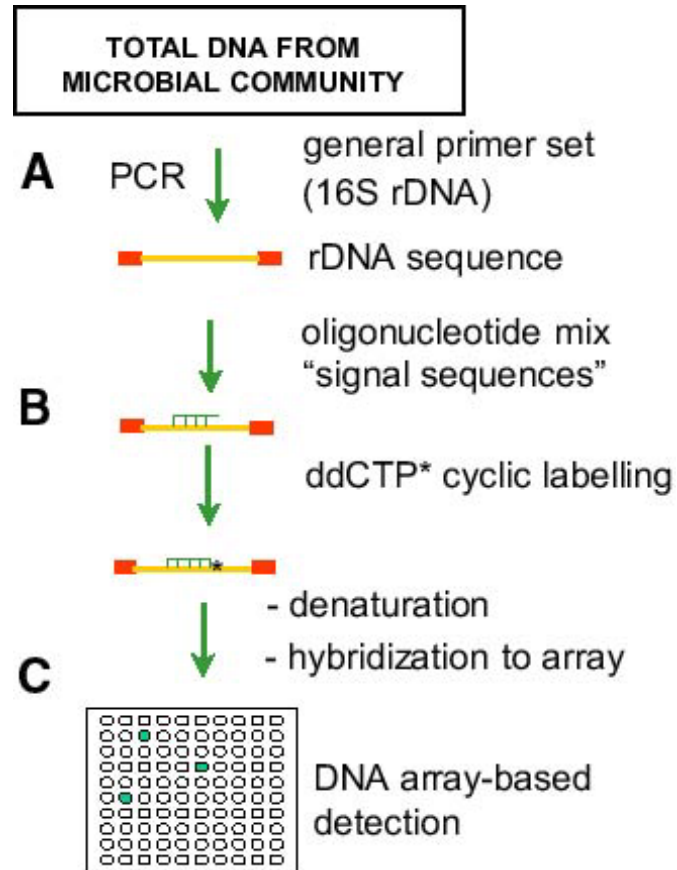


Application of sequence-specific labeled DNA probes in combination with array for fingerprinting and microbial community analyses

