



COST 853

WG5: Environmental Monitoring

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DNA micro-array facility

Objectives:

fabrication of weak and medium density DNA chips

Help to result analysis

Training courses

- Access modalities:

Common service of UCB-Lyon 1 (DTAMB)

Open to external researchers (academics and private companies)

Access after project examination by a scientific committee

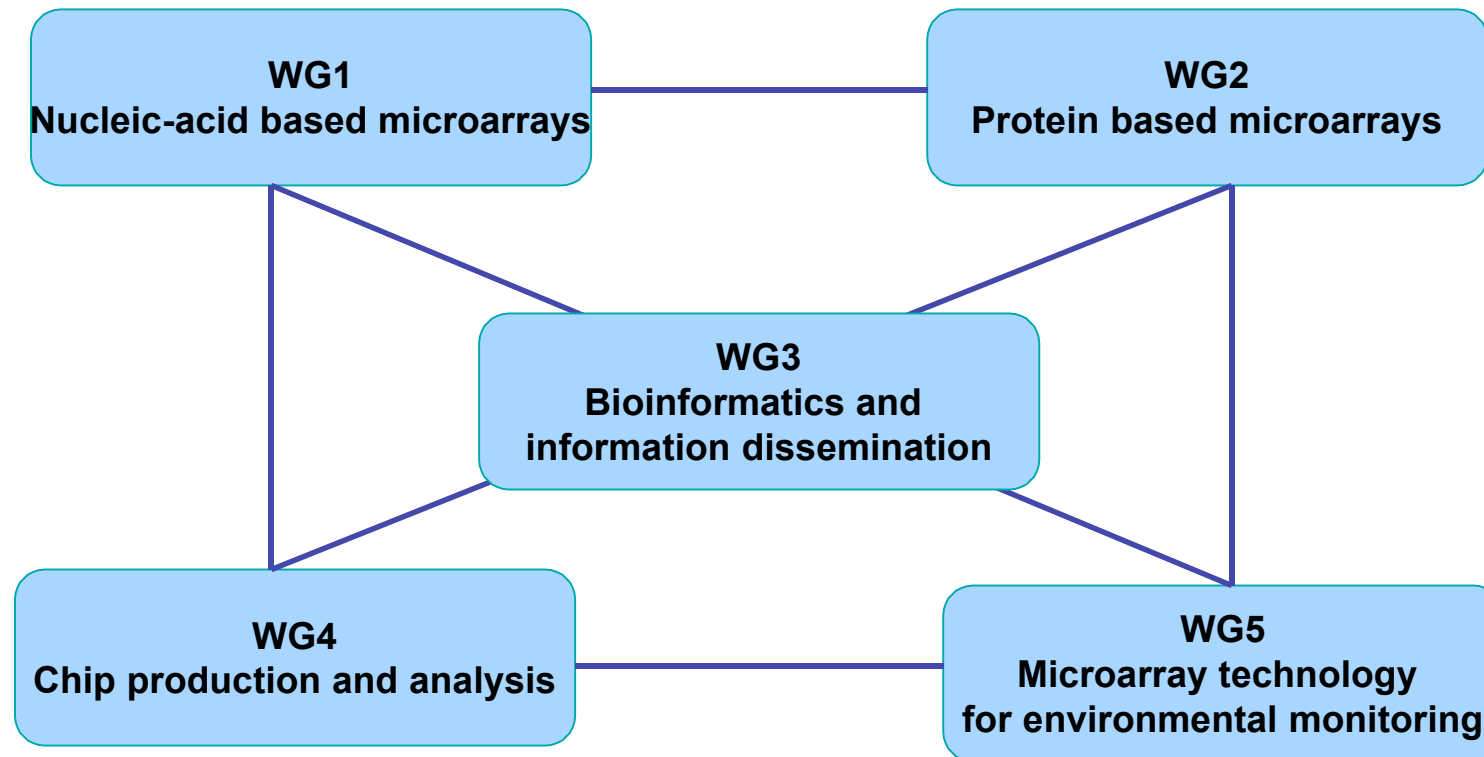
<http://www.ens-lyon.fr/genopole-rhone-alpes/>





COST ACTION 853

Agricultural Biomarkers for Array Technology





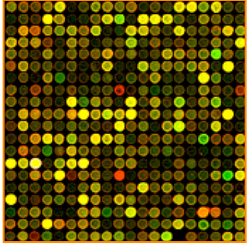
COST ACTION 853

Agricultural Biomarkers for Array Technology

Working Group 5: Micro-Array Technology for Environmental Monitoring

The objective of this Working Group is to collect information to develop MT for environmental monitoring in agro-ecosystems as required for instance to evaluate the impact of phytosanitary or other pollutant input, of agricultural practices or of other relevant ecological factors. For this purpose, MT may be used to measure the effect on three environmental indicators:

- *community diversity (meta-genome fingerprinting)**
- *key gene expression of functional communities (marker function)**
- *whole genome expression of selected strains (bio-marker expression).**



Why DNA chips ?

