

Monitoring Bioremediation with Molecular Tools

Hauke Smidt

Laboratory of Microbiology
Wageningen University

Fate of Environmental Pollutants

- ✓ Biodegradability largely depends on
 - ✓ chemical structure
 - ✓ environmental conditions

&

Purifying Capacity of microbiota

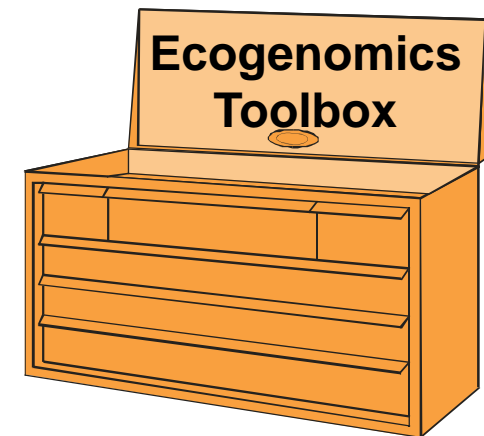
Fate of Environmental Pollutants



Opening up the black boxes

How to open the black box

- ✓ Molecular Toolbox to assess and predict
 - ✓ Phylogenetic identity / complexity (WHO)
 - ✓ Catabolic capacity (POTENTIAL)
 - ✓ Catabolic activity (ACTION)



- ✓ Exploring and defining the self-purifying capacity of natural and man-made environments

Today's Talk: Focus on Organohalides

✓ Application and use:

✓ Manufacture:

- Wood preservation
- Intermediate in organic synthesis
- Oils, lubricants, and dye-carriers

✓ In several pesticides & fungicide

✓ Combustion product

✓ Properties:

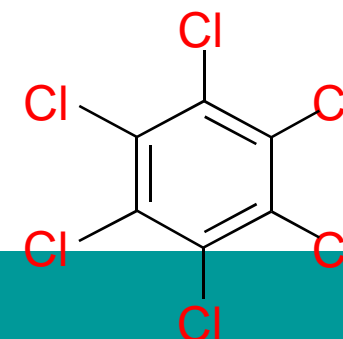
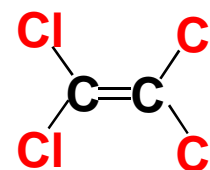
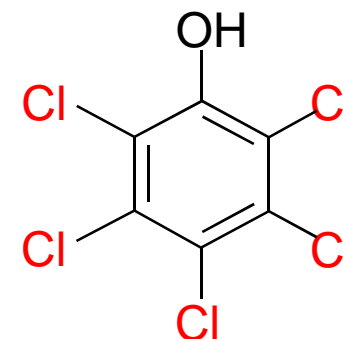
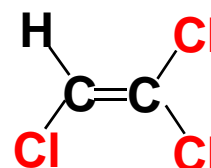
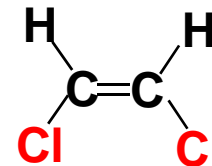
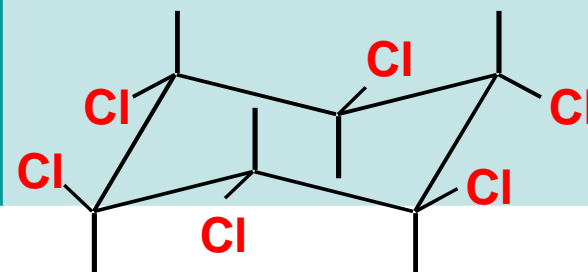
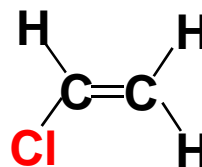
✓ Stable

✓ Mostly low in solubility

✓ Tendency to sorb to organic matter

✓ Bioaccumulation

✓ Toxic/carcinogenic

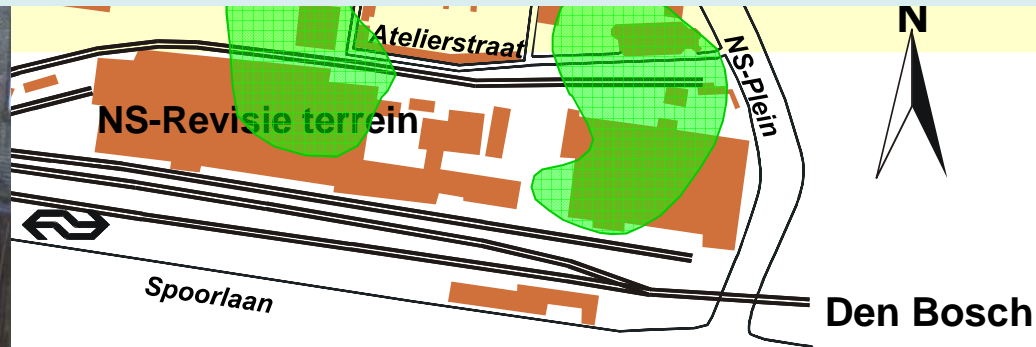


Bioremediation of Aquifers and Sediments

✓ Industrial Sites NL



✓ Large number of polluted locations in the Netherlands



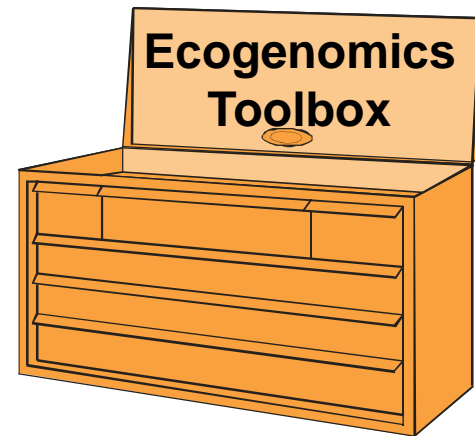
Chlorinated Aliphatic Hydrocarbon (CAH) pollution

Biological cleanup of chlorinated compounds

- ✓ Biodegradability of halogenated hydrocarbons largely depends on
 - ✓ chemical structure
 - ✓ environmental conditions
 - ✓ **Purifying capacity of microbiota**
- ✓ **Reductive dehalogenation** is often crucial step for initial biodegradation in anoxic environments
 - ✓ dechlorination by specific enzymes
+ energy conservation : **organohalide respiration**

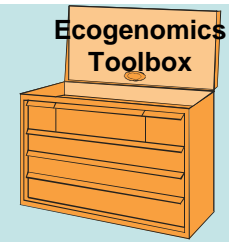
How to open the black box

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 - ✓ Catabolic capacity
 - ✓ Catabolic activity

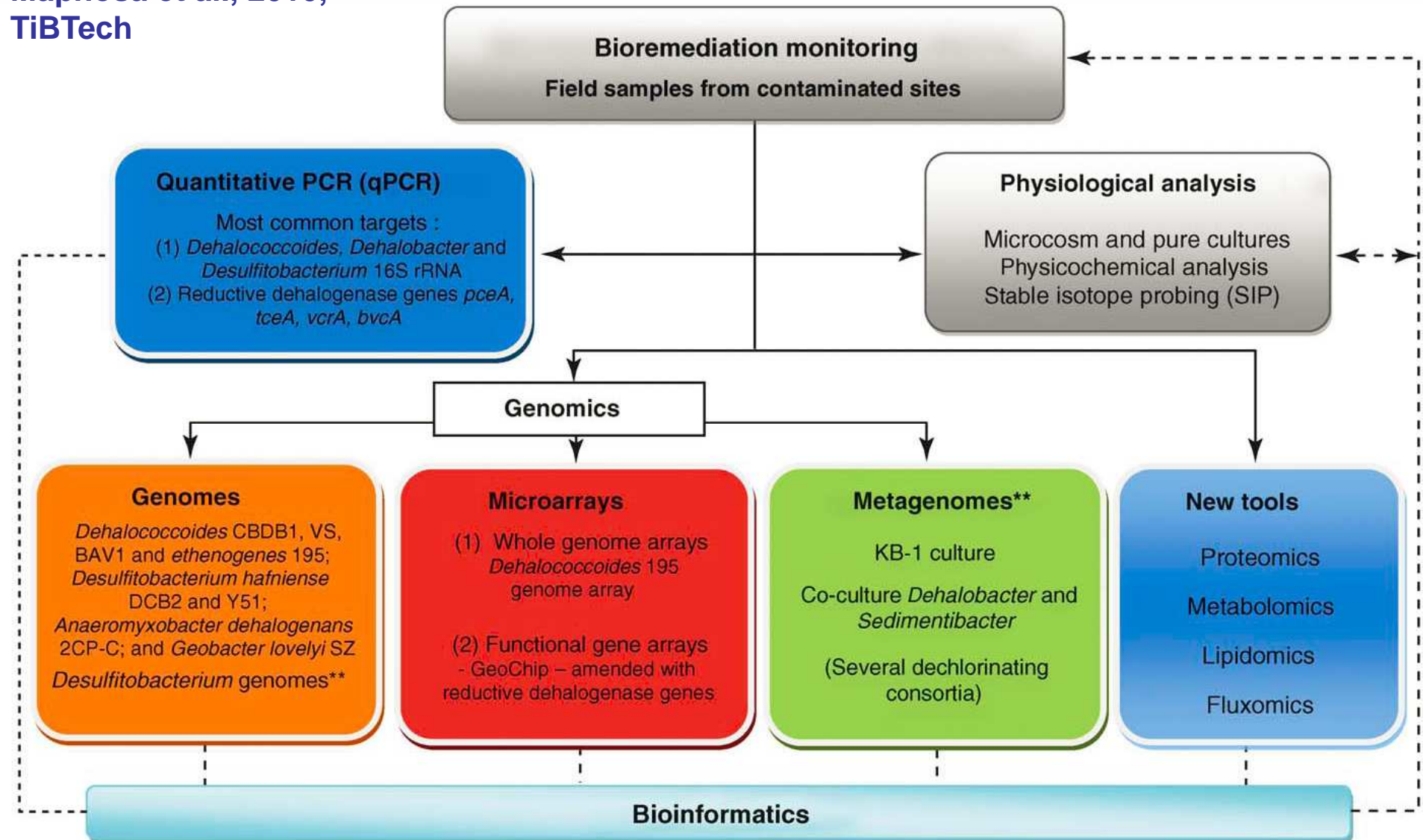


- ✓ Exploring and defining the self-purifying capacity of natural and man-made environments

