

Developing metabolic and taxonomic microarrays for environmental applications

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Development of Two Types of Microarrays

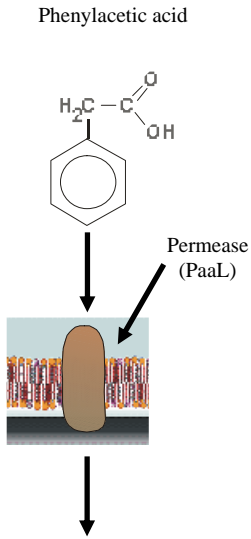
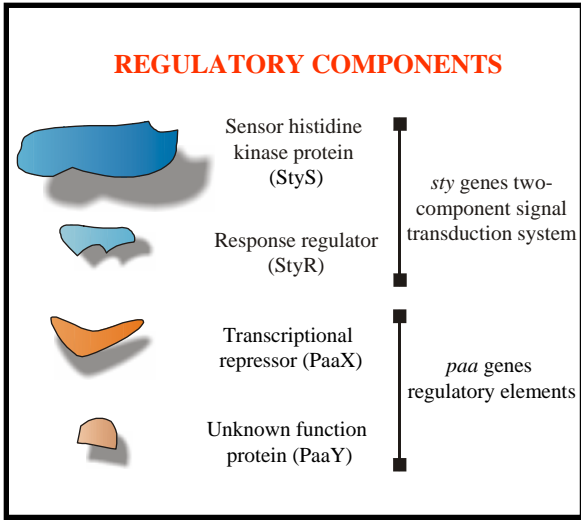
- Taxonomic: profiling microbial community phylogeny, biodiversity
Two targets: 16S rDNA and *cpn60*
- Metabolic / Functional: profiling pollutant transformation, ecosystem health
Multiple targets: catabolic, metal resistance and biogeochemical cycle

Environmental Applications of Microarrays

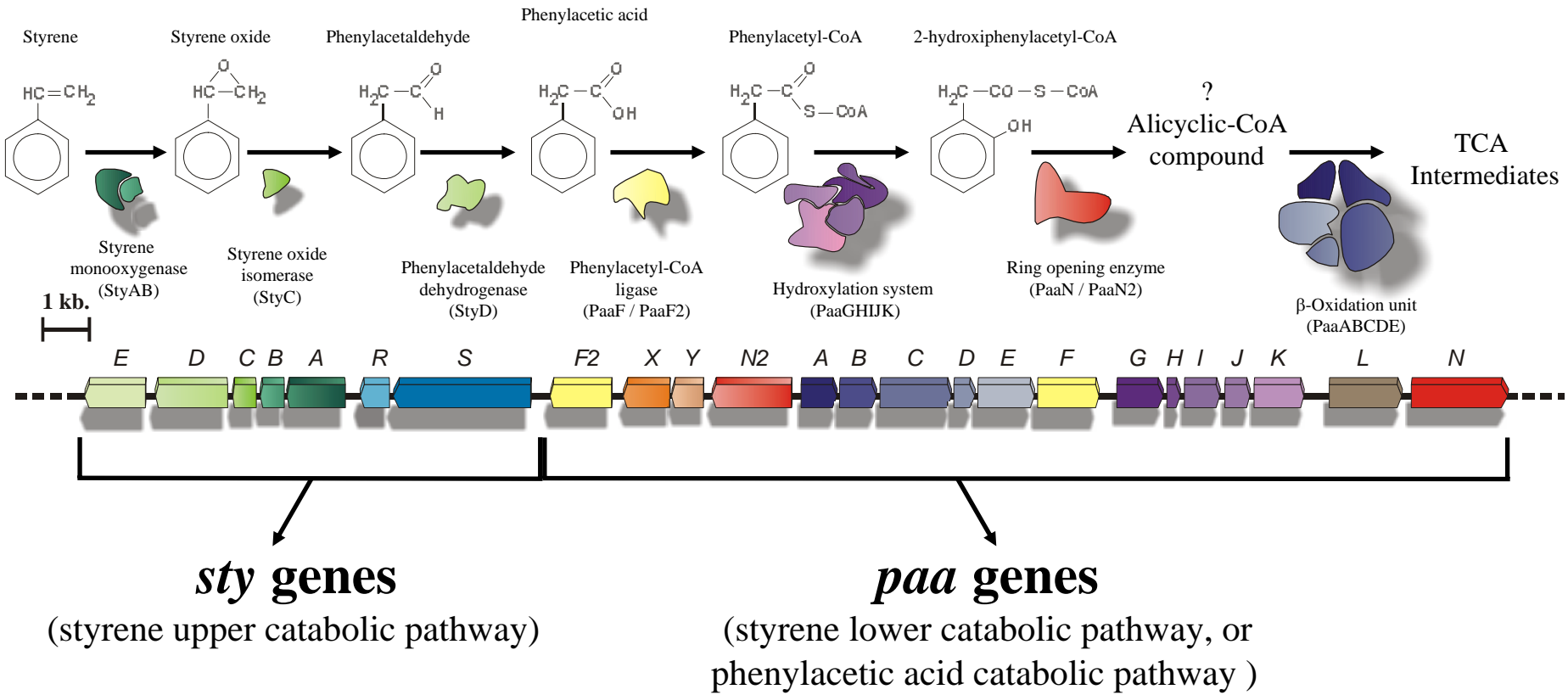
- expression analysis of specific biodegradation pathways: response of individual strains, consortia or natural microbial communities to different substrates
- analysis of the functional capacity of specific environmental samples: temporal and spatial analyses (pollutant degradation, biogeochemical cycles, etc.)
- characterization of the structure and composition of natural microbial populations
- profiling the response of natural microbial communities to stress and stimulation