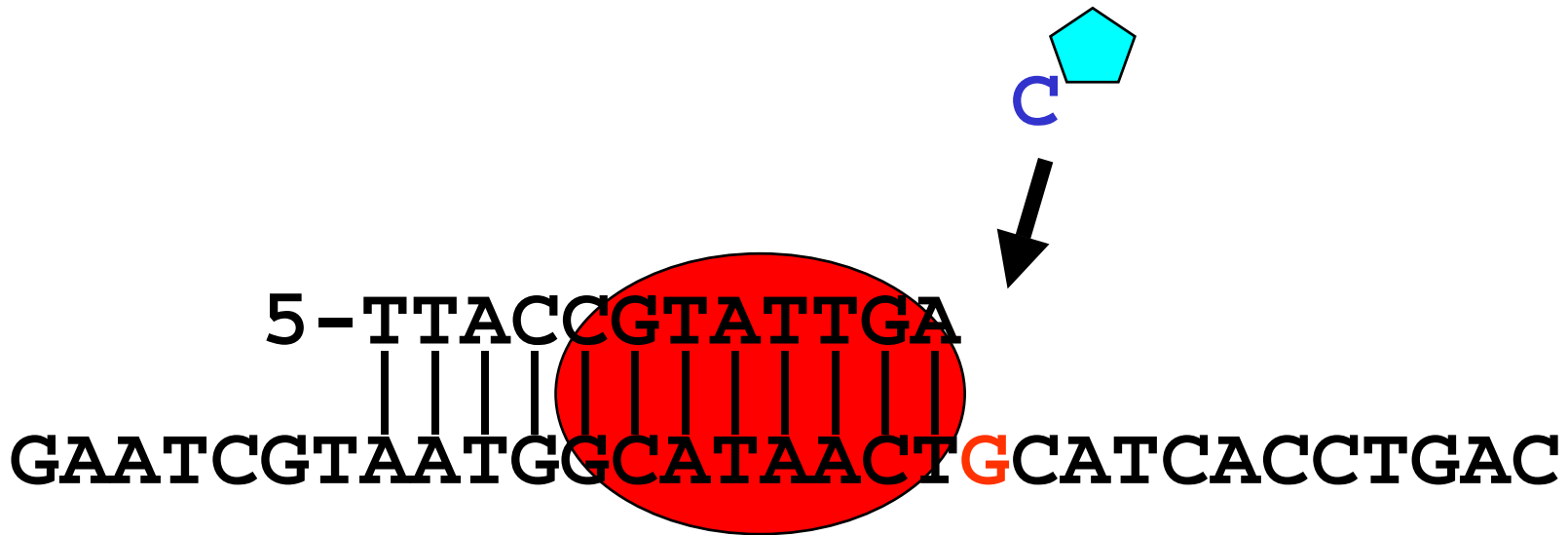
Knut Rudi

MATFORSK Norwegian Food Research Institute

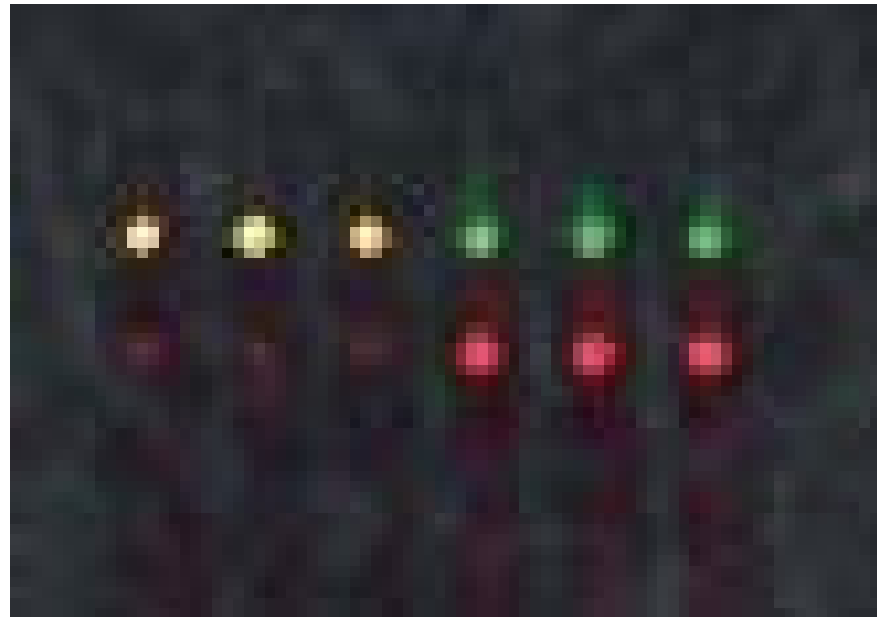
16S rDNA ARRAY ANALYSES



SEQUENCE-SPECIFIC LABELING

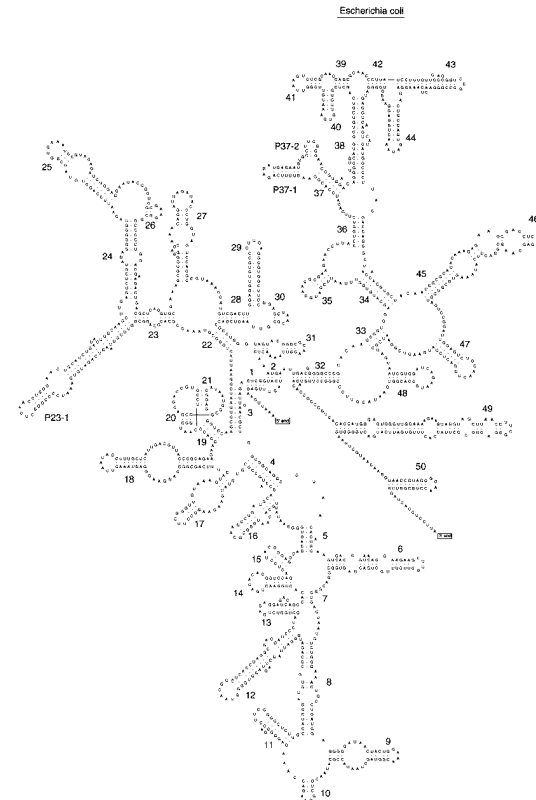


DNA microarray data



