Application of Molecular Tools to Optimize Bioremediation

California Department of Toxic Substances Control, Remediation Technologies Symposium

May 15, 2008

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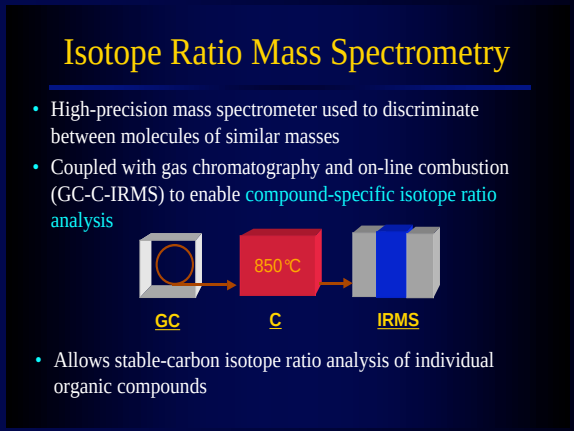
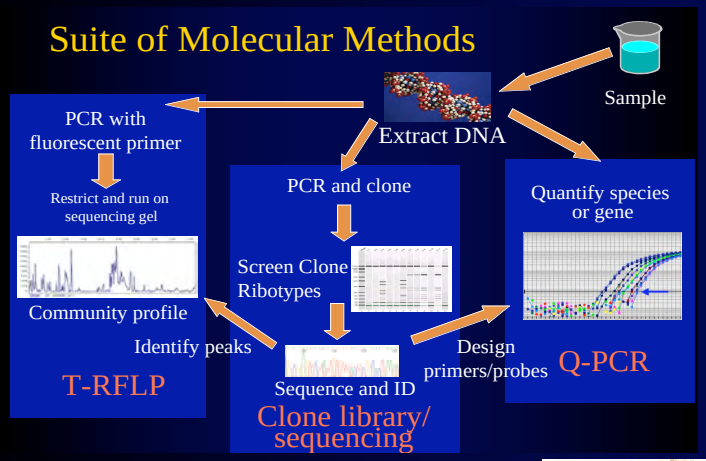
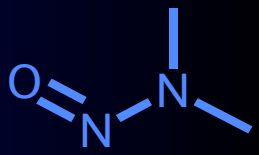
Laboratory Themes

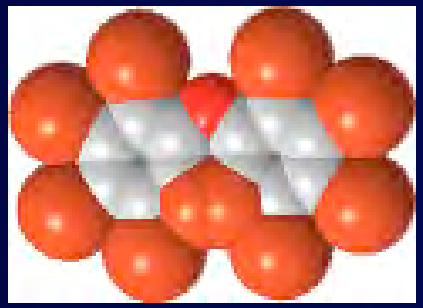
- Molecular and Isotopic Tools to Quantify Bioremediation