For high throughput analysis !

- Massive availability of sequence data
 - complete genomes (**± 150 bacteria / archeae, TIGR**)
 - systematic gene sequencing (**± 140 000 16S, Genebank, RDP**)
 - huge clone libraries (**± 100 000 metagenomic cosmids or BACs**)
- Massive analysis of hybridation data (*megadata*)
 - need for automated data processing (from handycraft to industry)
 - need for informatic & bioinformatic
 - a long term effort

The 16S chip project

- Analysis of bacterial communities

Rapid and accurate determinations of the taxon content of bacterial microflora and using these descriptions to characterize spatio-temporal variations or effects of perturbing factors.

Great number of taxa to be distinguish and quantified simultaneously

- * RISA, DGGE, SSCP, T-RFLP ...

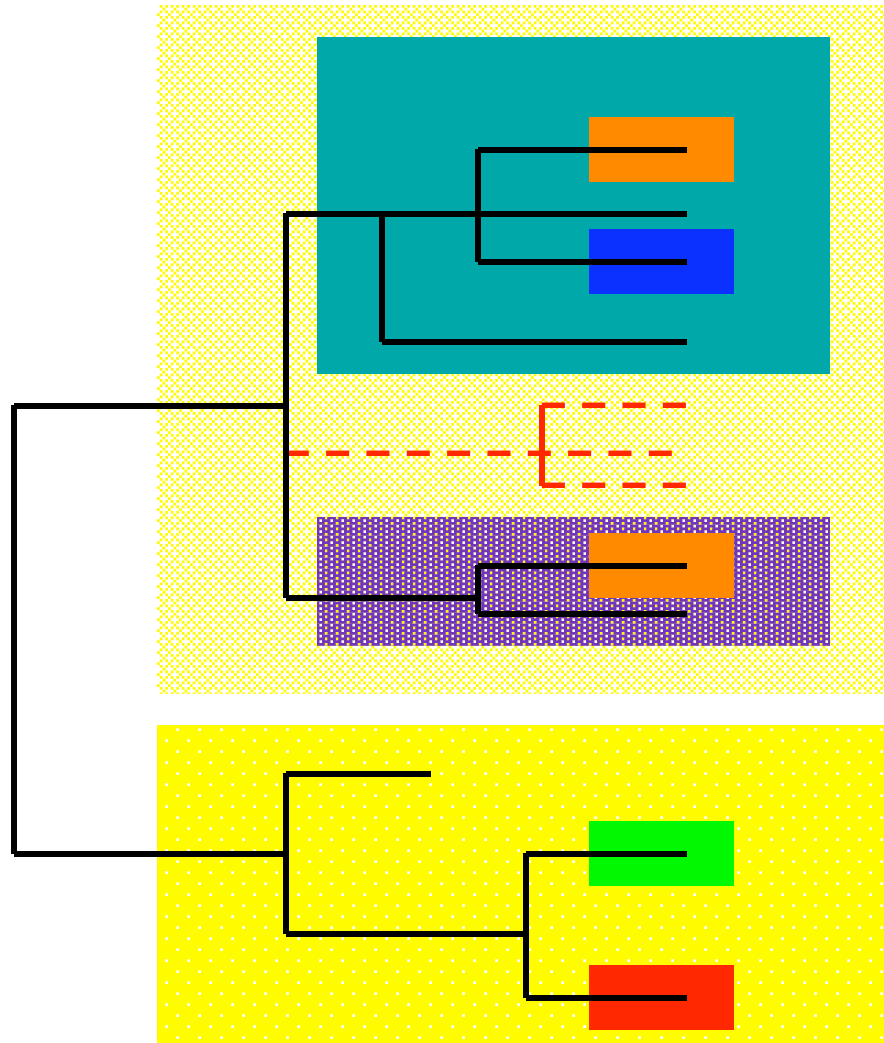
- * DNA chips: massive parallel analysis

Distinguishing in parallel and specifically
taxa at the different taxonomic levels