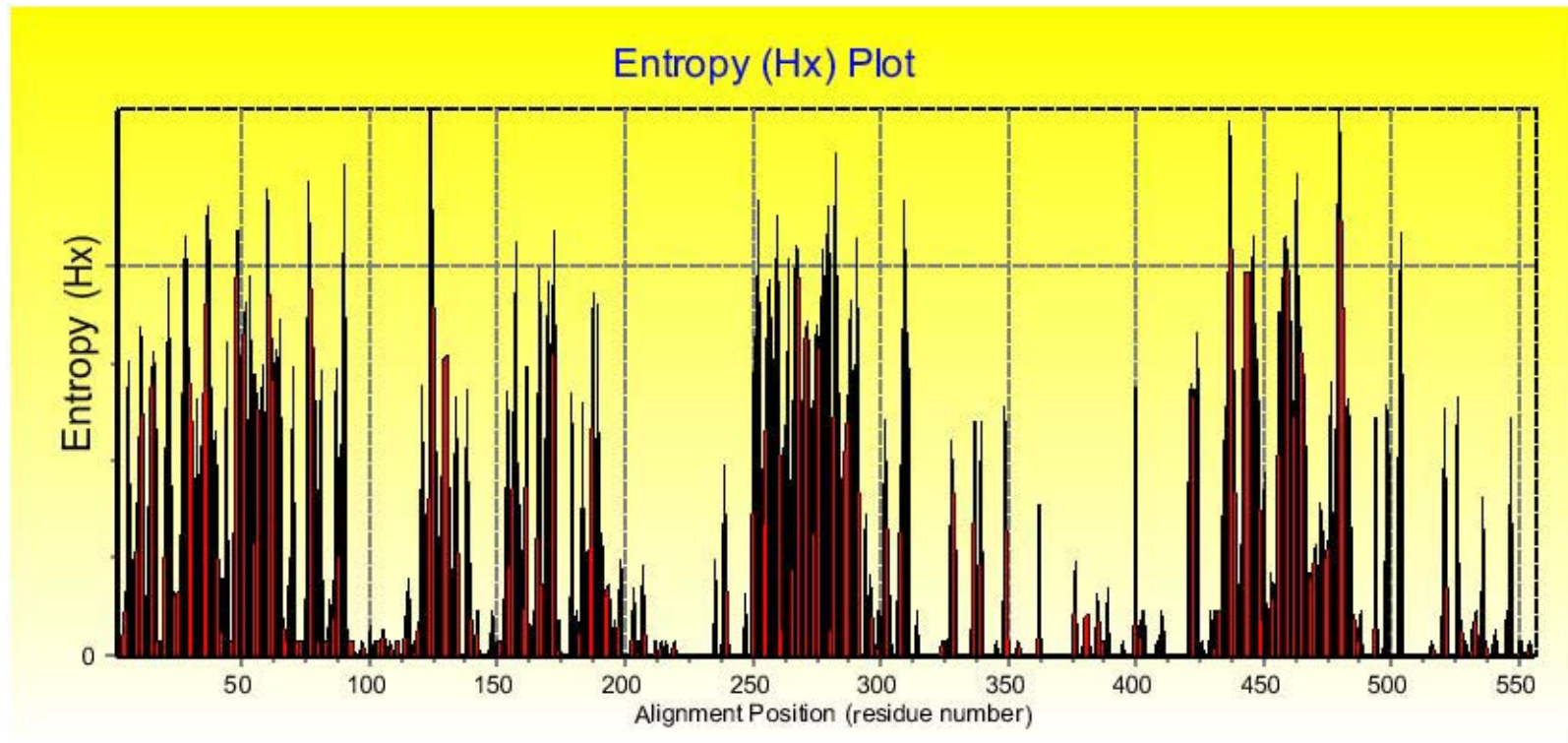
MULTIPLEX ANALYSES OF MICROBIAL COMMUNITIES BY 16S rDNA

- ◆ Analyses of salad matrix
- ◆ Measuring autolysis
- ◆ Fish spoilage



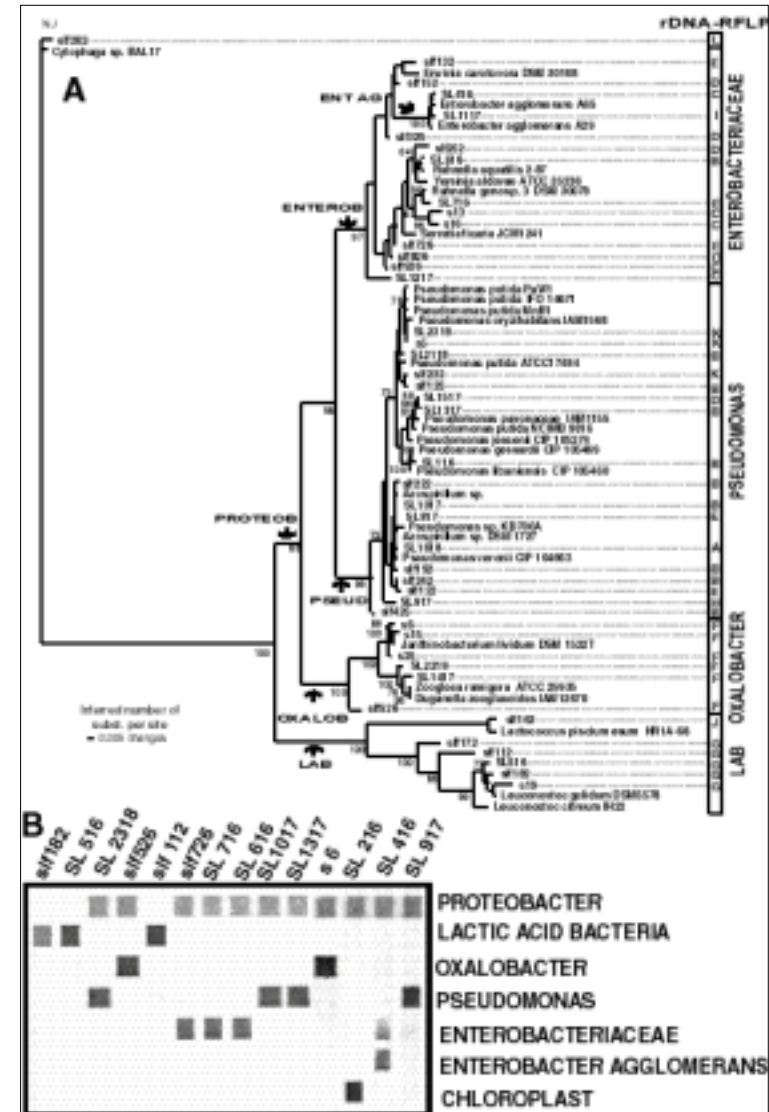
Secondary structure of 16S rRNA

PHYLOGENETIC INFORMATION IN 16S rDNA