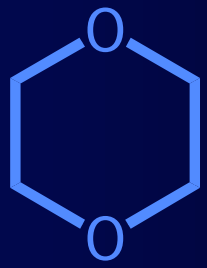


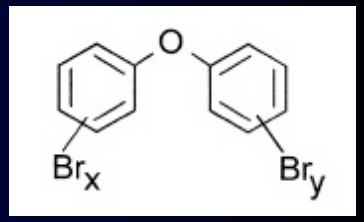


- Biodegradation of Emerging Contaminants

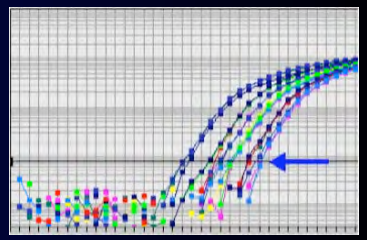




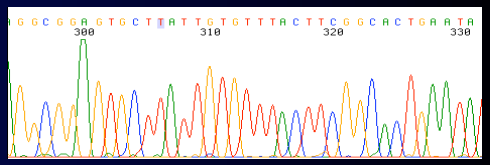




Outline

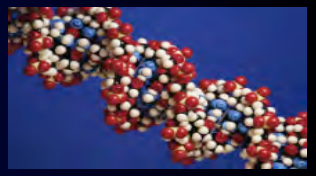


- Bioremediation of solvents
- Molecular Tools to Optimize Bioremediation

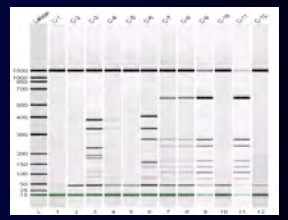


  - Gene detection/quantification - ribosomal and functional genes

  - Quantification of expression



  - Microarrays for transcriptomics and community analysis



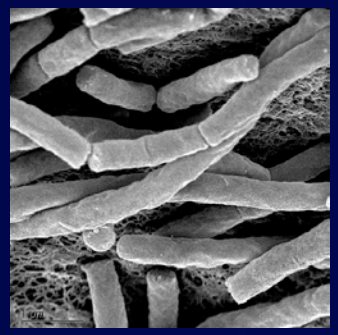
What’s the Problem?

- Leaking Underground Tanks are common

One Solution - In Situ Bioremediation

In situ → In place

Bio → microbial



Remediation → method to fix

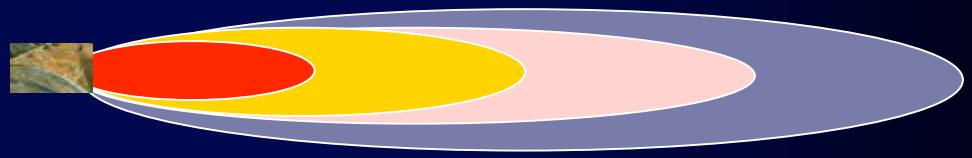
Using microorganisms to clean up pollution in the environment

How does it work?

How does in situ bioremediation clean up pollution?

- Soil microbes are remarkably versatile!
- They eat (electron donor) and breathe (electron acceptor) many types of organic and inorganic chemicals to generate energy to live.
  - (Gasoline, oil, chromium, iron, solvents, perchlorate, nitrate, ammonia, MTBE, etc)

- They live in complex communities capable of degrading contaminants by “bucket brigade”.



claybennett.com

Applying *In Situ* Bioremediation

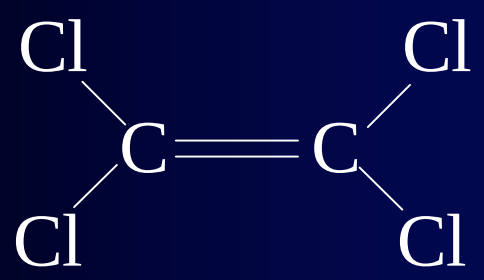
- Natural Attenuation - biotransformation occurs naturally: indigenous microbes present, substrates & nutrients present (can be MNA)

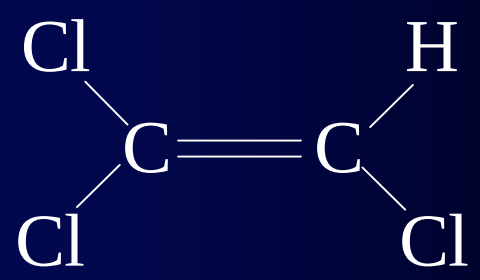
- Biostimulation - indigenous microbes present, substrates &/or nutrients must be added
- (a type of Enhanced Attenuation or PPT)

- Bioaugmentation - indigenous microbes not present, organisms must be added

alken-murray.hypermart.net/clearflo.htm

Per- & Tri- chloroethene (PCE, TCE)





- Suspected carcinogens
- Cause damage to kidneys, liver, lungs, central nervous system
- Most common GW contaminants at US Superfund sites