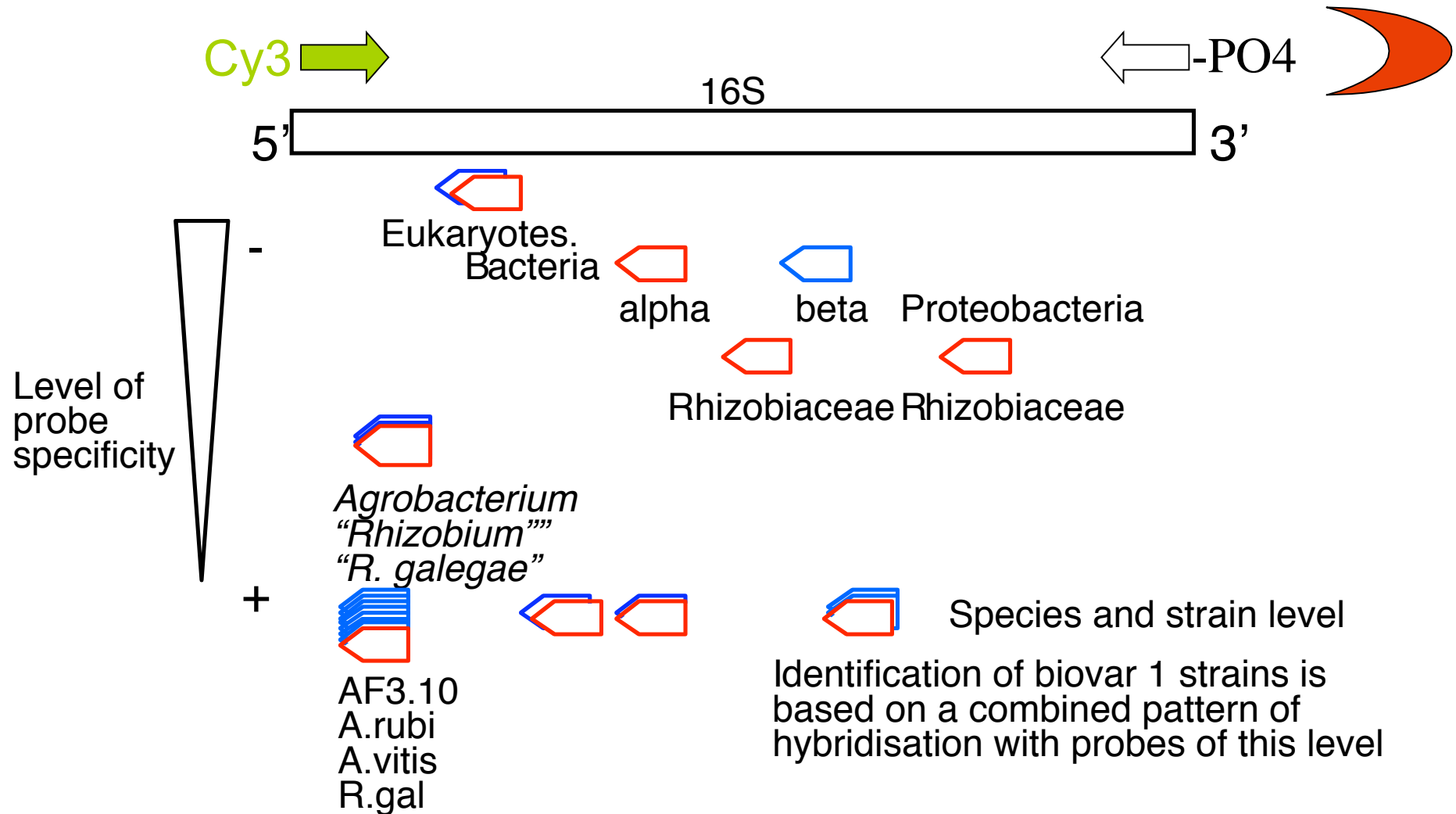
Pilot 16S chip

- **Bacterial identification**
 - 1 strain
 - Specificity
 - Qualitative response
 - Nested probes
 - Multiple probes
- **Analysis of bacterial communities**
 - N strains : high throughput
 - Qualitative AND quantitative response !

Nested probes



Position of probes along the 16S rRNA. Nested design.