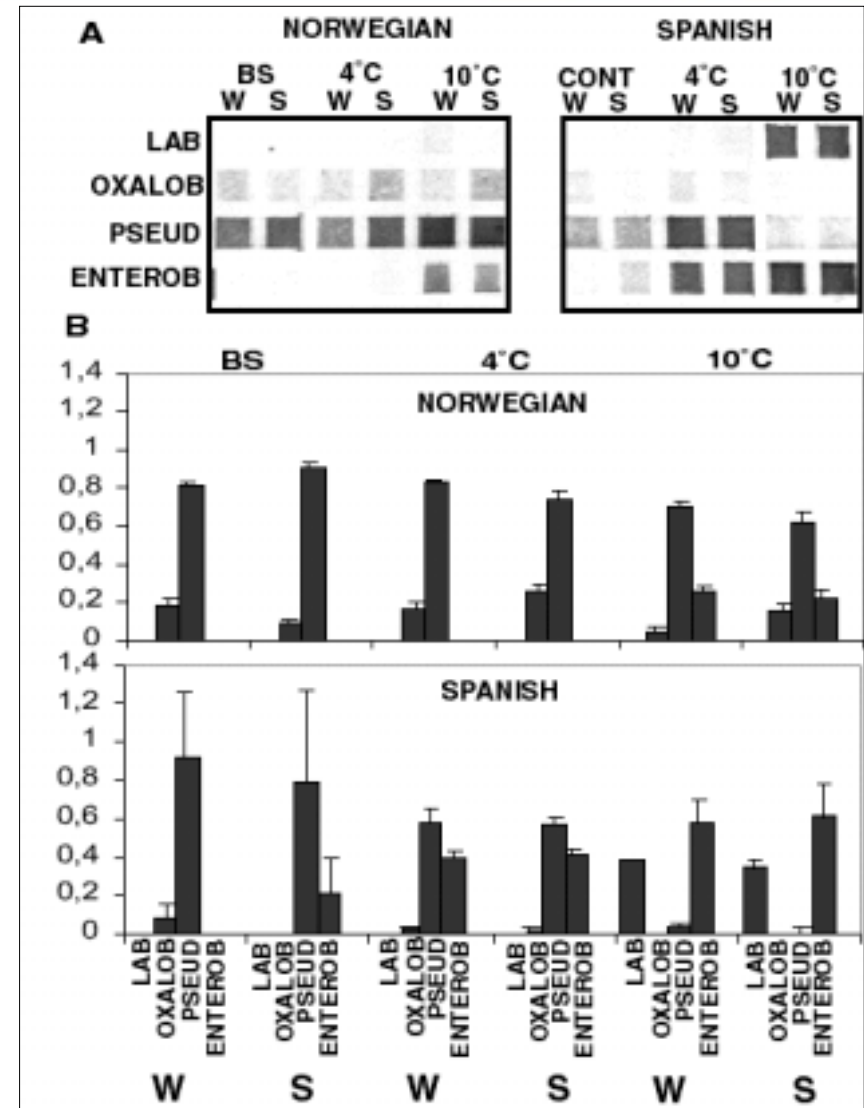
PROBE IDENTIFICATION

- ◆ Phylogenetic reconstruction
- ◆ Identification of point mutations in multiple alignments
- ◆ Testing of probes on pure isolates



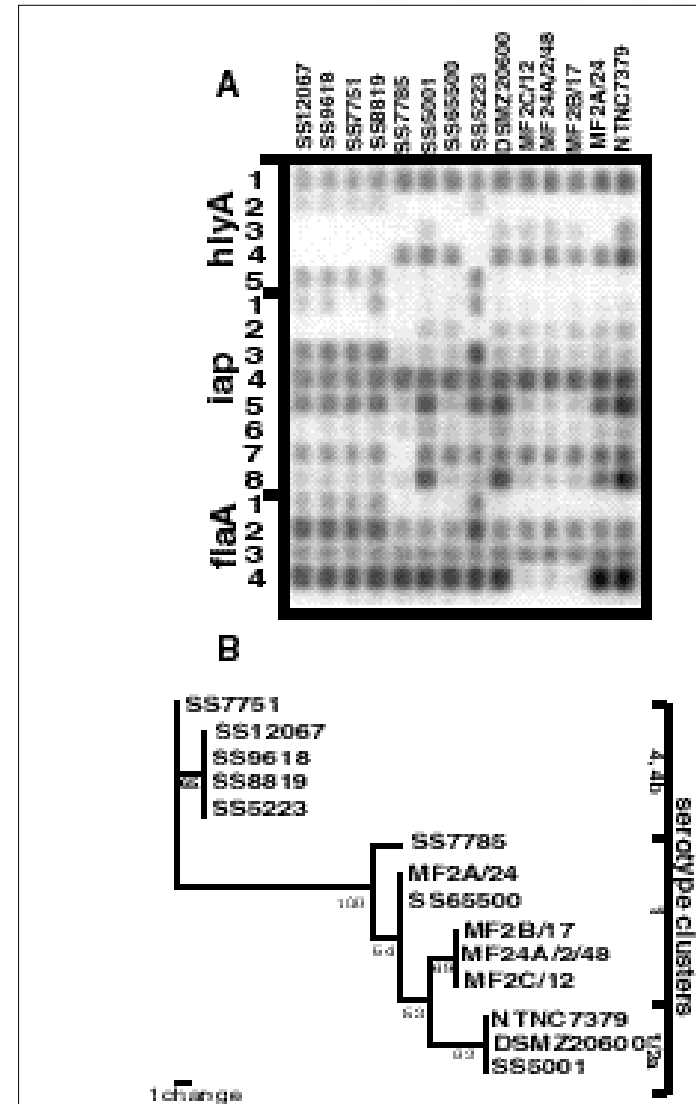
ANALYSES OF NATURAL COMMUNITIES