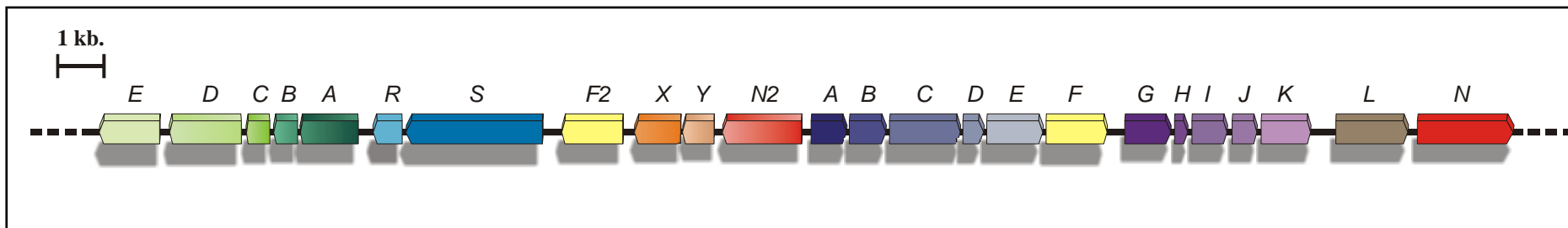
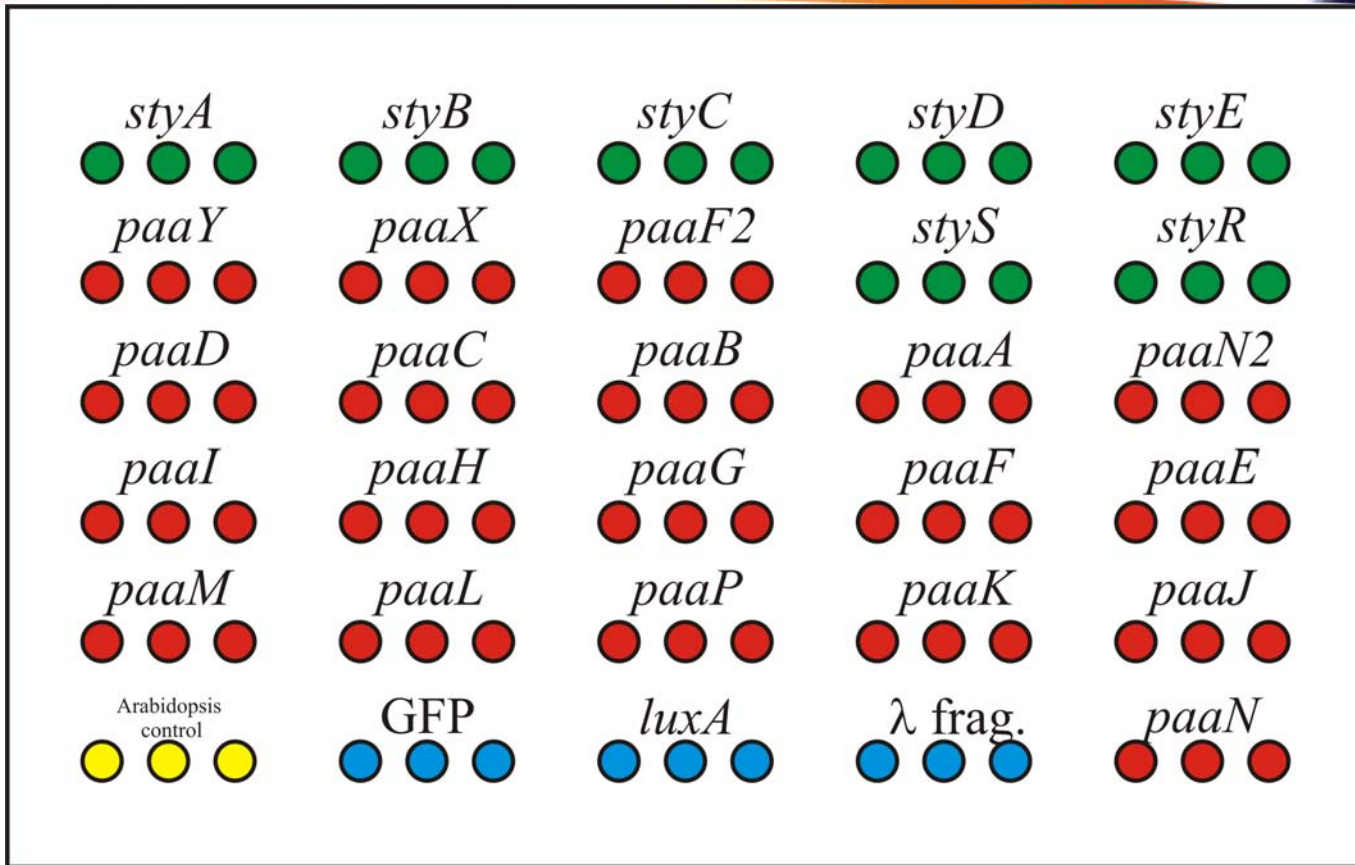
Analysis of the expression of genes
involved in the degradation of
styrene and phenylacetic acid



Pseudomonas sp.
strain Y2
sty and *paa* genes



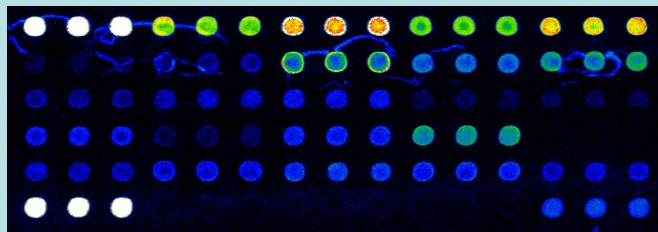
sty-paa v2 microarray layout



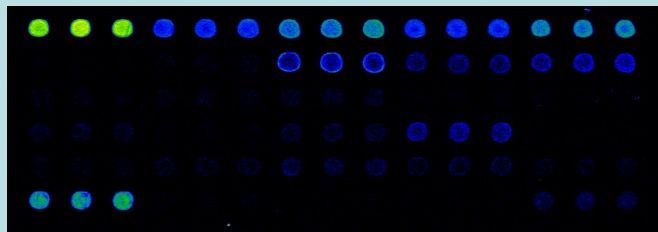
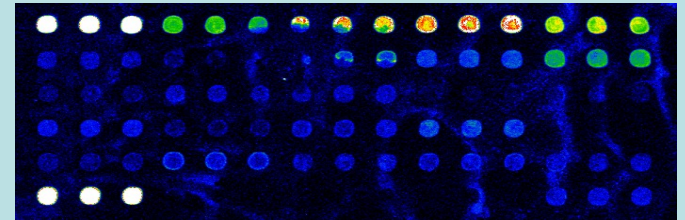
Induction of the *sty-paa* gene cluster in Y2