Criteria for probe design

- Target : 16S rRNA or the corresponding DNA strand
- Nested probes, based on the phylogeny
- ca. **20-mers** (+ variations)
 - 2 MM or more
 - As central as possible
 - T_m as homogeneous as possible (63-66°C)**
- no secondary structures

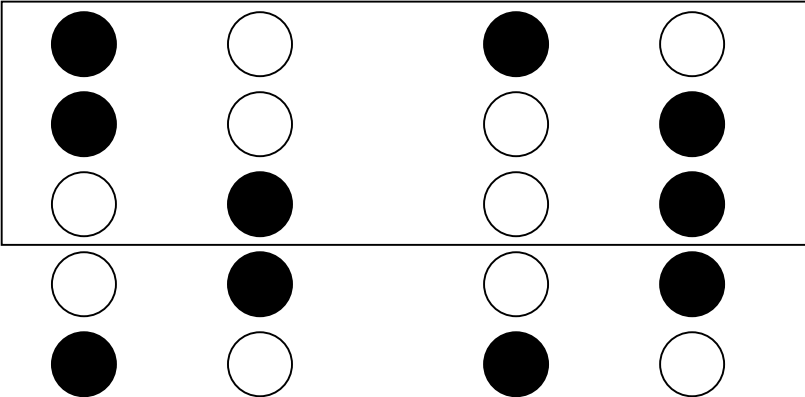
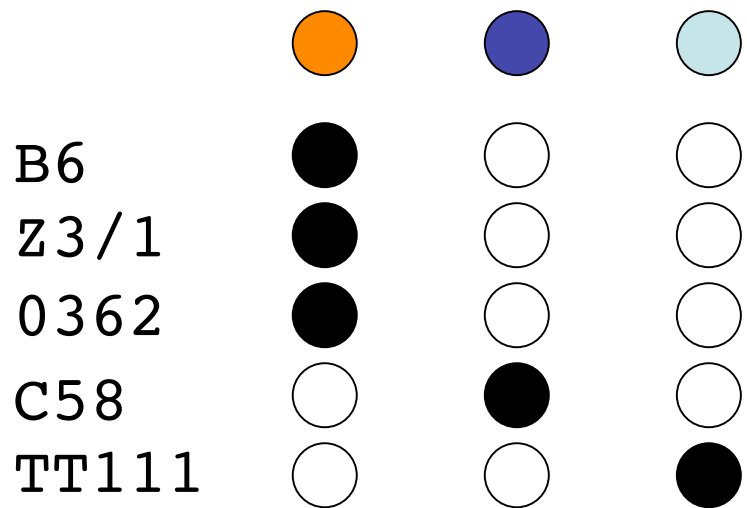
Combination of probes

Biovar1

B6 GCAGGAAAGGGTATGTTC
 Z3/1 GCAGGAAAGGGTATGTTC
 0362 GCAGGAAAGGGTATGTTC
 C58 GCAGAGATGGGTAGATTC
 TT111GCAGGGCACGGTAGATTC

GCT <u>CT</u> GG	GACA <u>AT</u> GTC
GCT <u>CT</u> GG	GACA <u>TC</u> GTC
GCT <u>GC</u> GG	GACA <u>TC</u> GTC

GCT <u>GC</u> GG	GACA <u>TC</u> GTC
GCT <u>CT</u> GG	GACA <u>AT</u> GTC



Conclusions of the pilot study

- It was possible to find probes that distinguish species at the finest level permitted with the 16S rRNA molecule.
- A combination of probes is sometimes necessary.
- Probes behaved as expected (when single-stranded PCR product was used for the hybridization)
- Different probes, same target, different signal levels

16S DNA chip project

Construction of a medium density 16S DNA chip

- * oligo design (300 ≠ features)
 - * great phyla
 - * Proteobacteria
 - Rhizobiaceae
 - * spotting (4*300=1200 spots)
- use of ARB
Ph. D. A. Herrera
Ph. D. A. H. Sanguin
" "

Present assays:

- * calibration with known bacterial mixtures
- * analyses of spatial & temporal diversity of ≠ microflores
 - * soils (mining vs. revegetalized soils)
 - * soil vs. Rhizosphere
 - * etc
- * comparison to alternative methods (RISA, 16S-SSCP, 16S-cloning-sequencing, etc)

- Training workshop for beginners (CNRS)
 - UMR 5122 « Microbiologie et Génétique »
 - UMR 5557 « Ecologie Microbienne »

19-20 june (theory, 80p)
23-26 june (practice, 24p) 2003

**"Techniques de Puces à ADN
pour le génotypage et l'analyse
du transcriptome des micro-organismes"***

* *"DNA chip techniques for genotyping and transcriptome analysis of micro-organisms"*



Seminar and bioinfo (ARB) training session by L. Bodrossy