How to open the black box

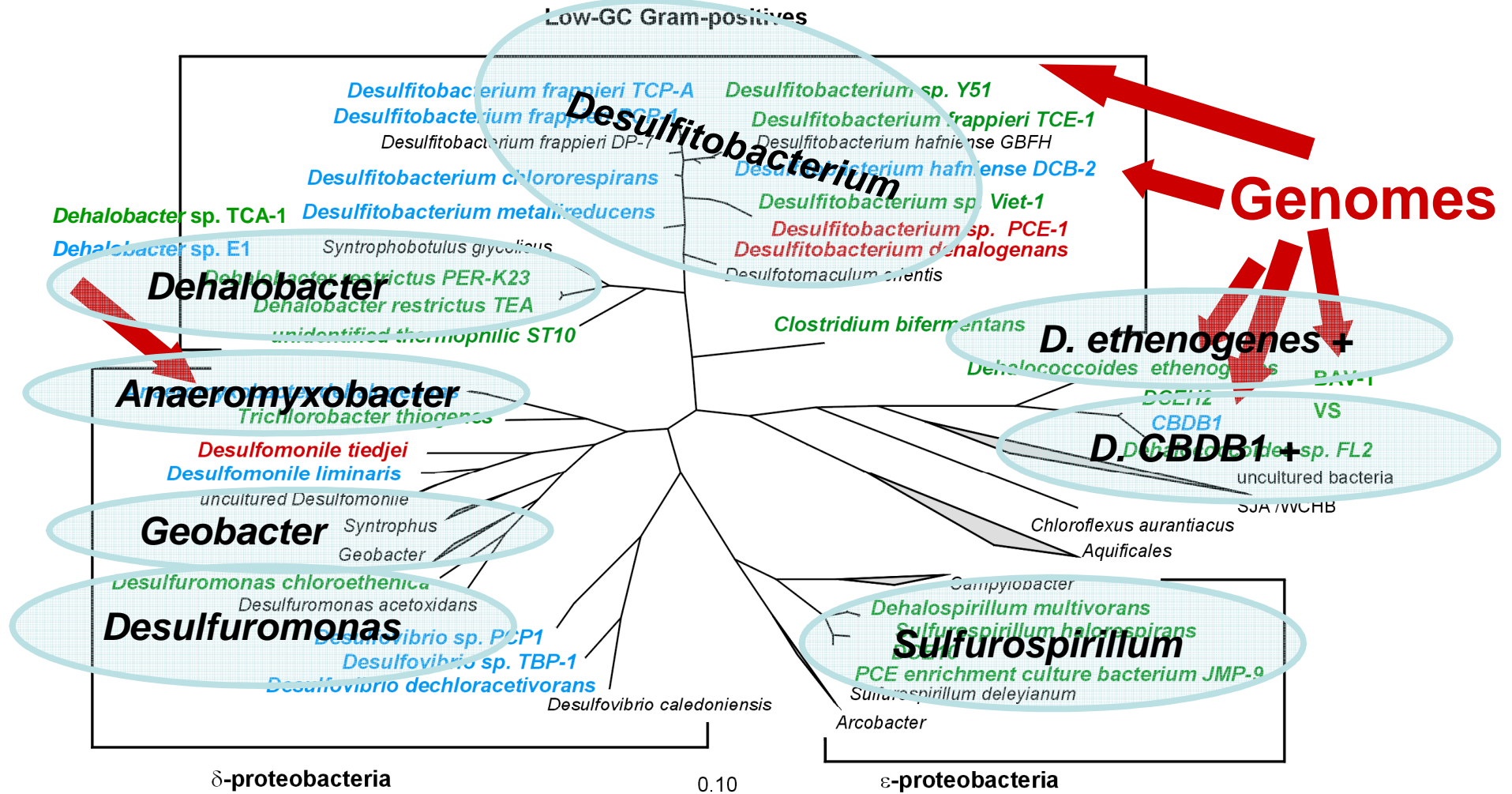


Maphosa et al., 2010,
TiBTech



Organohalide Respiring Bacteria

Diversity of isolates (16S rRNA)



■ Halo-aromatics

■ Halo-aromatics
■ Halo-aliphatics

■ Halo-aliphatics

Organohalide Respiring Bacteria

What do they live on

Restricted metabolism

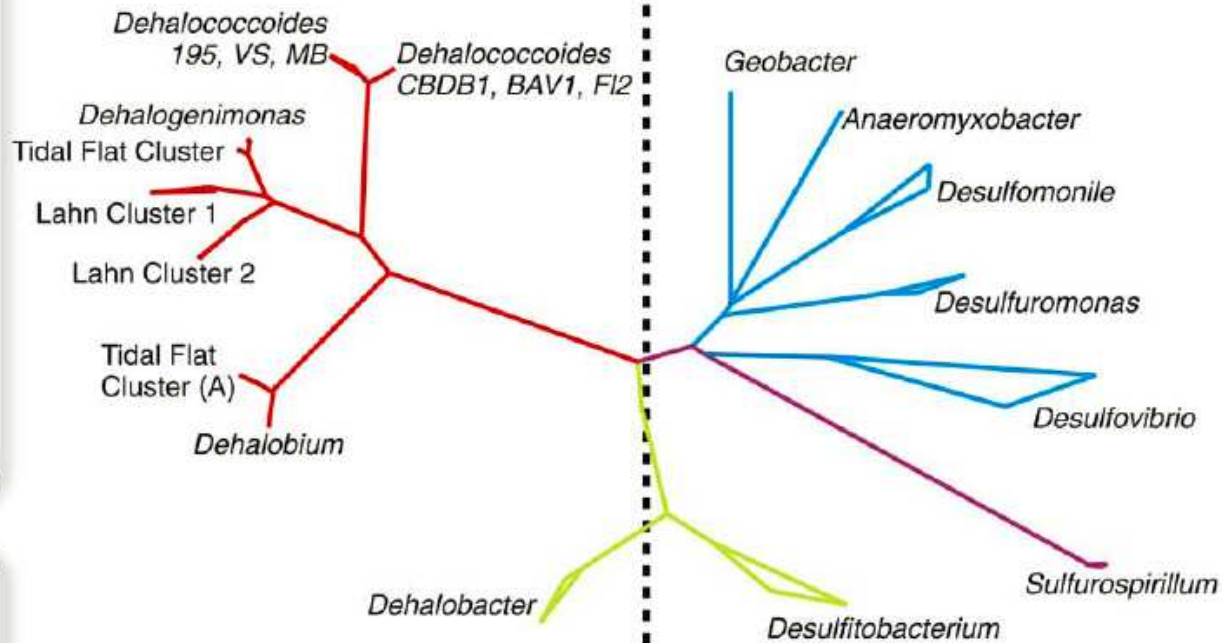
Difficult to culture, slow growth rates
 -need Vitamin B12
 -often maintained and studied in mixed cultures

Versatile metabolism

Relatively easy to culture

- Electron acceptors**
- PCE
 - TCE
 - DCE
 - VC
 - TCA
 - DCA
 - CF
 - HCH
 - chlorophenols
 - chlorobenzenes
 - dioxins
 - PCBs

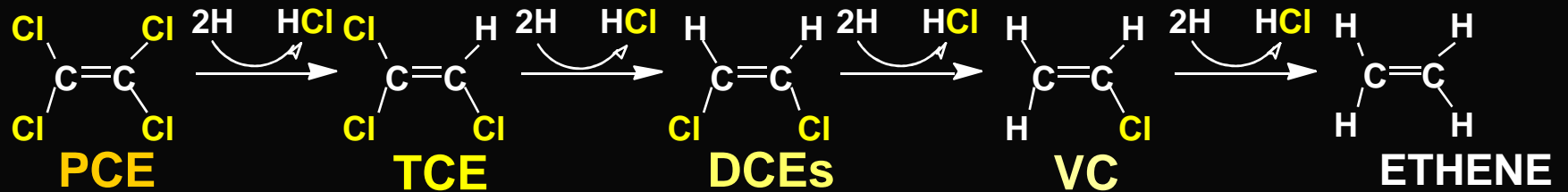
- Electron donors**
- H₂
 - acetate



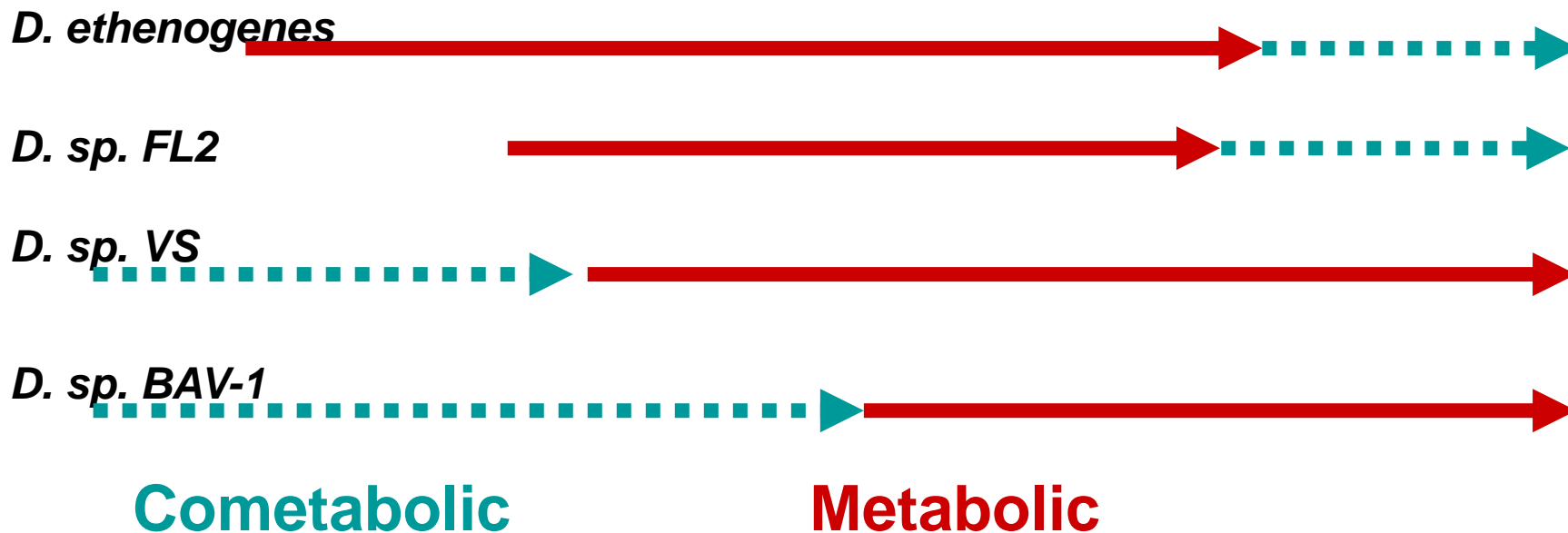
- Electron acceptors**
- PCE
 - TCE
 - DCA
 - chlorobenzenes
 - chlorophenols
 - fumarate
 - nitrate
 - nitrite
 - DMSO
 - sulfite
 - thiosulfate
 - As(V)
 - Fe(III)
 - Mn(IV)

- Electron donors**
- H₂
 - formate
 - acetate
 - pyruvate
 - ethanol
 - lactate
 - butyrate
 - succinate
 - phenyl methyl ethers

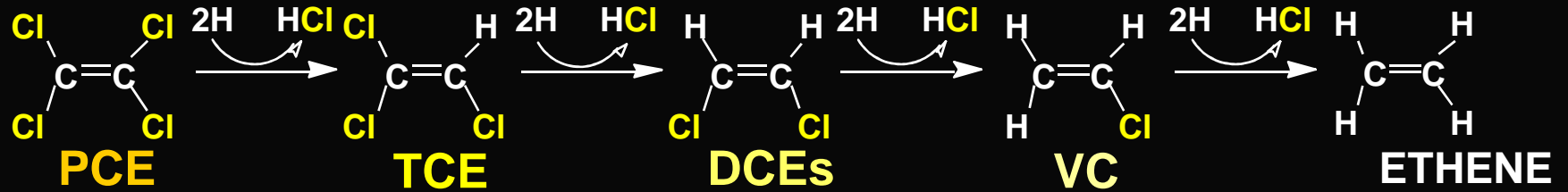
Stepwise Dehalogenation & Microdiversity



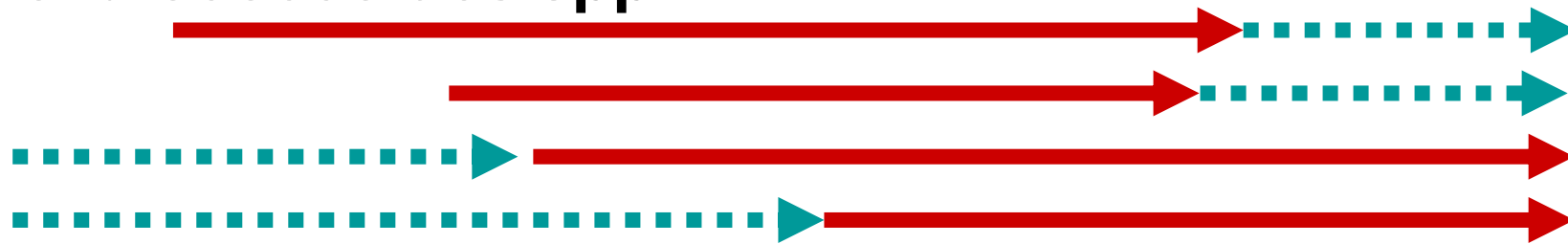
What *Dehalococcoides* spp. can do:



Stepwise Dehalogenation & Microdiversity



***Dehalococcoides* spp.**



Clostridium sp.