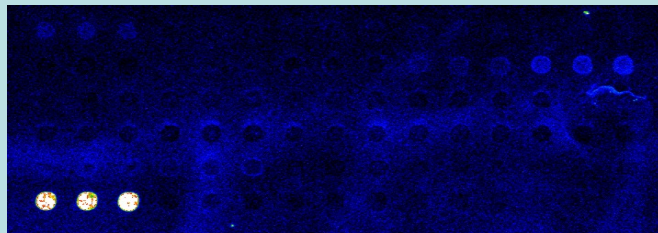
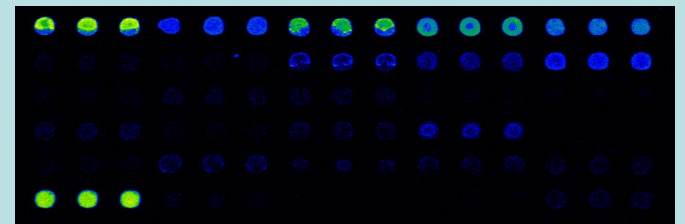
Glycerol-Cy3



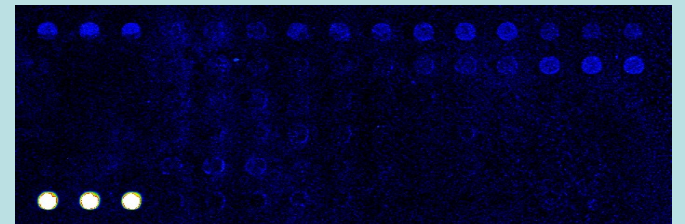
Styrene-Cy5



Styrene-Cy3



Glycerol-Cy5

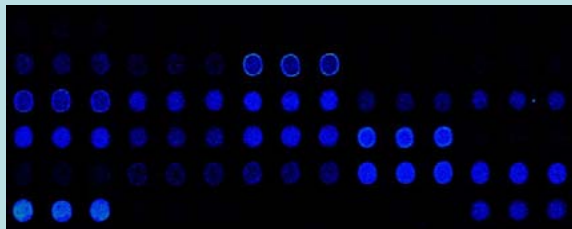
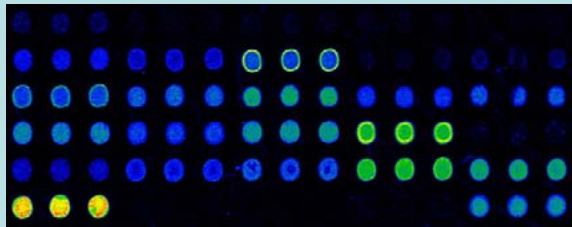


10mg Total RNA

mRNA (from 10mg Total RNA)

Induction of the *sty-paa* gene cluster in Y2 and C1

Y2



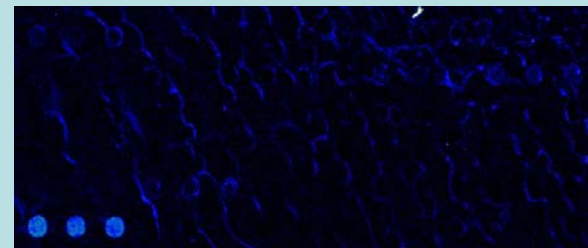
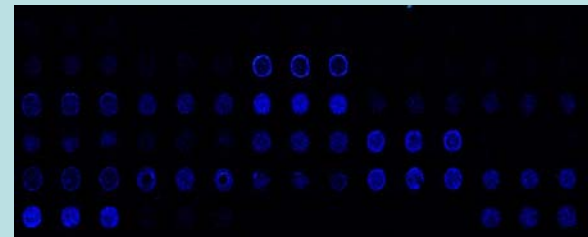
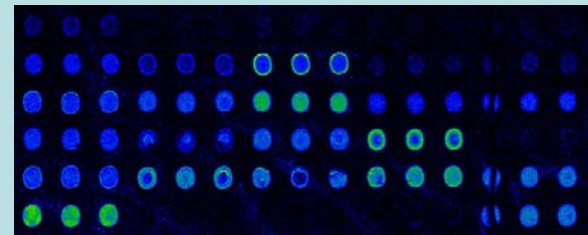
Glycerol-Cy3


PAA-Cy5

PAA-Cy3

Glycerol-Cy5

C1





Analysis of natural microbial
populations in a petroleum
hydrocarbon contaminated site
in the Canadian high Arctic

Potential Environmental Targets

Catabolic indicators

Organic pollutant degradation

- aliphatic and aromatic hydrocarbons
- chloro-organics
- nitro-organics
- pesticides