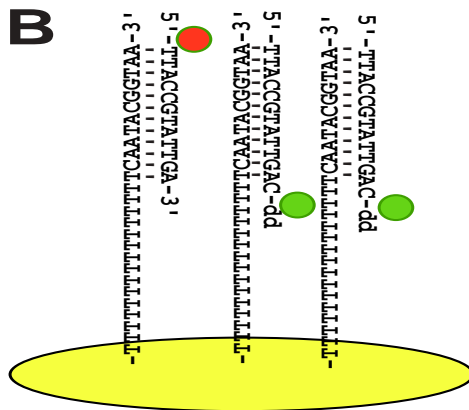
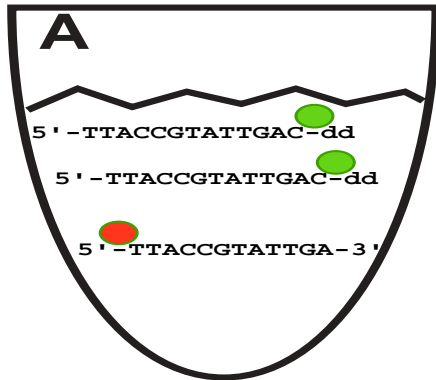
- ◆ DNA purification directly from natural samples
- ◆ Analyses of the intact communities with specific probes
- ◆ Genetic profiles for entire communities are obtained