Dehalobacter spp.

Desulfitobacterium spp.

Desulfuromonas spp.

Sulfurospirillum spp.



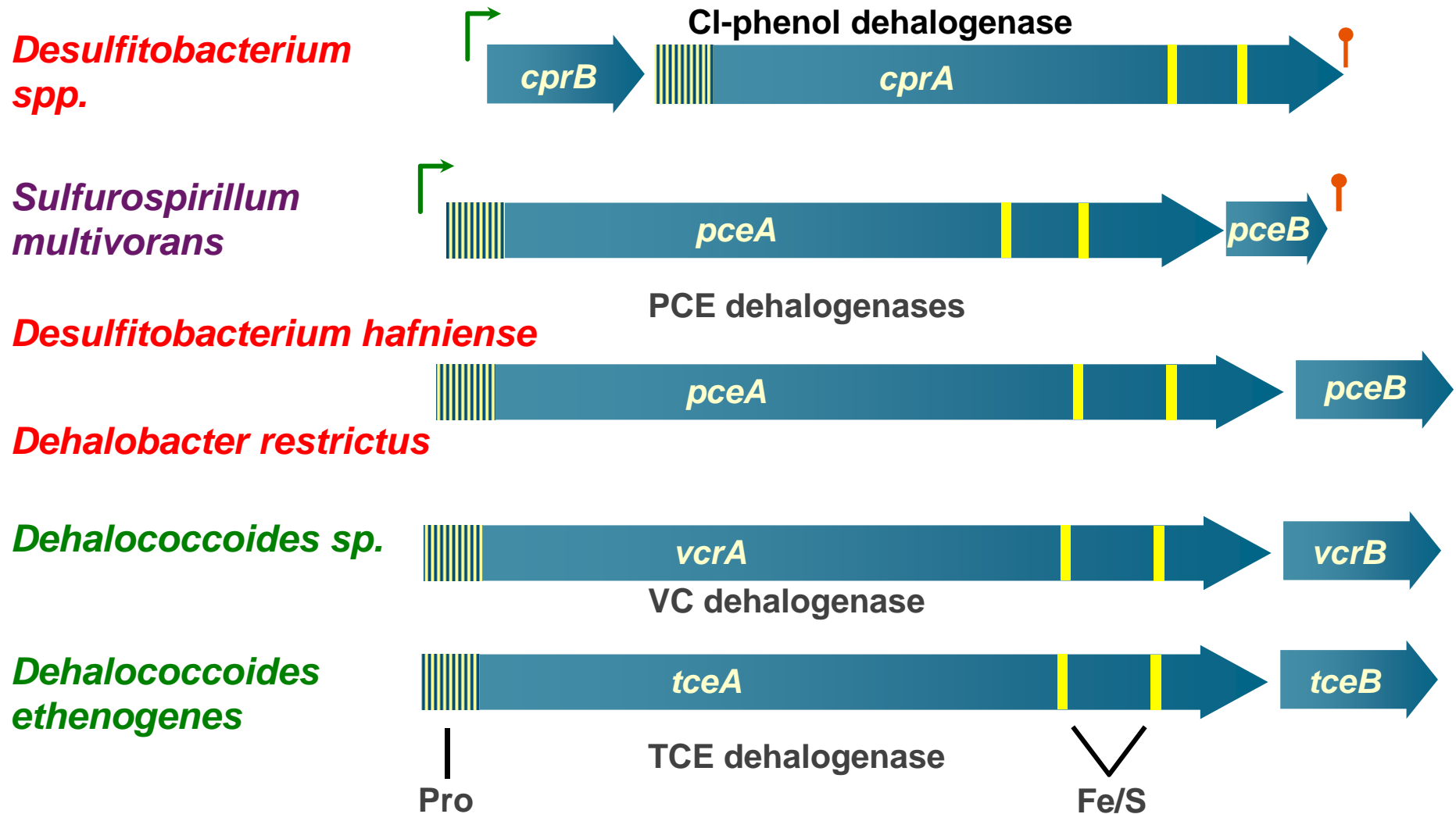
Lessons from Isolates

- ✓ Reductive dehalogenation **widespread across bacterial domain**
- ✓ **Functional microdiversity** – Different activities in closely related strains
- ✓ **Non-dehalogenating strains** within organohalide respiring genera

Catabolic Gene Markers

Reductive dehalogenase genes

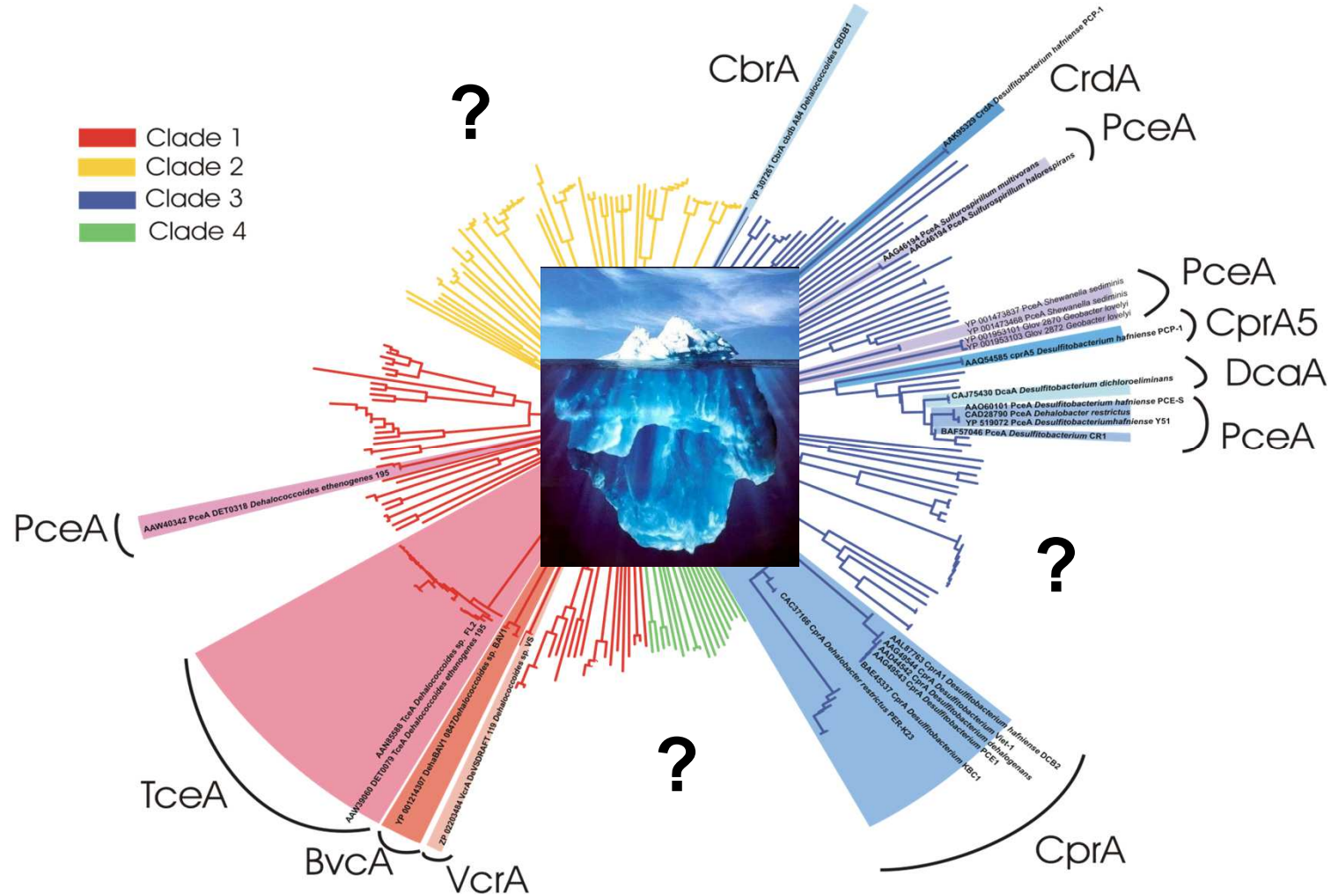
Conserved motifs



Organohalide Respiring Bacteria

Current Diversity of Dehalogenases

Some known
Many unknown



Assessing Activity of OHRB Study Site: The Ebro Basin

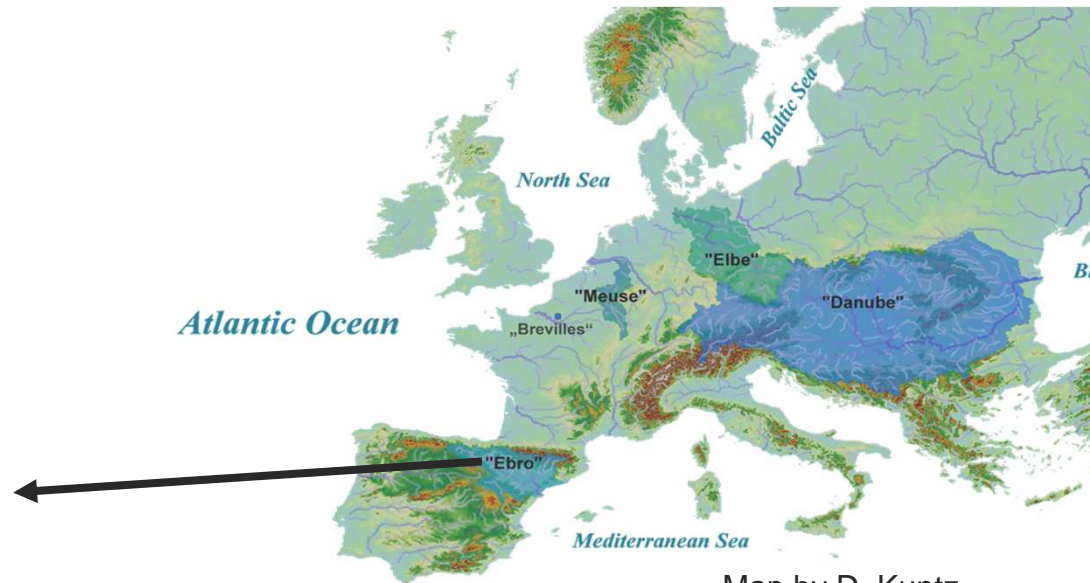


Ebro

pesticides
HCHs
DDT
PCBs
BFRs

Chlorinated organics

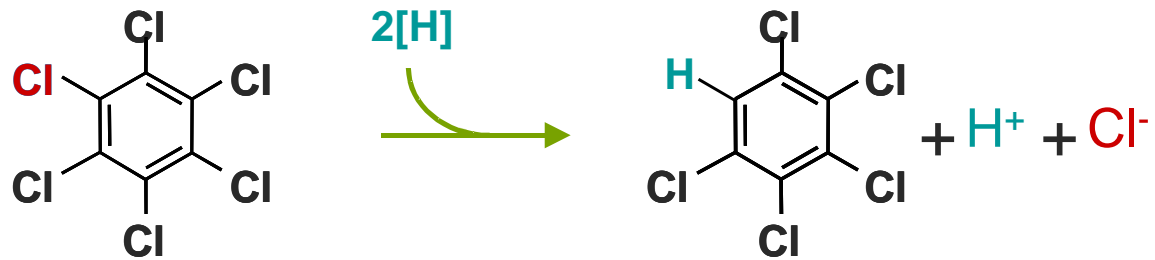
Heavy metals
(Hg,Cd,Zn,As)