Inorganic transformation

- metal resistance
- dissimilatory

Community indicators

Nitrogen cycle genes

- ammonia oxidation
- denitrification

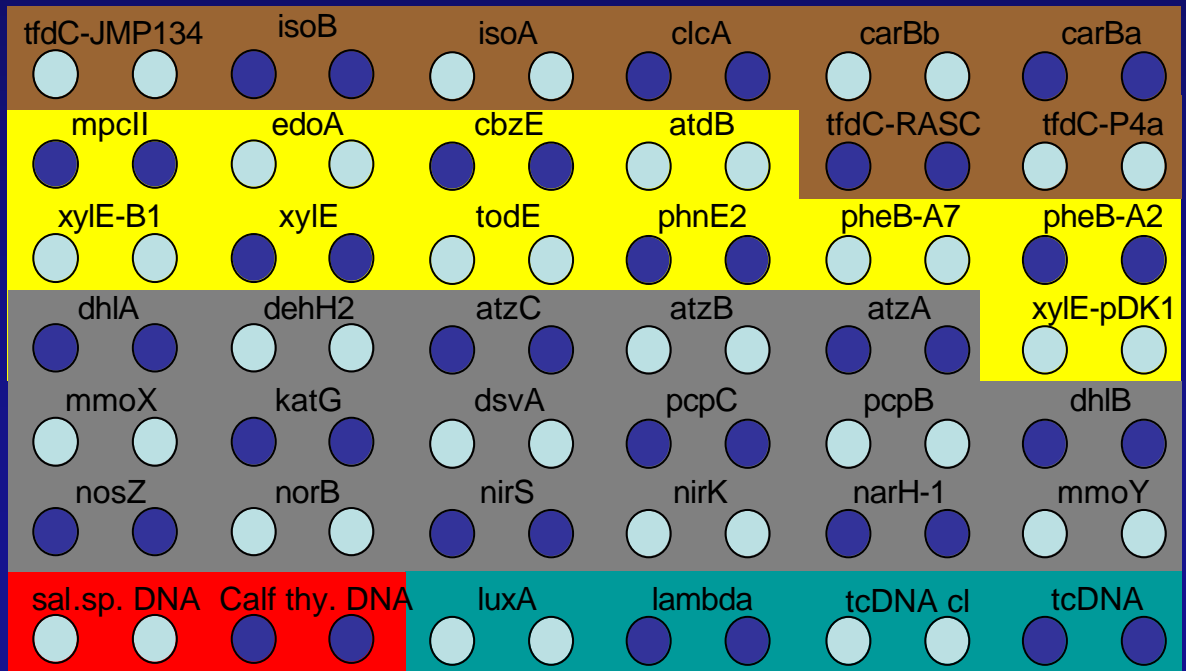
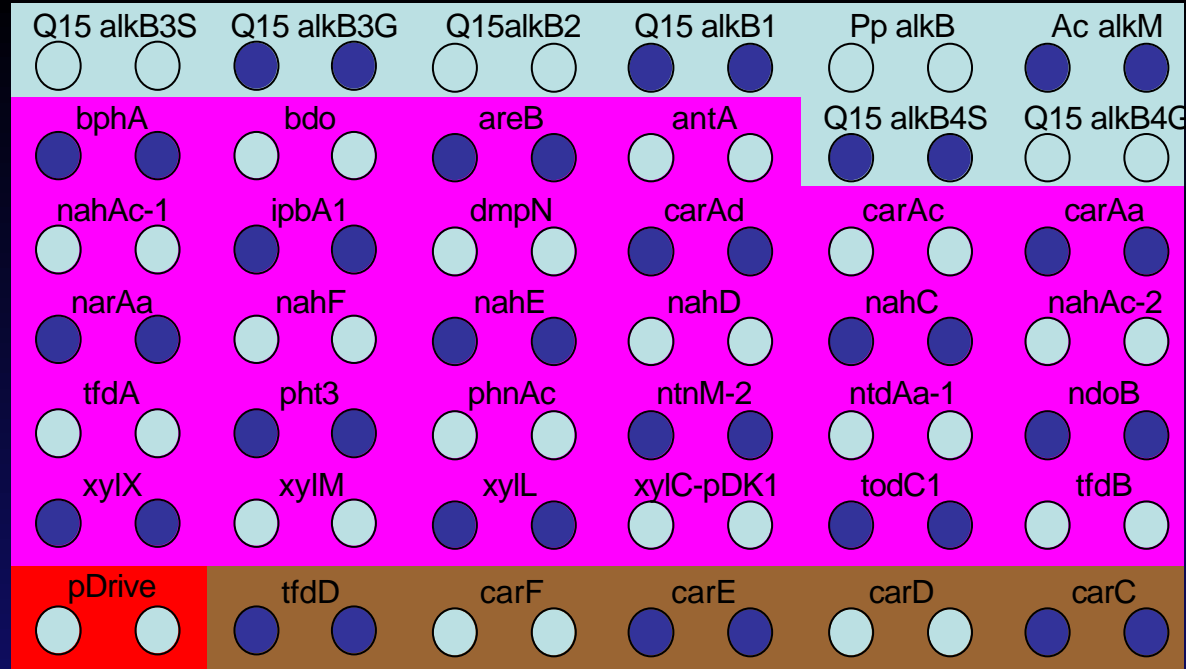
Sulfur cycle genes

