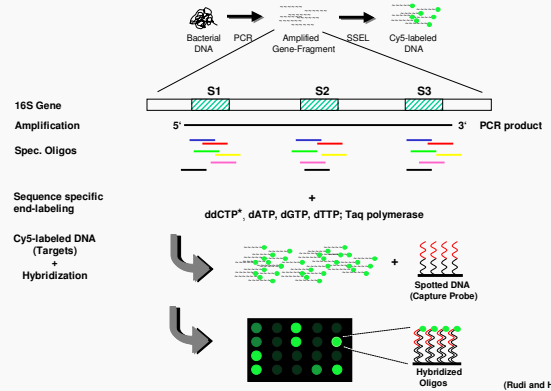
Results: Test of Hybridization Conditions



Hybridization temperature and formamide concentration were tested. Highest hybridization signals and lowest background were achieved at 42°C, 20% Formamide versus 55°C, 10% Formamide.

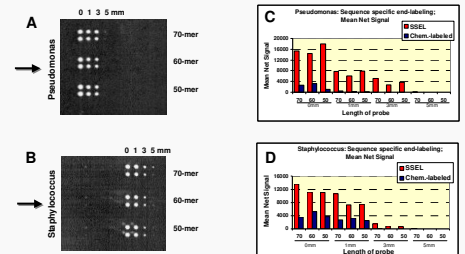
B. Test of Labeling Conditions

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.

Results: Test of Labeling Conditions (SSEL)



Hybridization of pilot array with Cy5-labeled oligos (SSEL), A. *Pseudomonas* specific and B. *Staphylococcus* specific (Fig A+B) in comparison to chemically-labeled oligos (Fig. C+D).

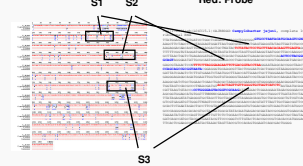
C. Design of Probes

Probe Design 16S rDNA: *C. jejuni*

Multiple Sequence Alignment (Multalin): *C. jejuni*, *C. upsaliensis* und *C. fetus*

16S ribosomal RNA: *Campylobacter jejuni*

Blue: Primer
Red: Probe



Conclusions

- Capture probes shorter than 70-mers can be used on Zeptosens platform
- Sequences with identities of less than 80-85% can be discriminated on the array (tested for a 24- and 33-mer)

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2. Phase: Array for up to 40 Bacteria

- 170 loci on array → ca. 4-5 probes/MO
- Development and testing of multiplex 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