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## Aims

To compare different labeling methods for the preparation of target DNA for microarray experiments.

## Introduction

Nucleic acid microarrays are a recent advance in molecular technologies, offering the possibility to analyze an entire array of microorganisms, concerning their presence or absence in a particular environmental sample, in a single experiment.

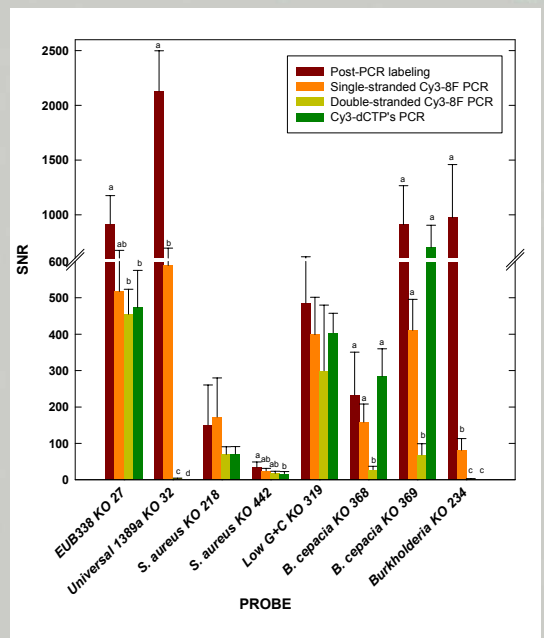
Composting is an aerobic process by which organic materials are degraded by successive groups of microorganisms. In a previous study, we designed and developed an oligonucleotide microarray for the investigation of microbial communities in compost. The compost microarray was systematically optimized by comparing different microarray fabrication parameters. In this study, the efficiency of different methods of labeling 16S rDNA for compost microarray hybridizations are compared.

## Materials and Methods

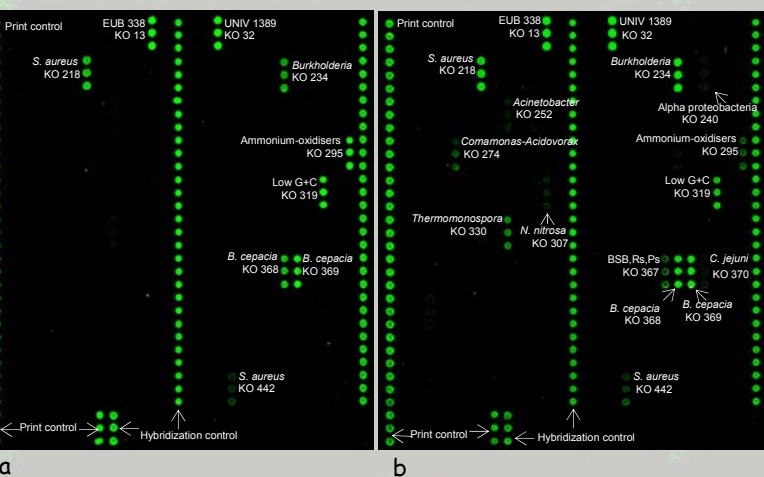
- Design and evaluation of oligonucleotide probes
- Microarray manufacture and processing
- Preparation of labeled *Burkholderia cepacia* and *Staphylococcus aureus* DNA using:
  - Post-PCR labeling process and a DecaLabel DNA labeling kit
  - Cy3-labeled forward primer to generate double-stranded target DNA
  - Cy3-labeled forward primer and Lambda exonuclease enzyme to generate single-stranded target DNA
  - Cy3-dCTP substitutes in PCR
- Hybridization, Scanning of array, Statistical analysis

## Results

- Highest signals obtained with post-PCR labeling but → most non-specific hybridizations (Fig. 1, Fig. 2)
- ss-PCR products labeled with Cy3-forward primer gave the next highest signals for most probes
  - less non-specific hybridizations (Fig. 2)
- ds-PCR product (labeled with Cy3-forward primer, or with Cy3-dCTP's) resulted in acceptable SNR ratios for all probes except probes located toward the 3' end of the 16S rRNA gene
  - Possible partial renaturation of two strands resulting in inaccessibility of 3' end target site
  - Possible steric hindrance effect as result of partially ds-PCR product



**Figure 1:** Signal intensities of probe-target duplexes obtained using the different labeling methods. Columns represent average SNR values from triplicate experiments with triplicate spots. Error bars indicate the standard deviation from the means of each probe. The bars are labeled with the letters a, b and c to indicate statistically significant differences within each probe group at  $P \leq 0.05$ .



**Figure 2:** Fluorescence images showing hybridization of Cy3-labeled 16S rRNA products to the microarray. The microarray with immobilized probes was hybridized with (a) Cy3-8F-labeled PCR product and with (b) Post-PCR process Cy3-labeled PCR product. In both experiments, only *B. cepacia* and *S. aureus* DNA was labeled. Spots indicate positive hybridization signals from the probes.

## Conclusions

Comparison of different DNA labeling methods revealed that labeling via the Cy3-forward primer approach is the most appropriate for the preparation of labeled target DNA for our purposes. It is a time- and cost-effective method of DNA labeling for microarrays.

## Acknowledgements

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