- sulfate reduction

Carbon cycle genes

- cellulose degradation
- methane oxidation

Functional Gene Microarray - P4





Treatment area



Potable water supply



Treatment area



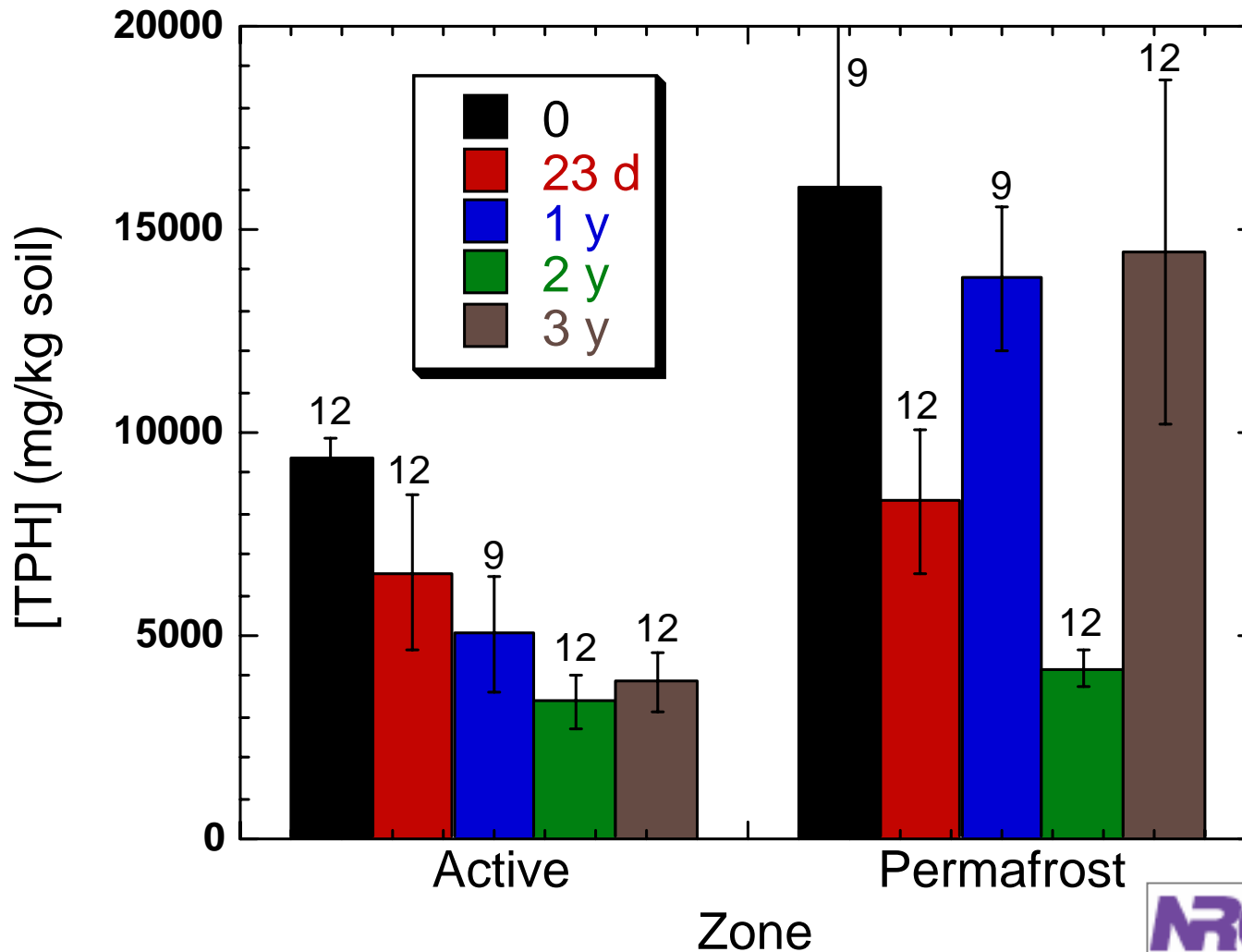
Nutrient solution reservoir



Application of liquid nutrients



Residual Hydrocarbons in Eureka Soils



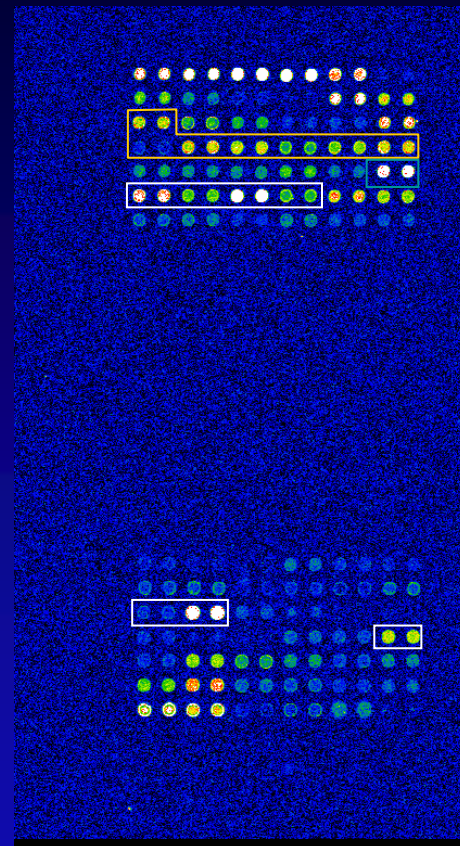
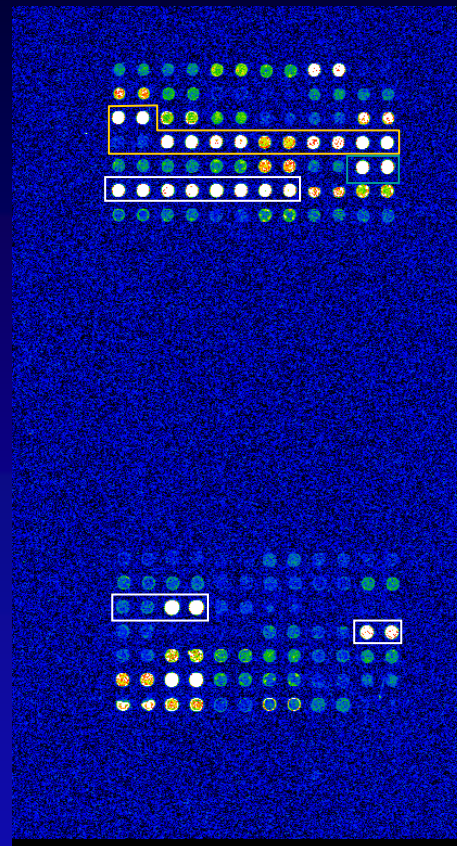
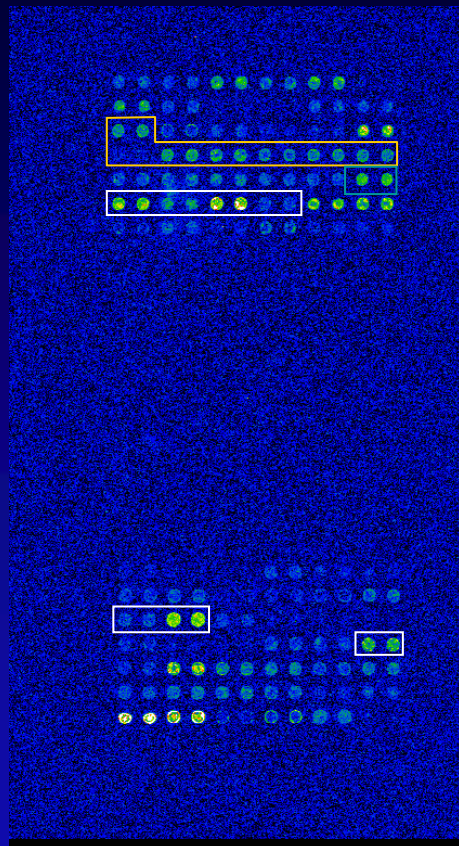
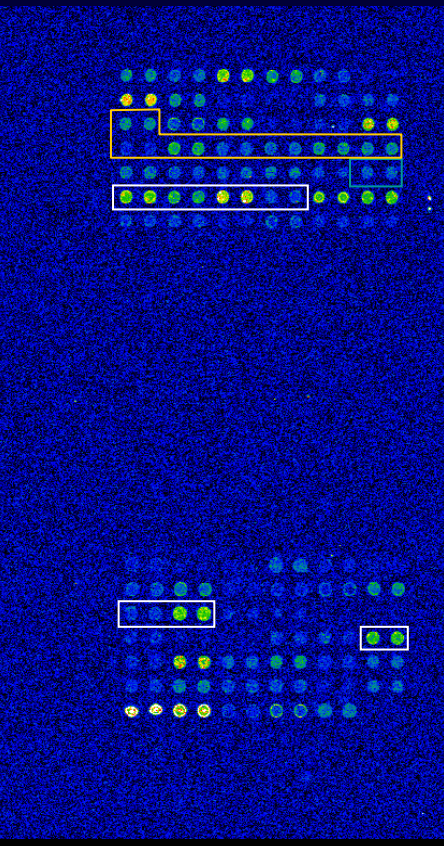
Eureka - Aromatics