Map by D. Kuntz

Focus on hexachlorobenzene

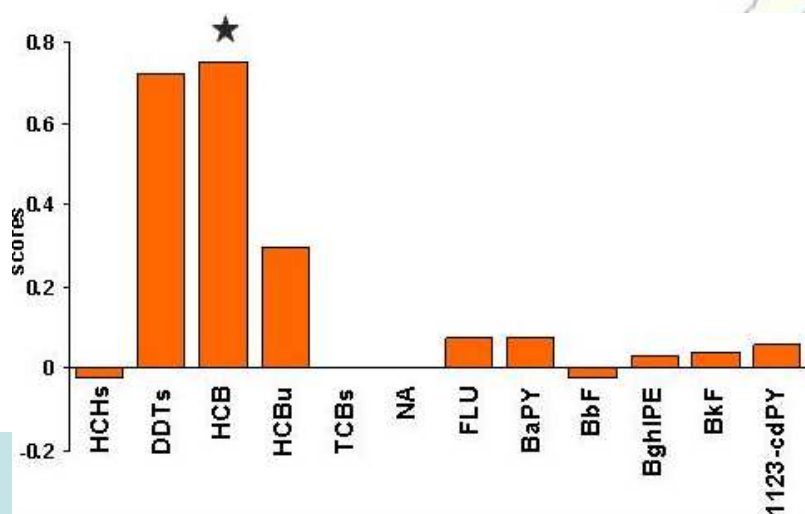
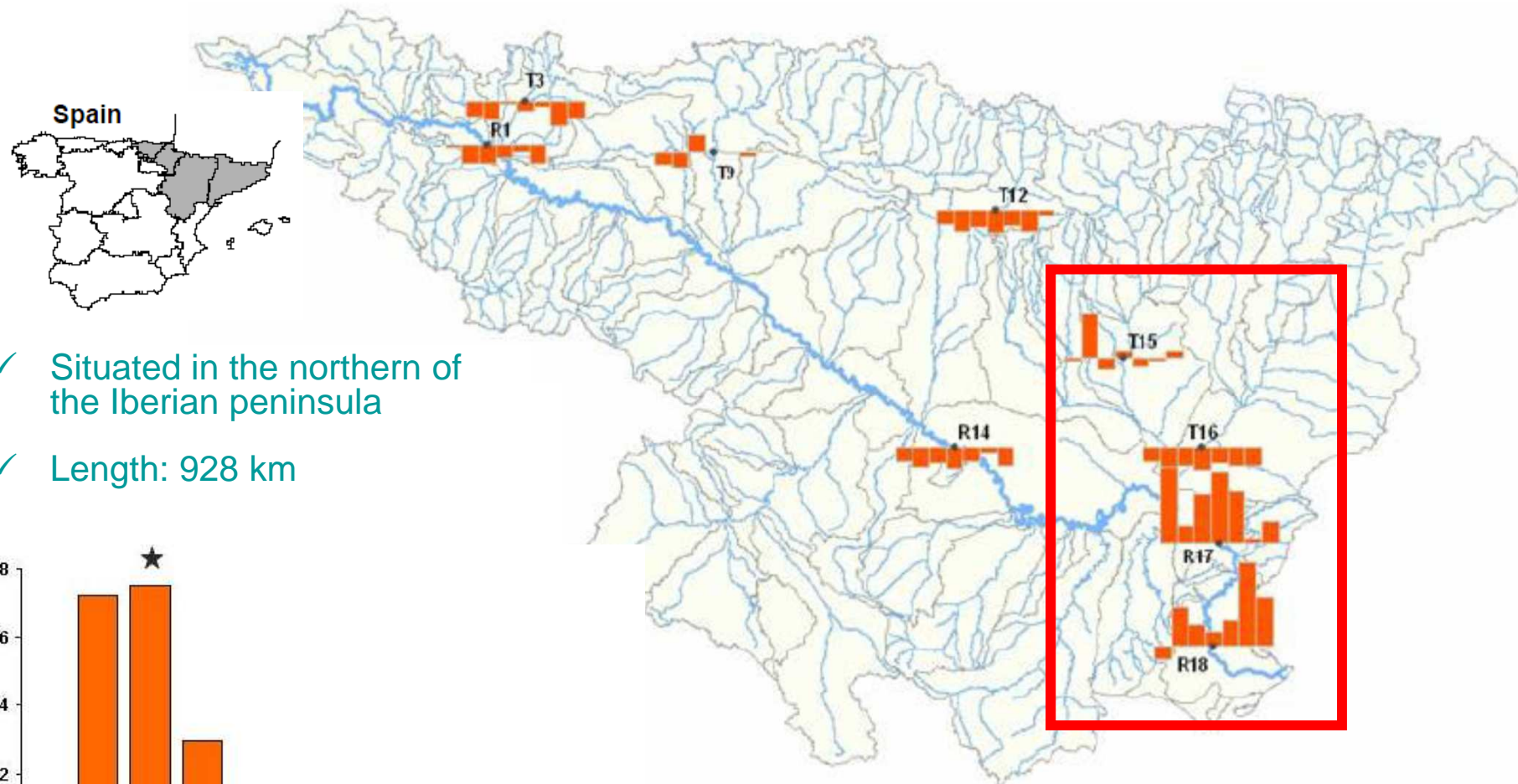
Hexachlorobenzene (HCB)



- ✓ Also here: stepwise dehalogenation
- ✓ Functional microdiversity in closely related strains of *Dehalococcoides*

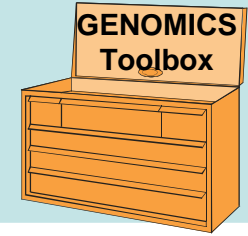
AQUATERRA sites

EBRO

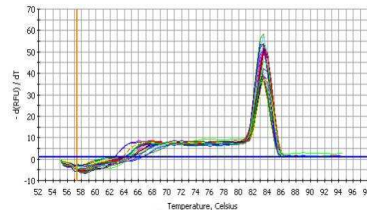


Terrado, M. Tauler R. and Barceló D., 2006, "GIS and chemometric modeling of historical data", AquaTerra Deliverable R2.10

Genomics Toolbox @ Work

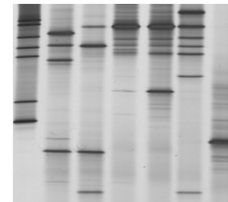


RNA & DNA isolation from sediment samples



Quantitative RT-PCR

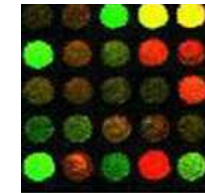
Dehalococcoides spp.
Desulfitobacterium spp.
Dehalobacter spp.
Universal Bacteria



DGGE

Dehalococcoides spp. specific DGGE assay

Sequencing

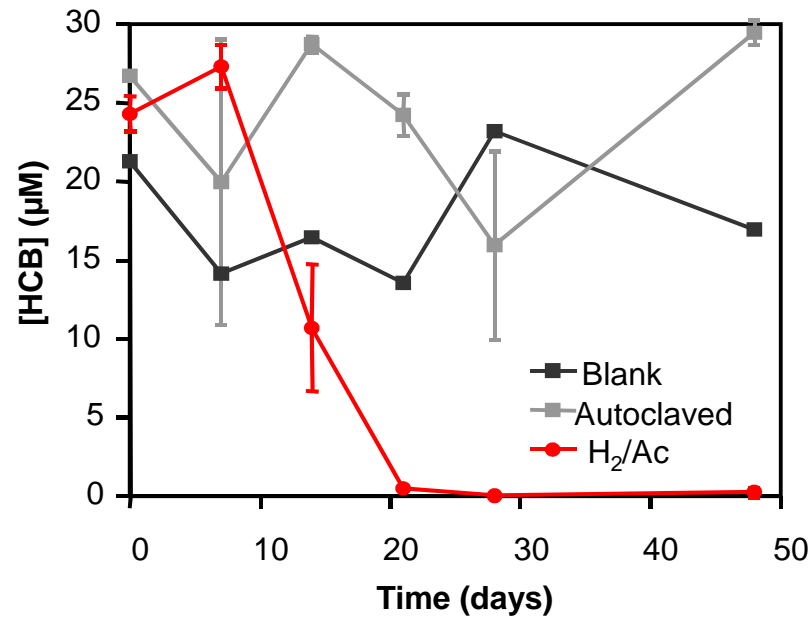


Functional Gene Microarray

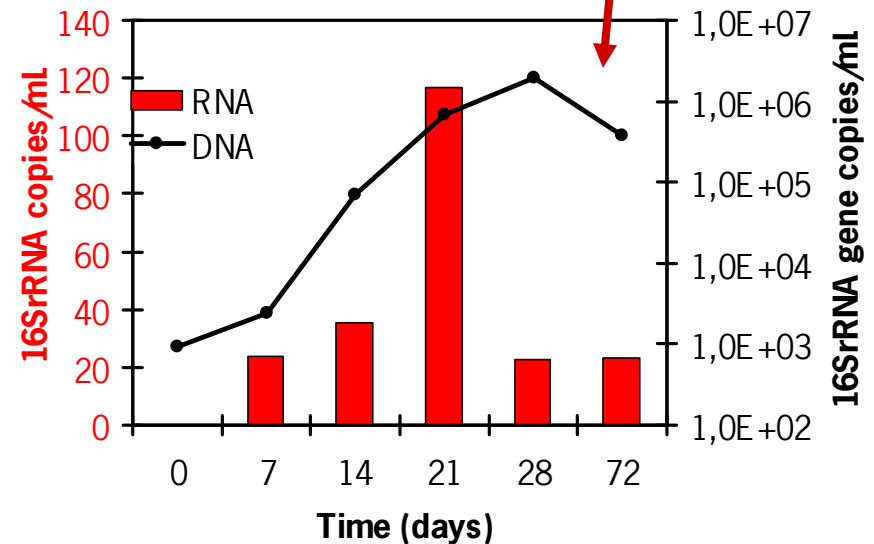
Statistical Analysis
(R & CANOCO)

Combination of physiological and molecular ecology data

Flix sediment



DNA stays much longer



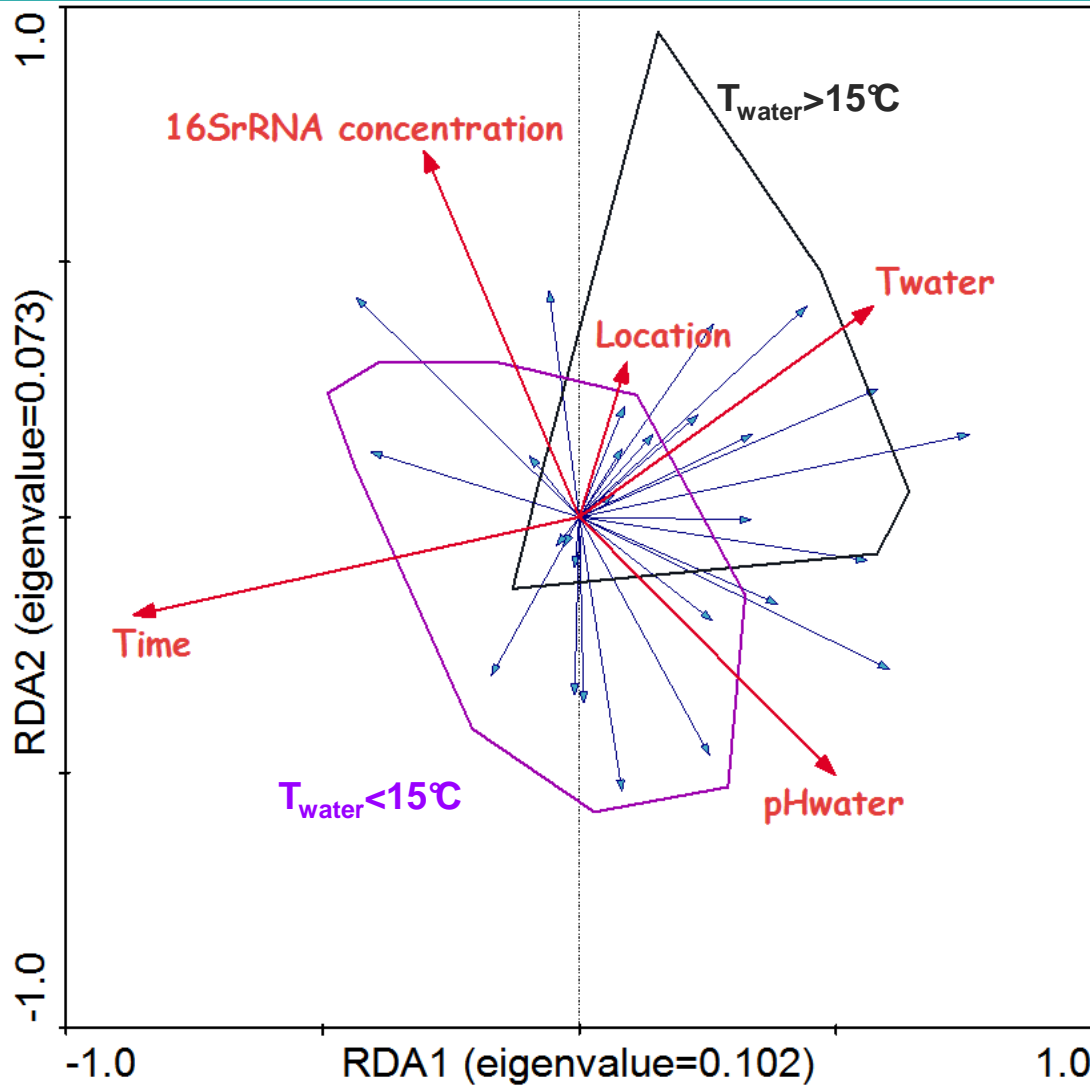
- Profile of rRNA copy numbers concomitant with degradation