FINGERPRINTING BY DNA ARRAYS

- ◆ *Listeria monocytogenes*
- ◆ Multi Locus Sequence Typing
- ◆ Targets
 - hly A
 - iap
 - fla A

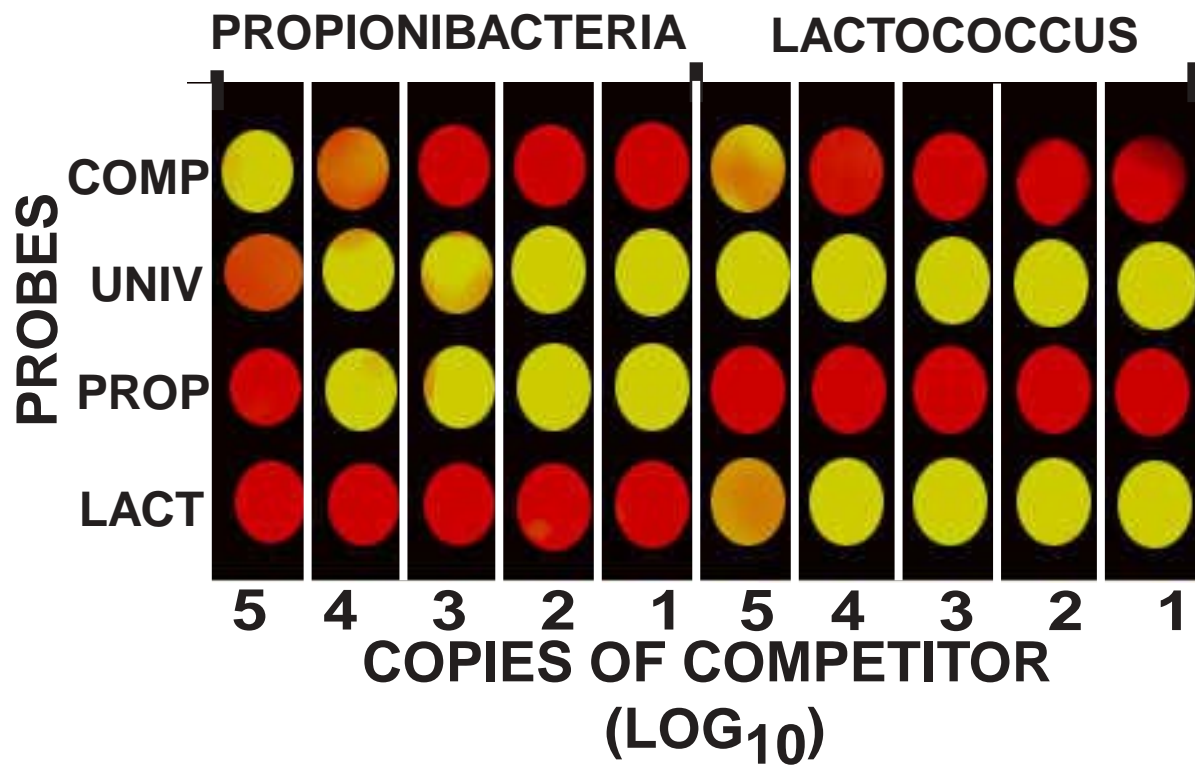


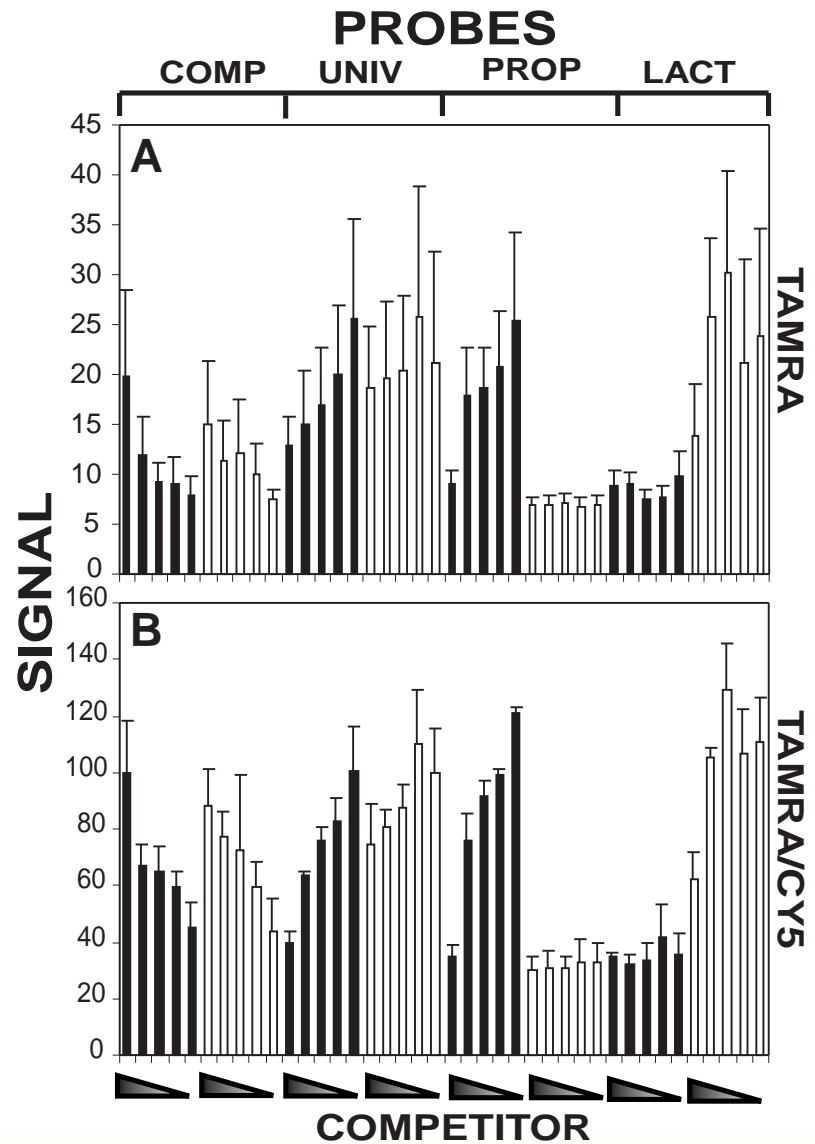


NORMALIZATION OF DNA MICROARRAYS

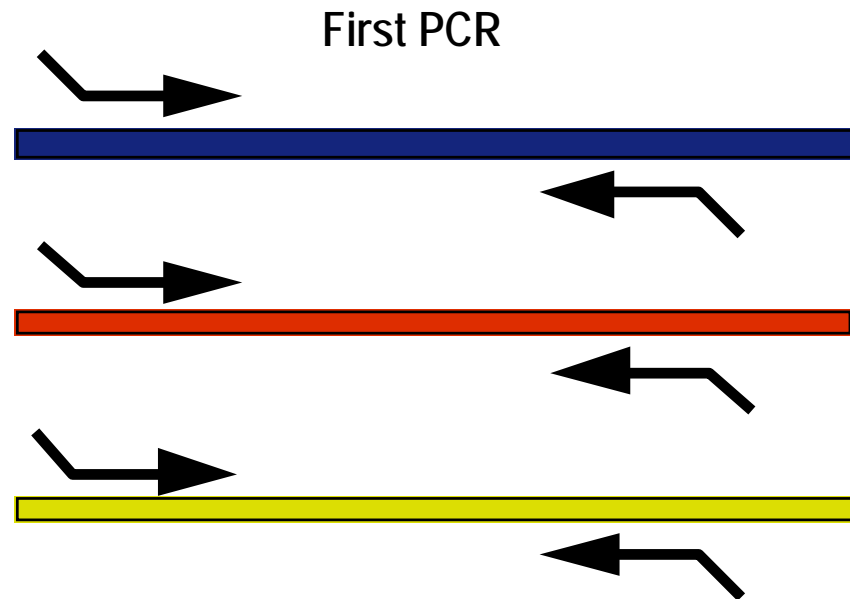
- ◆ Internal control in known concentration
- ◆ Quantification hybridization independent

Rudi et al. BioTechniques (in press)