T=0

T=23 days

T=1 yr

T=2 yrs



Culturable probe positive(%)

ndoB
0.5

ndoB
1.0

ndoB
9.5

ndoB
0.7

Naphthalene mineralization

-

++

++++

+++

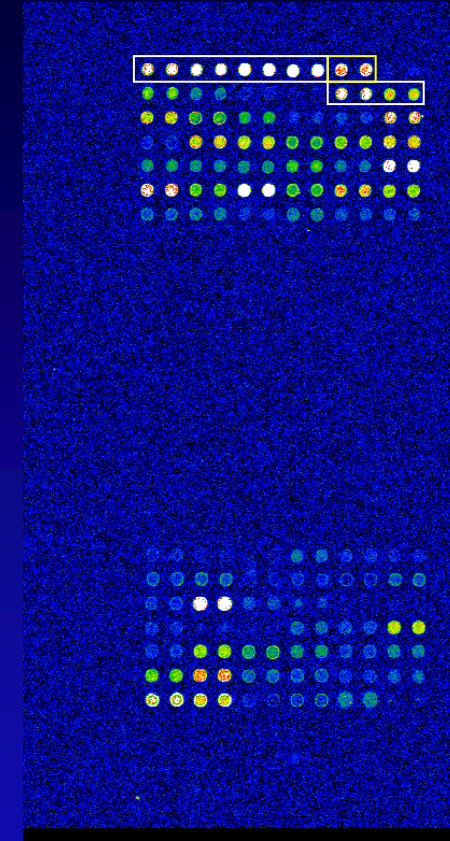
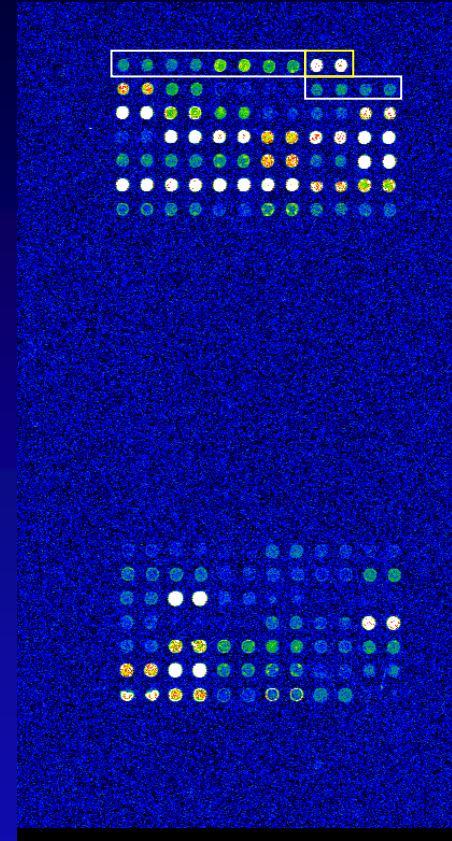
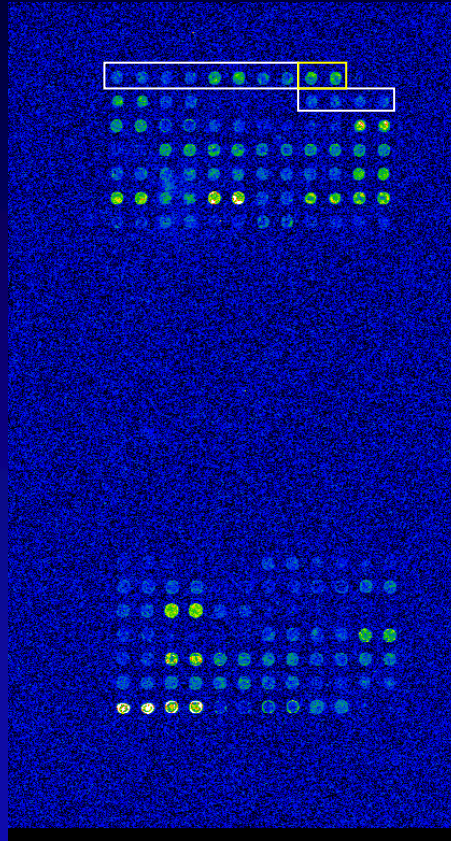
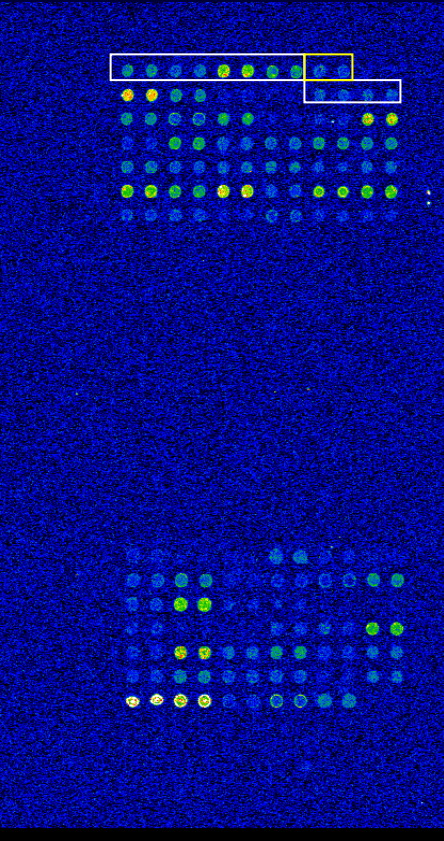
Eureka - Aliphatics