Dehalococcoides sp. specific qPCR

EBRO

Dehalococcoides Diversity

DGGE fingerprinting



Conditional Effects

Variable	P
Time	0.002
16SrRNA Conc.	0.004
T_{water}	0.092
pH _{water}	0.054
Location	0.190
Depth	0.146

SPECIES

(or DGGE Bands)



SAMPLES



— $T_{\text{water}} > 15^{\circ}\text{C}$



— $T_{\text{water}} < 15^{\circ}\text{C}$

ENV. VARIABLES

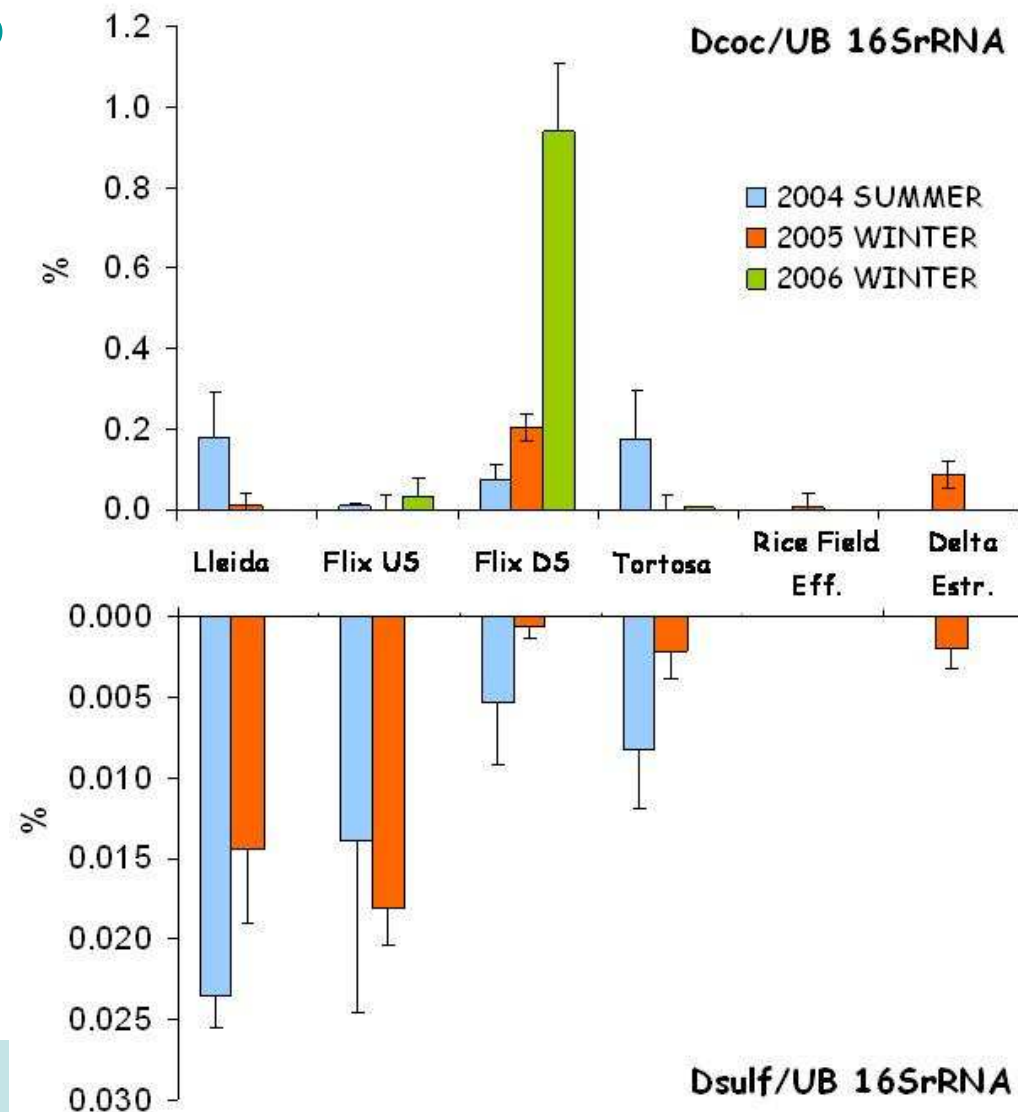


Multivariate statistics (RDA)

Quantitative PCR profiling

rRNA Bacteria & OHRB

- ✓ Ratio of 16SrRNA of OHRB to bacteria as a measure of activity
- ✓ *Dehalococcoides* spp. appear as most active OHRB
- ✓ Activity of *Desulfitobacterium* spp. in upstream locations



Routine HTP Monitoring of Bioremediation

✓ Multiplex assays:

- ✓ Allow detection of multiple targets in single experiment
- ✓ Reduce testing time and costs

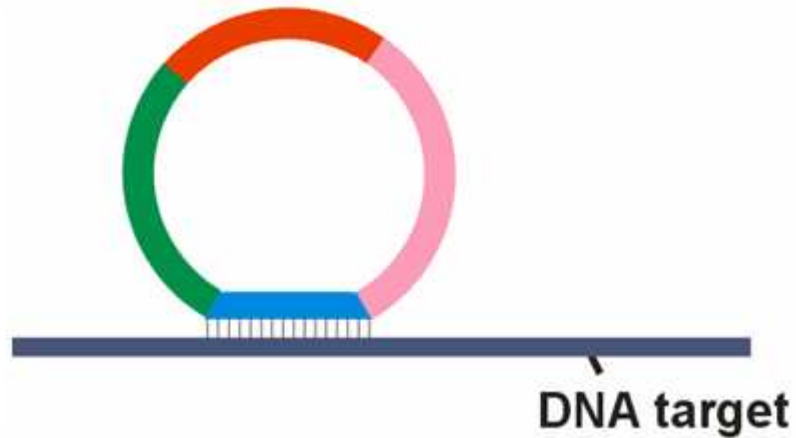
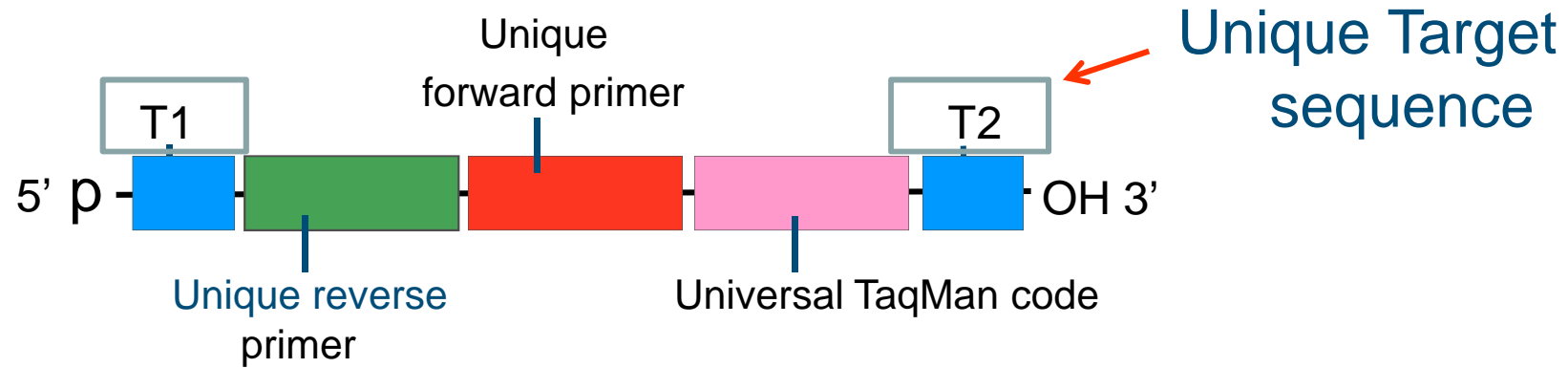
✓ Challenges

- ✓ High number of different targets
- ✓ Very specific detection required
- ✓ Targets might be present in a wide concentration range
- ✓ Low concentration target DNA in high concentration background DNA

Routine HTP Monitoring of Bioremediation

- Multiplex assays:
 - Allow detection of multiple targets in single experiment
 - Reduce testing time and costs
- Challenges
 - High number of different targets
 - Very specific detection required
 - Targets might be present in a wide concentration range
 - Low concentration target DNA in high concentration background DNA
- ✓ Technology
 - Circularization probe-based detection (Ligation probes)

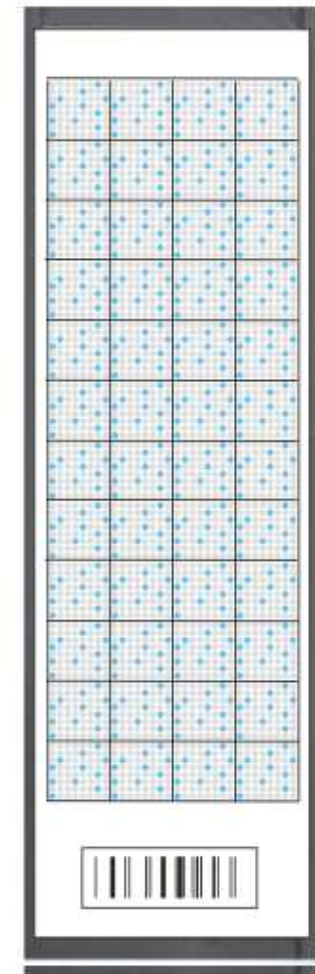
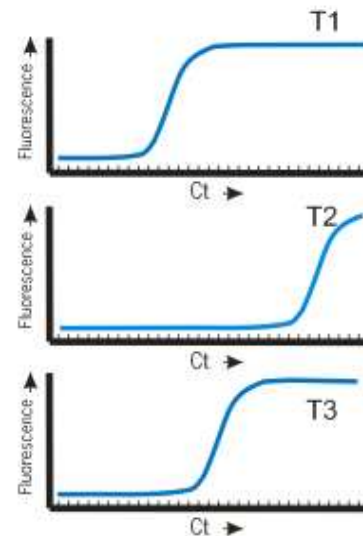
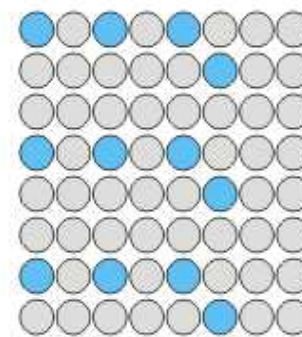
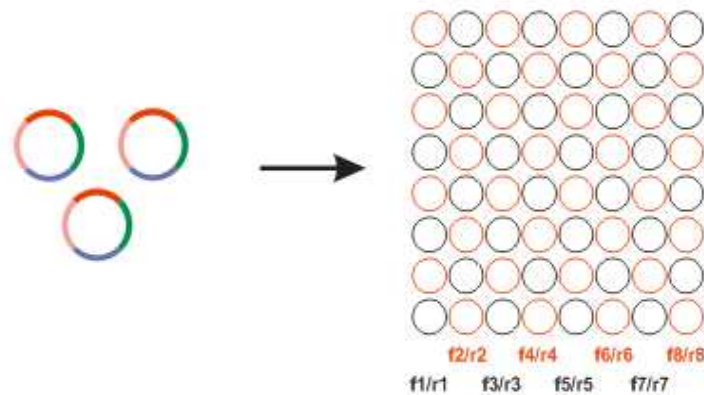
PRI-Lock probe principle