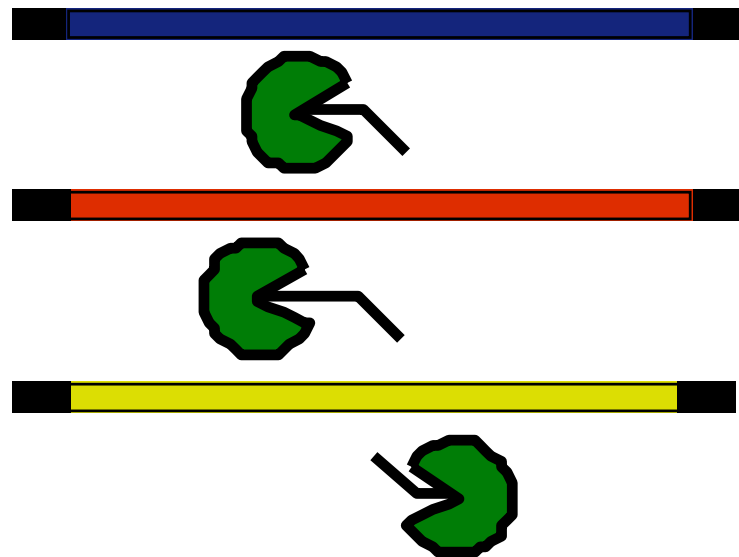


MULTIPLY QUANTITATIVE DETECTION METHOD



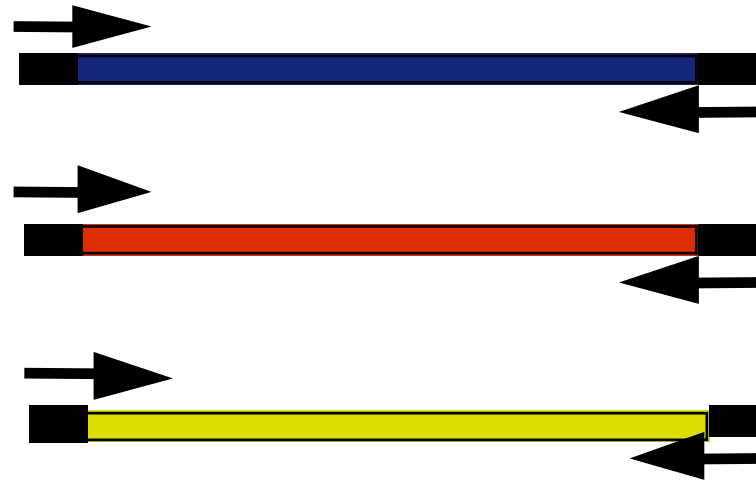
MULTIPLEX QUANTITATIVE DETECTION METHOD

Removal of primers by exonuclease



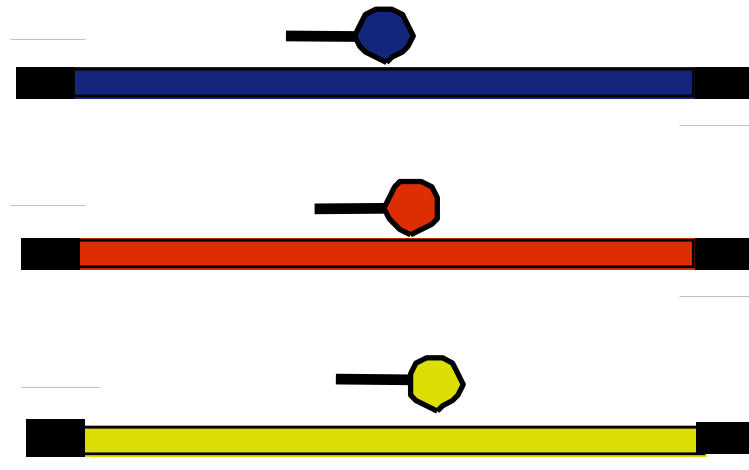
MULTIPLEX QUANTITATIVE DETECTION METHOD

Second PCR



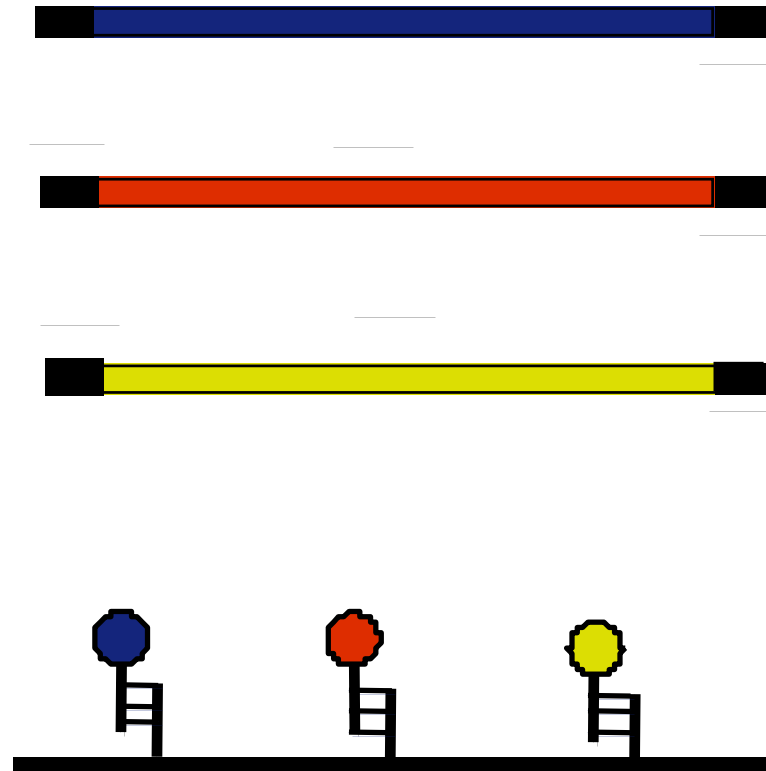
MULTIPLEX QUANTITATIVE DETECTION METHOD

Sequence-specific labeling