T=0

T=23 days

T=1 yr

T=2 yrs



Culturable probe positive(%)

Pp. alkB *Rh. alkB*
0.4 0.5

Pp. alkB *Rh. alkB*
2.0 1.8

Pp. alkB *Rh. alkB*
14.8 0.0

Pp. alkB *Rh. alkB*
1.2 1.0

Hexadecane mineralization

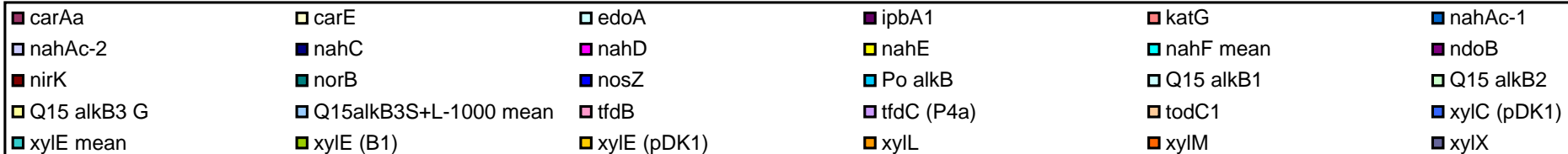
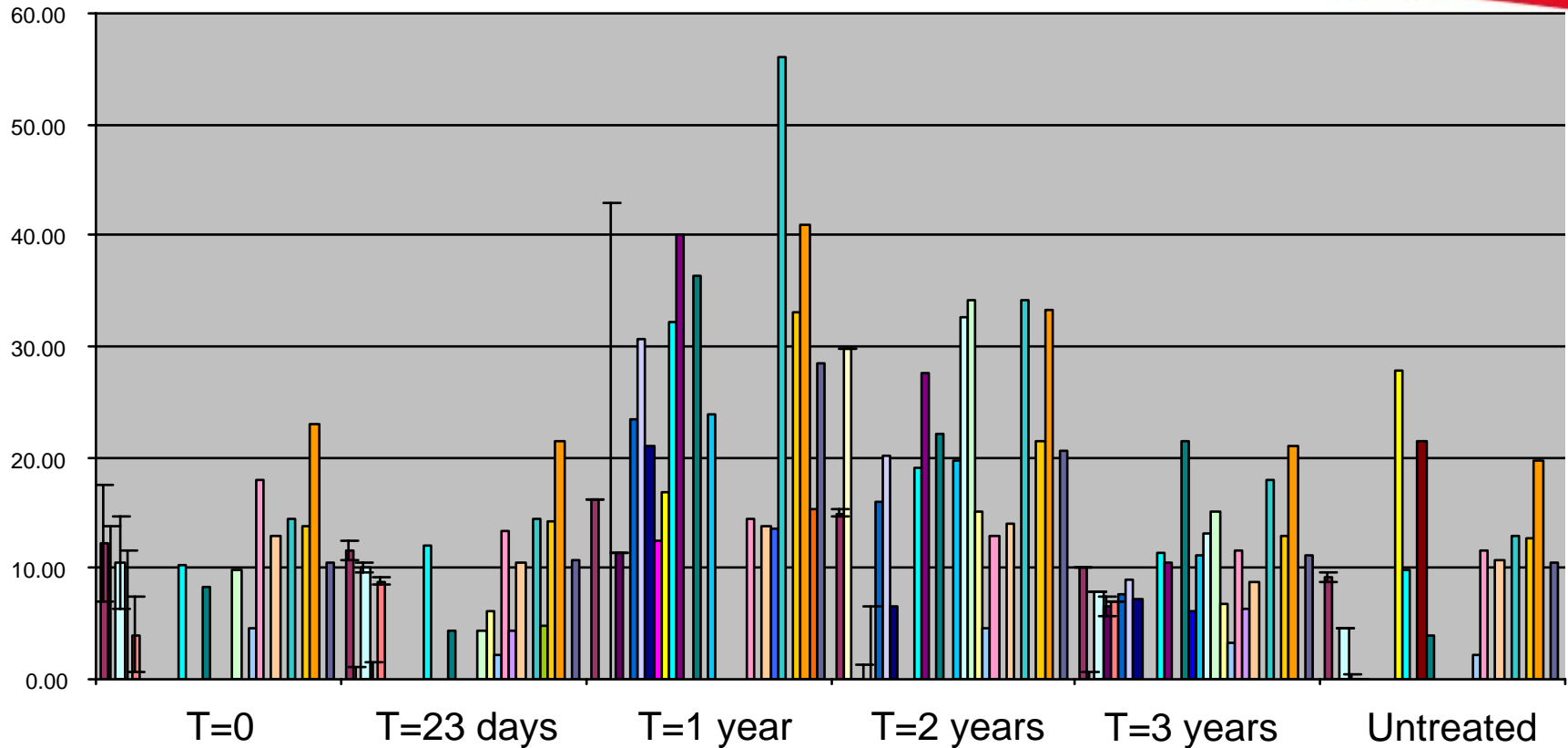
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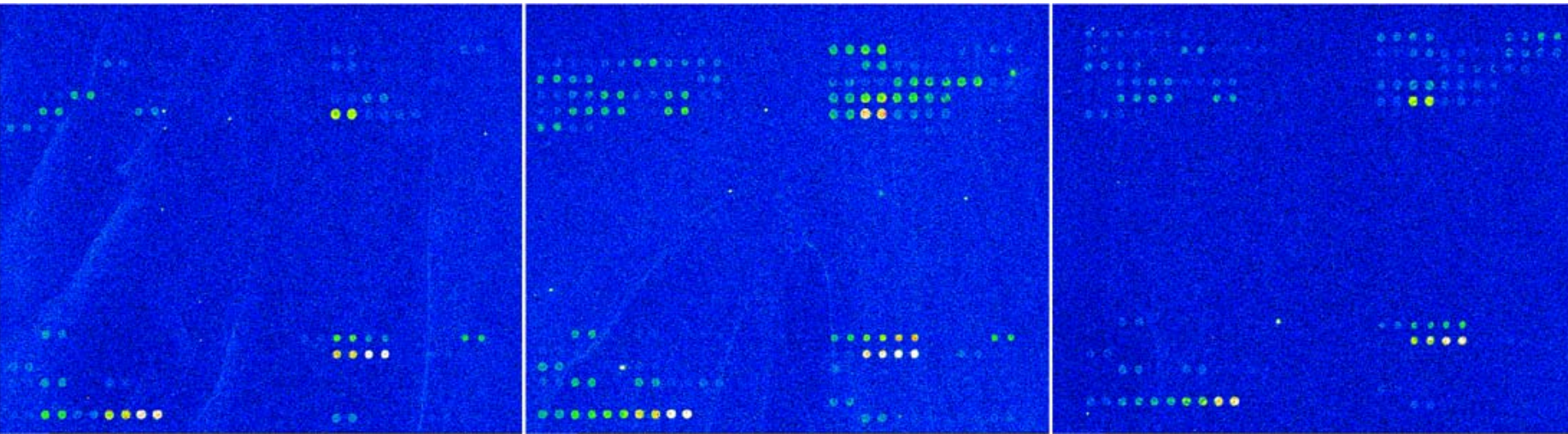
+/-