Hybridization
Ligation

Ligation Dependent

Quantitative multiplex platform



Multiplex PRI-lock ligation

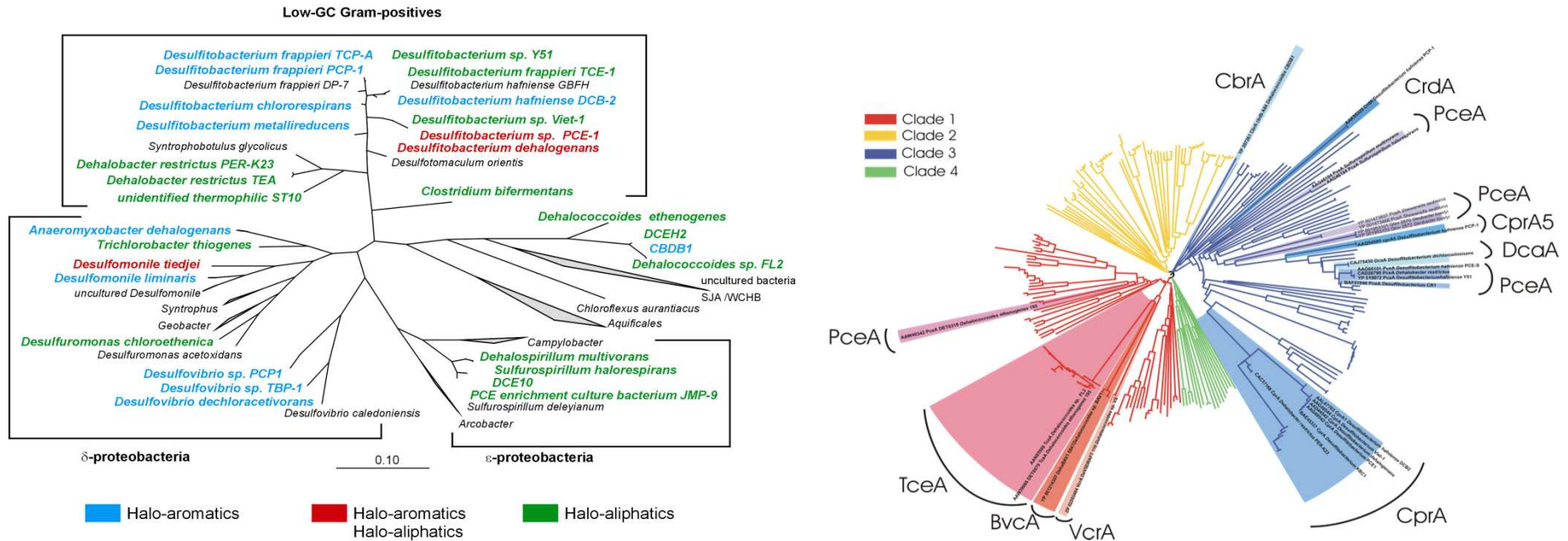
singleplex probe amplification

Biotrove

“33 nL Real-Time PCR array”

Quantitative multiplex platform

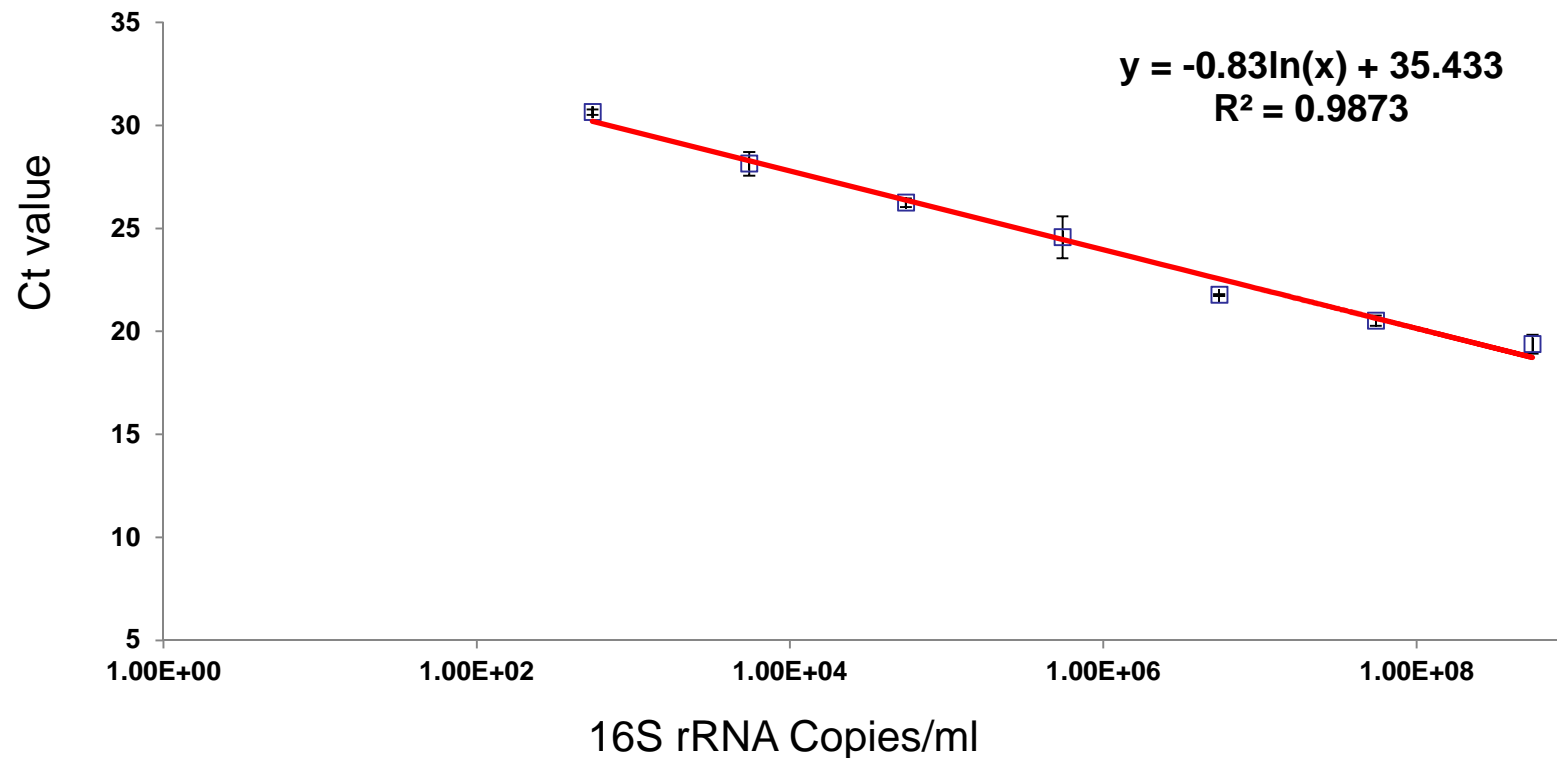
Targets



- Multiple PRI-lock ligation probes targeting representative
 - 16S rRNA genes
 - reductive dehalogenase genes