MULTIPLEX QUANTITATIVE DETECTION METHOD

DNA array hybridization



MULTIPLEX QUANTITATIVE DETECTION

Bt176

Bt11

Mon810

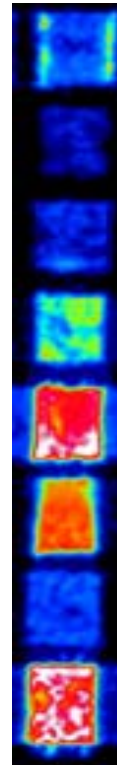
Amp

Nos

35S

IPC

Corn ref



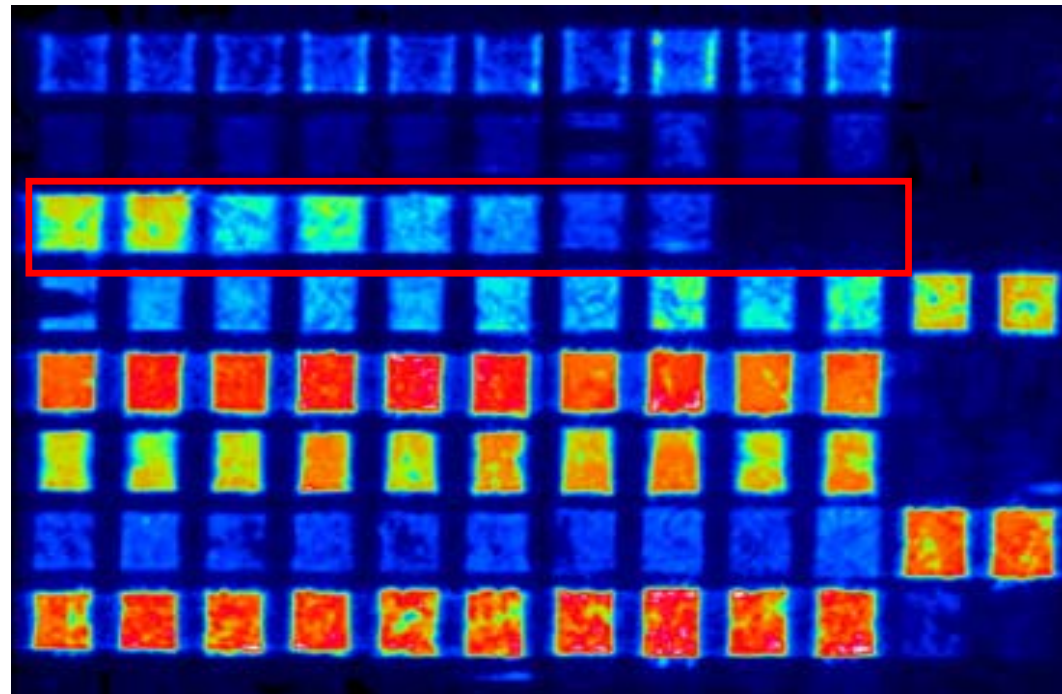
Scale



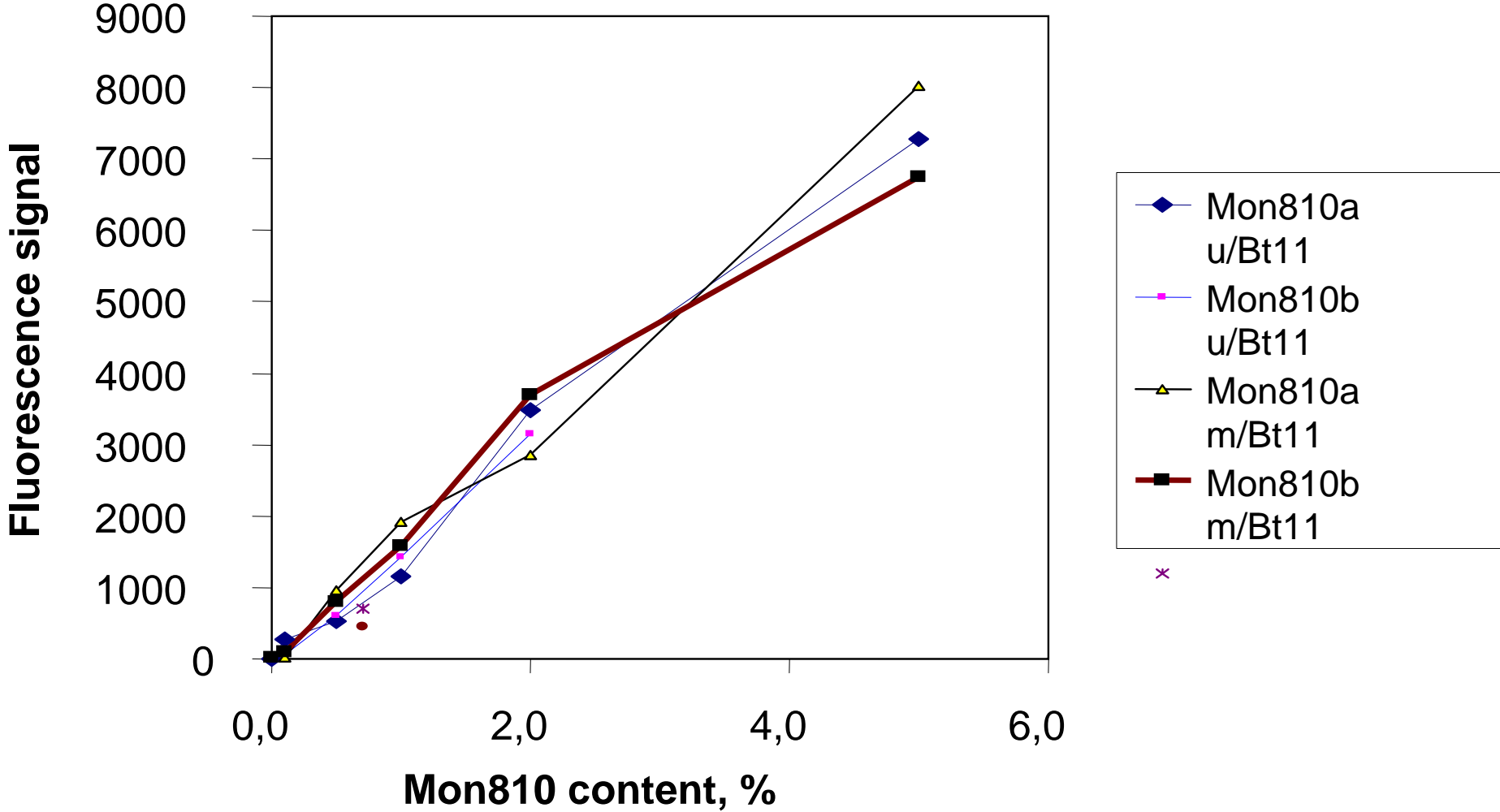
Mon810 maize (%)

2 1 0.5 0.2 0.0 Ctrl

Bt176
Bt11
Mon810
Amp
Nos
35S
IPC
Corn ref



MON810 SIGNALS VS MON810 CONTENT



REFERENCE SIGNAL