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Quantification of functional microarray data from Eureka



Catabolic oligo microarray analysis of Eureka site



T = 0

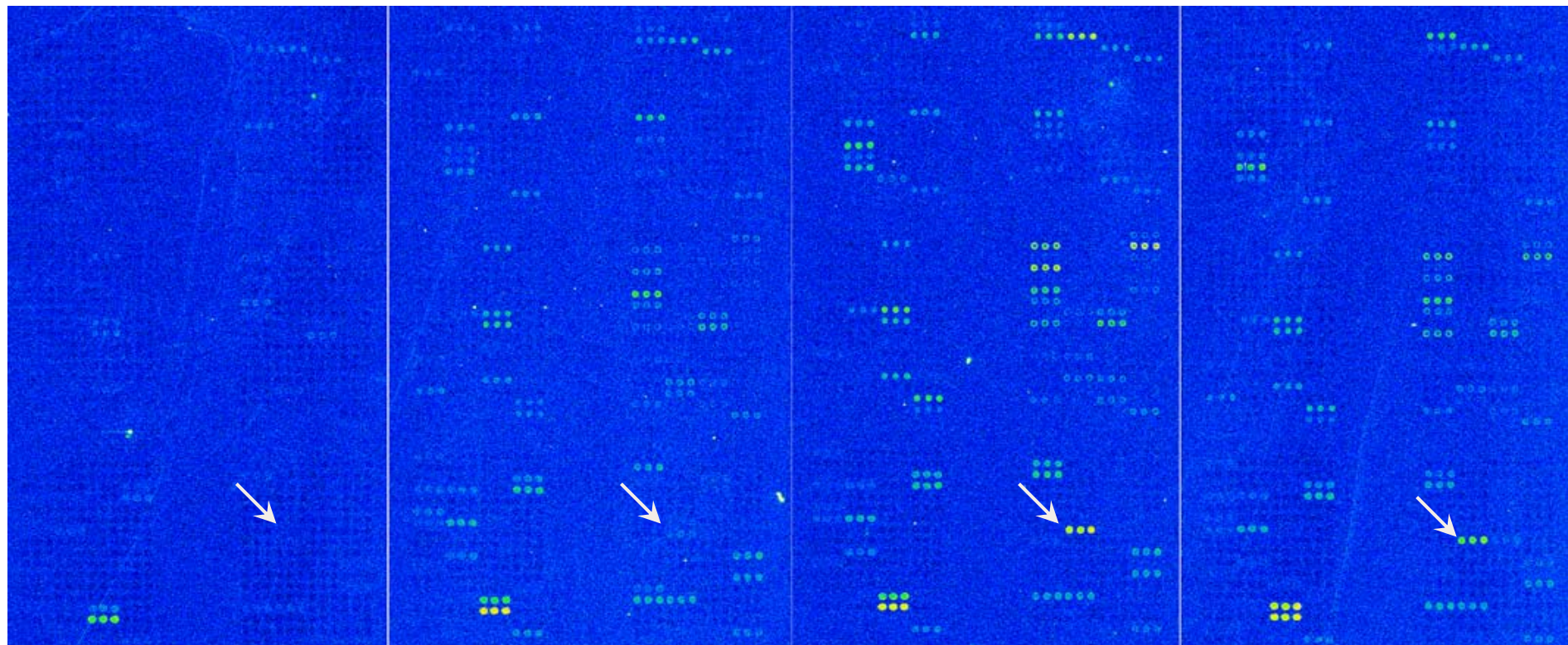
T = 1 year

T = 2 years

Design of Taxonomic Microarray

- dual target genes: 16S rDNA and *cpn60*
- total of 245 microorganisms, comprising all of the 218 microorganisms from the RDB backbone tree
- 118 of the microorganisms have both target genes represented
- 127 of the microorganisms have only 16S rDNA gene represented

Taxonomic microarray analysis of Eureka site



Untreated

T = 0

T = 1 year

T = 2 years



Technical Challenges to be Addressed

- quality and quantity of extracted nucleic acids
- optimization of labelling of extracted nucleic acids
- best targets: amplicons or oligonucleotides
- microarray printing: concentration and availability
- detection sensitivity and quantitation
- probe design and data management

Where to Next?

- increase number of amplicon probes for printing
- improve detection sensitivity
- evaluate RNA extraction methods
- analysis of more environmental samples
- quantify hybridization and compare to data from other analyses
- continue comparison of oligonucleotides with amplicons



Thanks to:

D. Juck

S. Sanschagrín

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J.R. Lawrence

L.G. Whyte

S. Hemmingsen

J. Hill

B. Goalen

M. Nahir

GHI-2, EC-STAGE,
EC-EMBRR