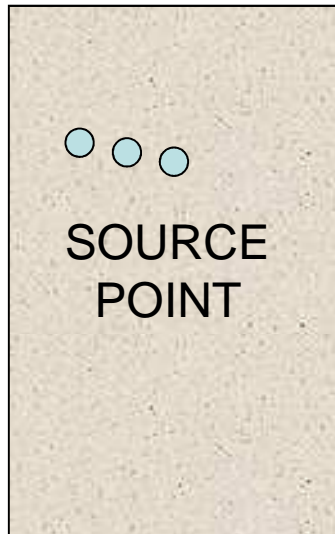
Probe Dynamic Range

Representative Example: Standard Curve for *Dehalococcoides* Probe

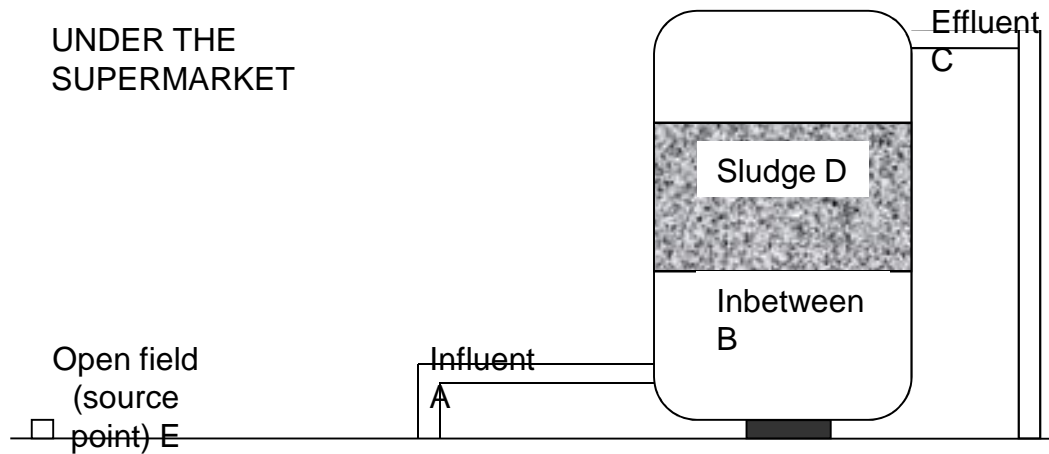


Detection Dynamic Range = 6 orders of magnitude

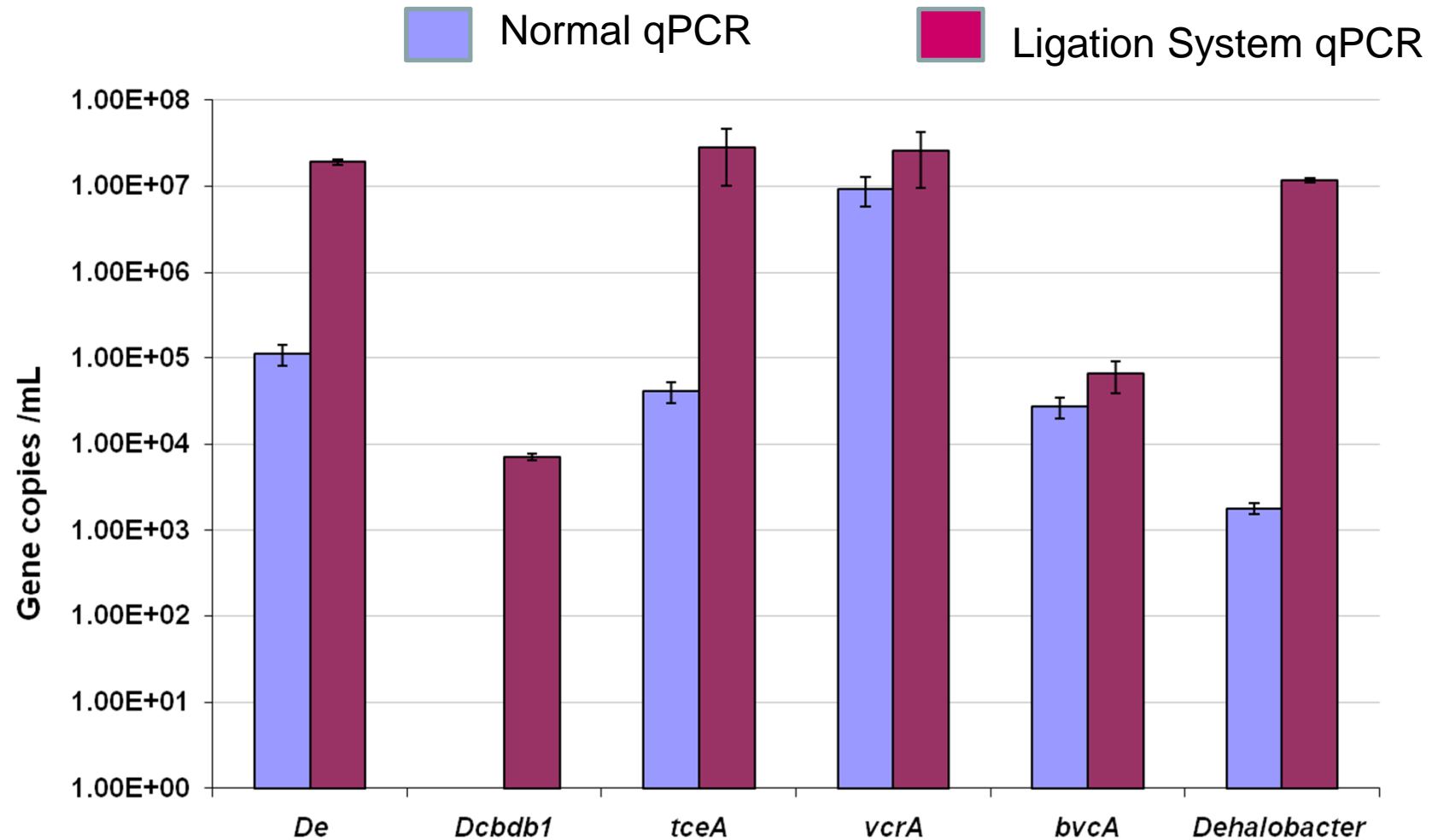
Cleaning Under The Supermarket...



UNDER THE SUPERMARKET



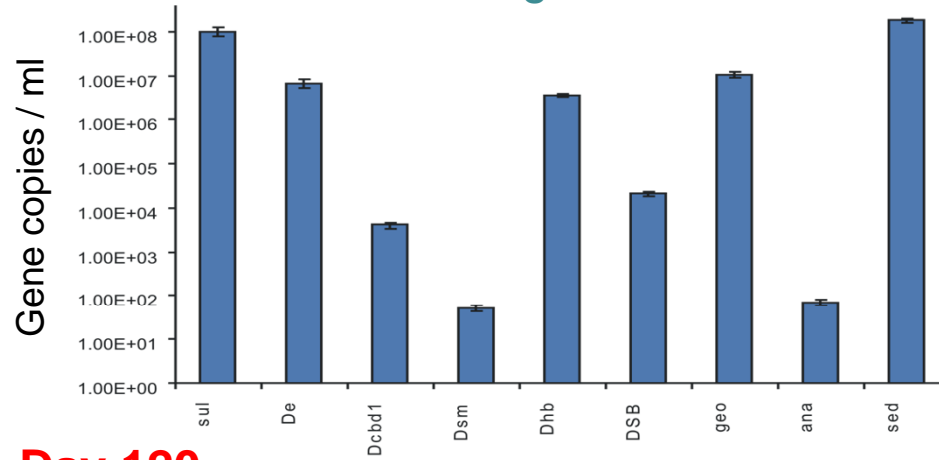
Profiling the Bioreactor SYBR qPCR vs Ligation System



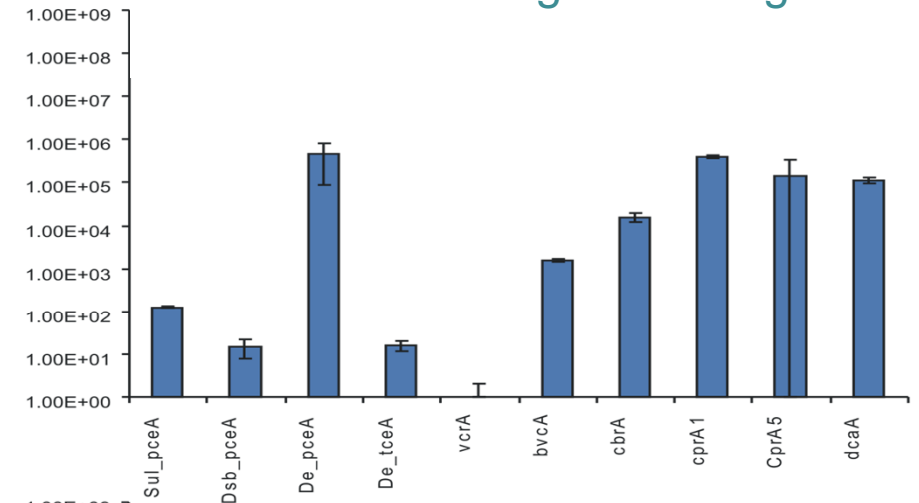
Monitoring Field Samples

Day 1

16S target

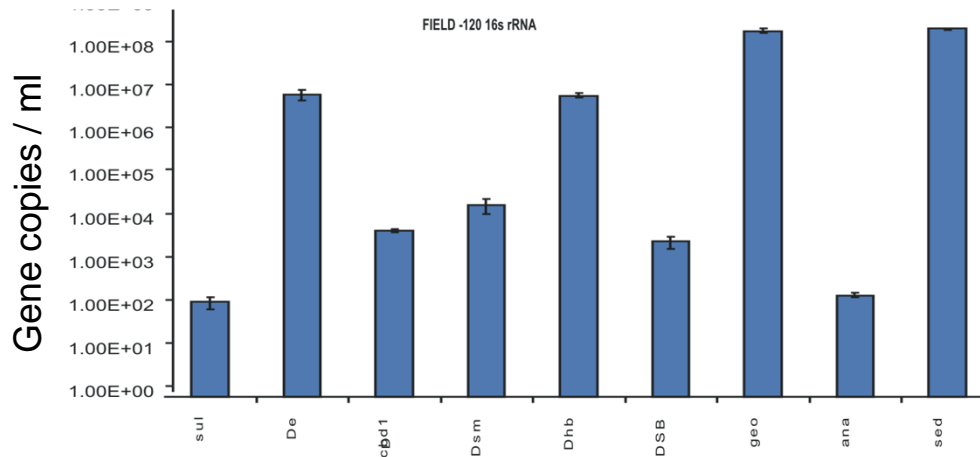


Reductive dehalogenase targets

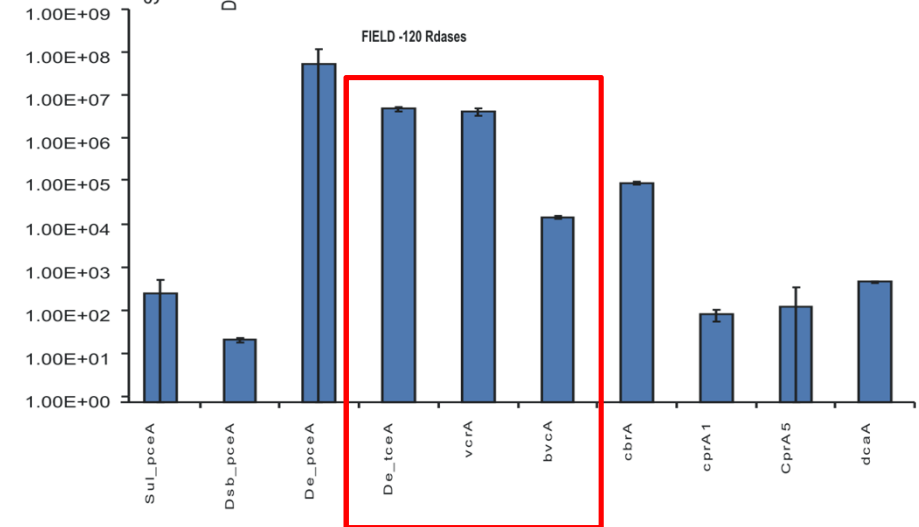


Day 120

FIELD -120 16s rRNA



FIELD -120 Rdases



- Bioaugmentation with Dechlorinating culture :
- Increase in Reductive dehalogenases for Vinyl Chloride degradation

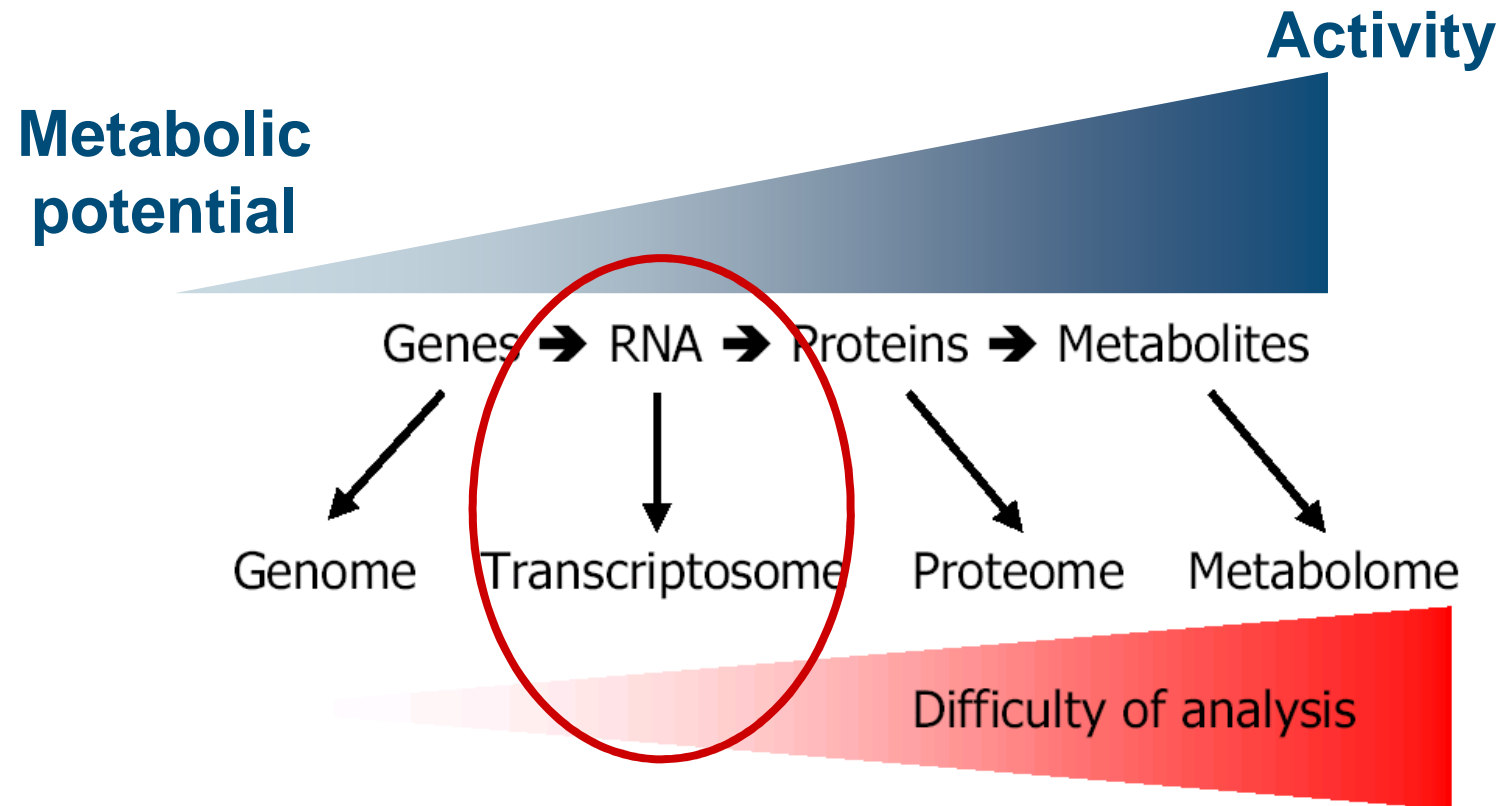
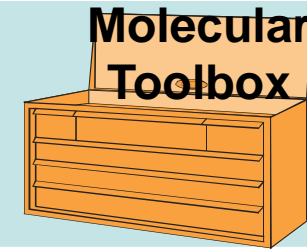
HTP Monitoring

Current State of the Art

Quantitative multiplex target detection

- PRI-Lock Probes --- High specificity
- Single and multiplex target detection independent
- Detection Magnitude : 6-7 orders dynamic range
- Universal Pre- amplification improves sensitivity
- Universal TaqMan / SYBRGreen PCR conditions
- Applicable to various environmental systems and biomarkers

Current Issues

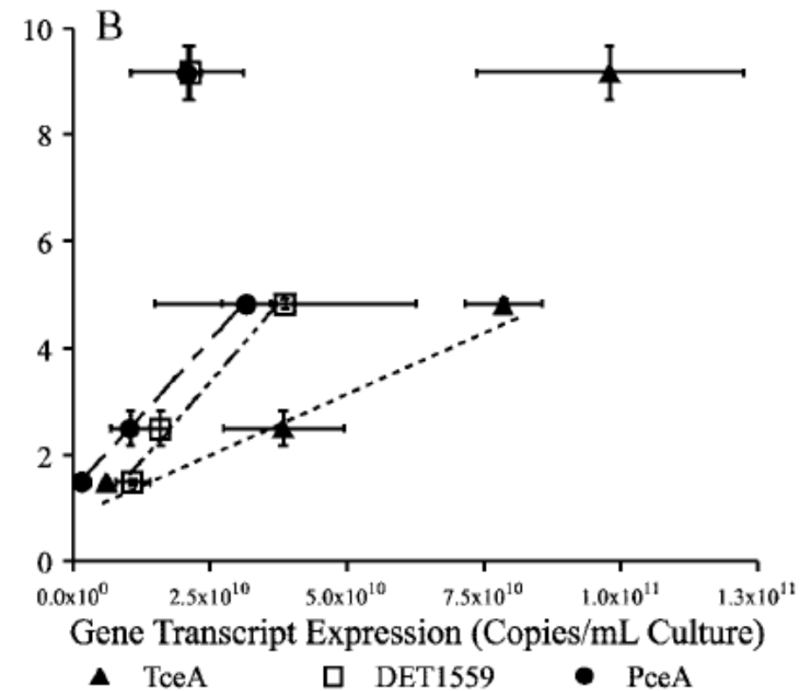
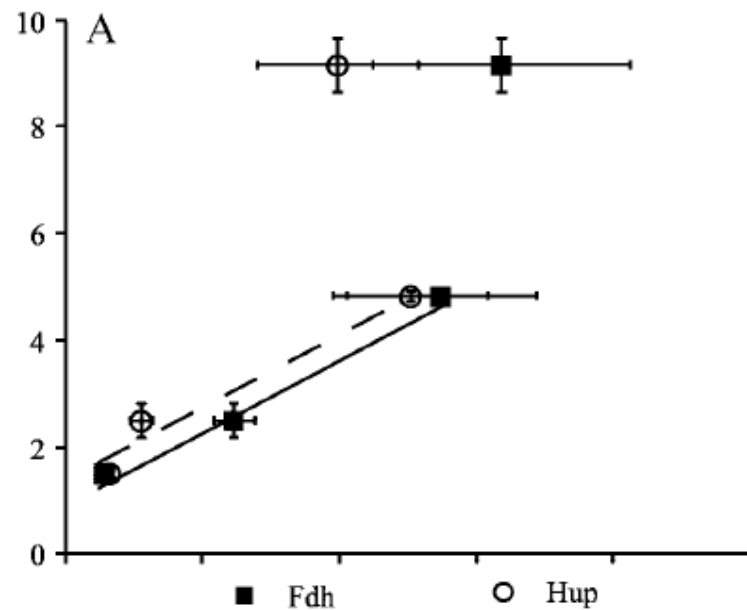


Can we actually deduce degradation rates?

Correlating gene expression levels to respiration rates

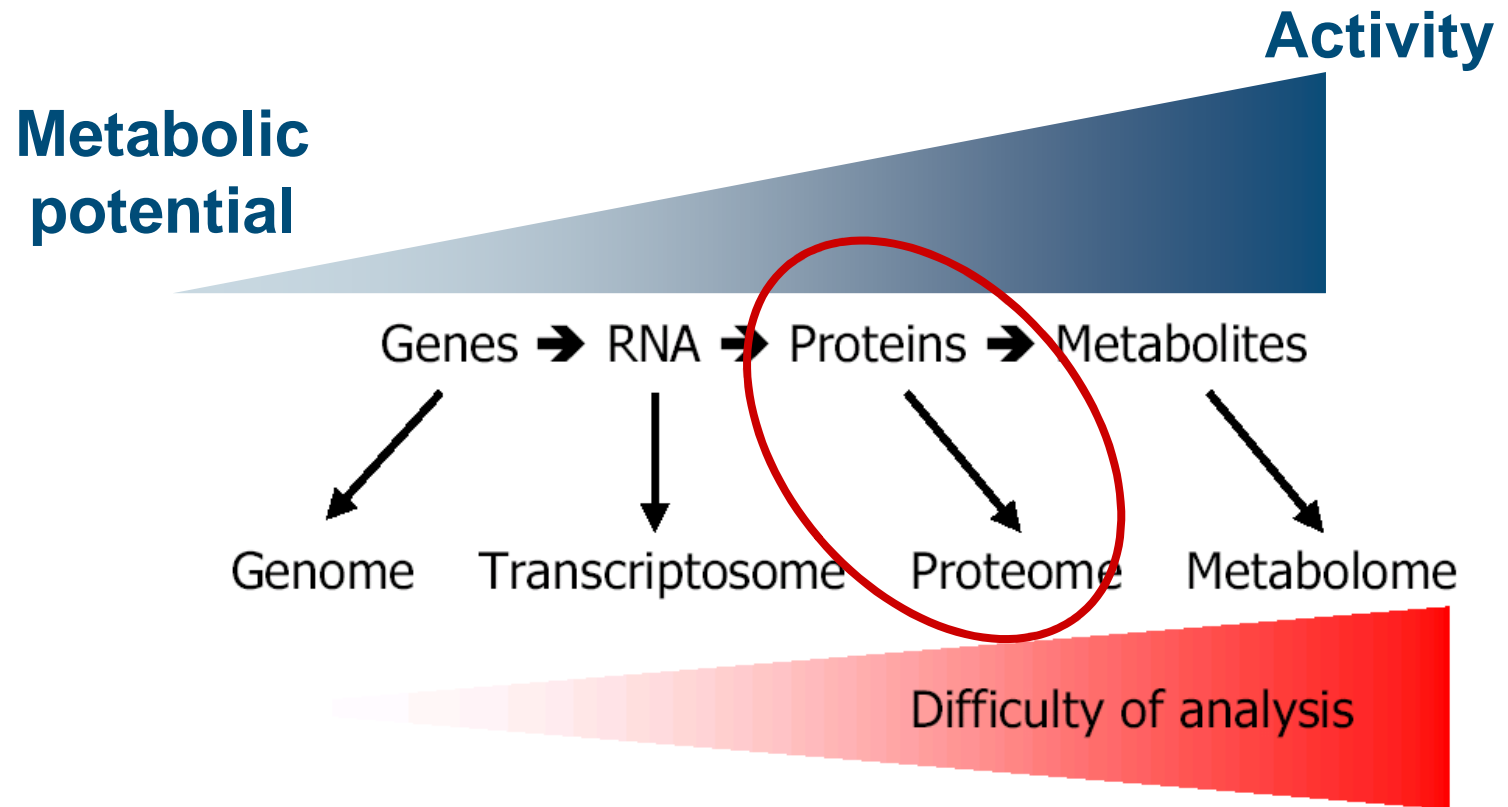
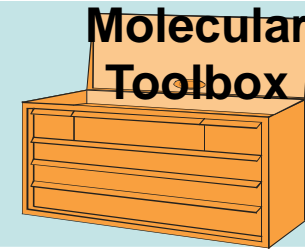
Rahm & Richardson
2008, EST

- ✓ *Dehalococcoides ethenogenes* 195
 - PCE continuously fed



- ✓ Linear correlation across limited range of rates
- ✓ BUT: Rates probably strain (enzyme) specific!!

Some Issues

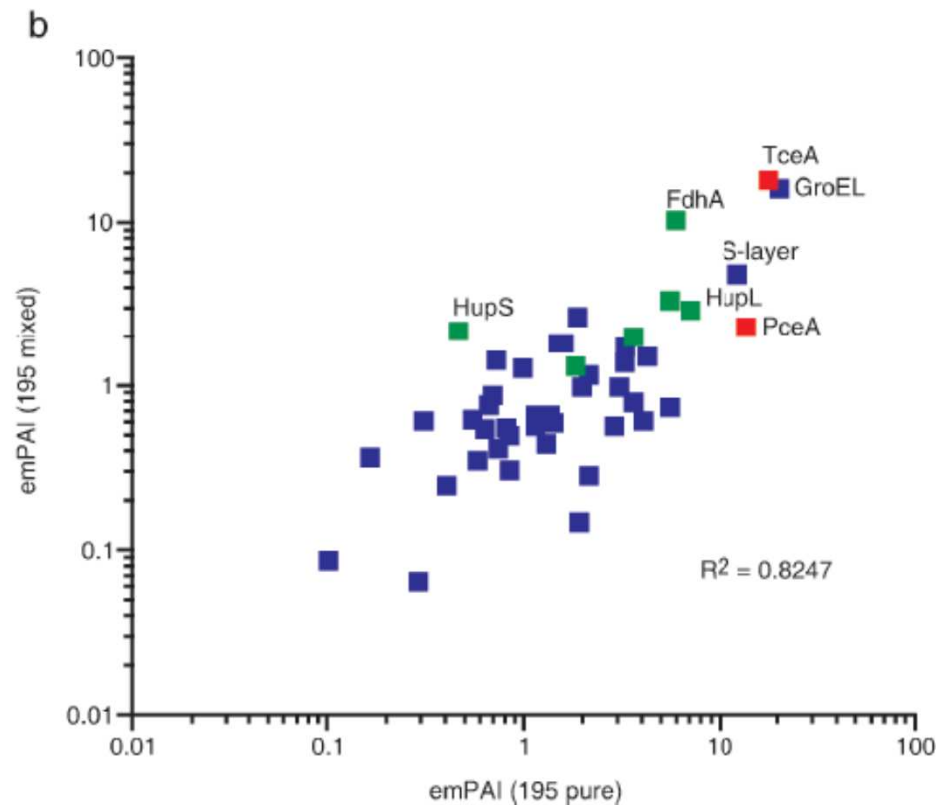


Can we look at proteins as the actual biocatalysts ?

HTP Proteomics

Morris et al.,
2007, AEM

- ✓ *D. ethenogenes* 195 pure culture and mixed culture
- PCE fed



Detection of respiratory enzymes (Rdh, H2ases, Fdh)

Special Thanks to ...

WU-Microbiology

Thomas Kruse
Farai Maphosa
Neslihan Tas
Ye Tian
Mark Sturme
Mark van Passel
Hans Heilig
Gosse Schraa
Willem M. de Vos



EU



PRI

Ronald v. Doorn, Cor Schoen,
Peter Bonants