Cleaning and degreasing

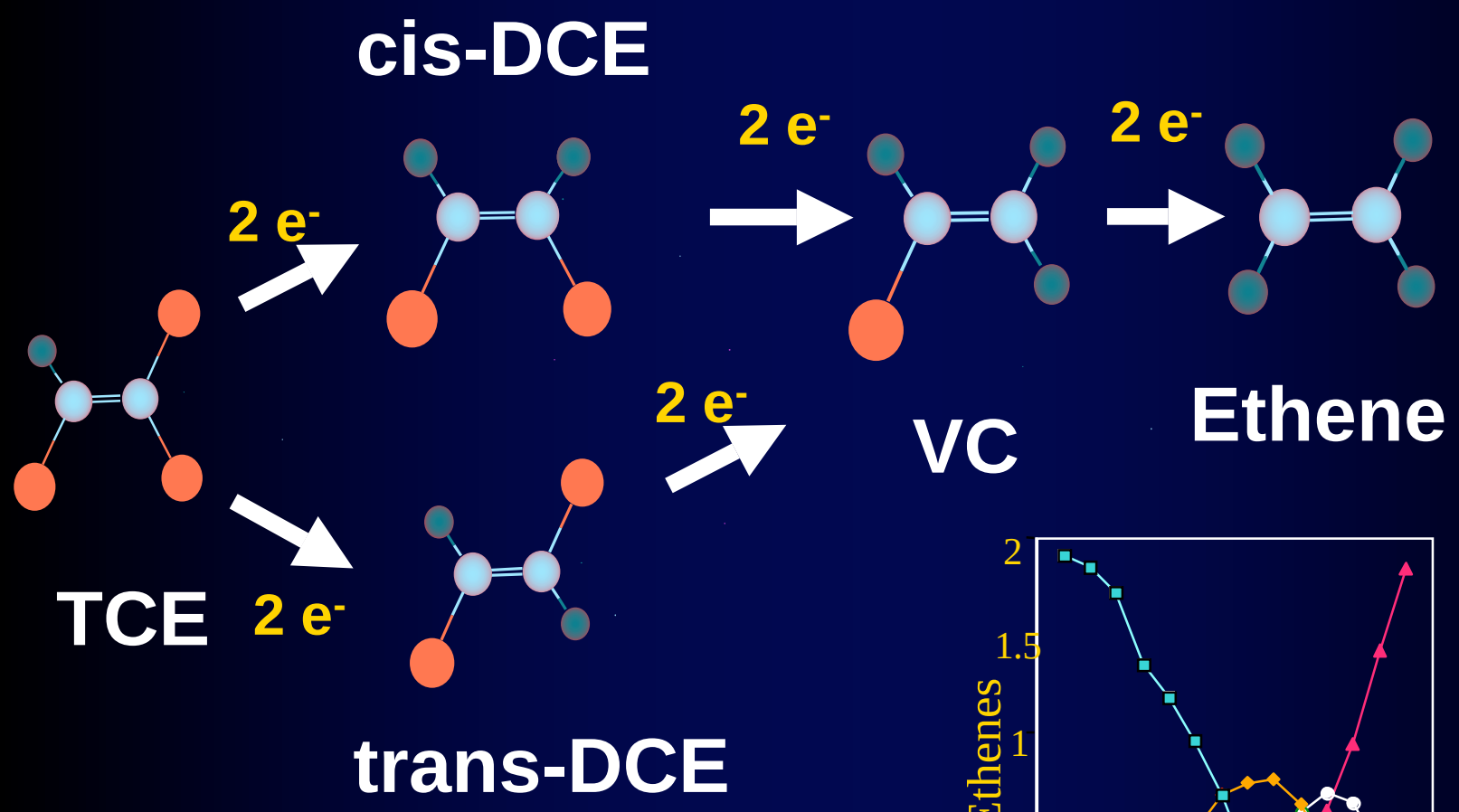
Chemical Processing

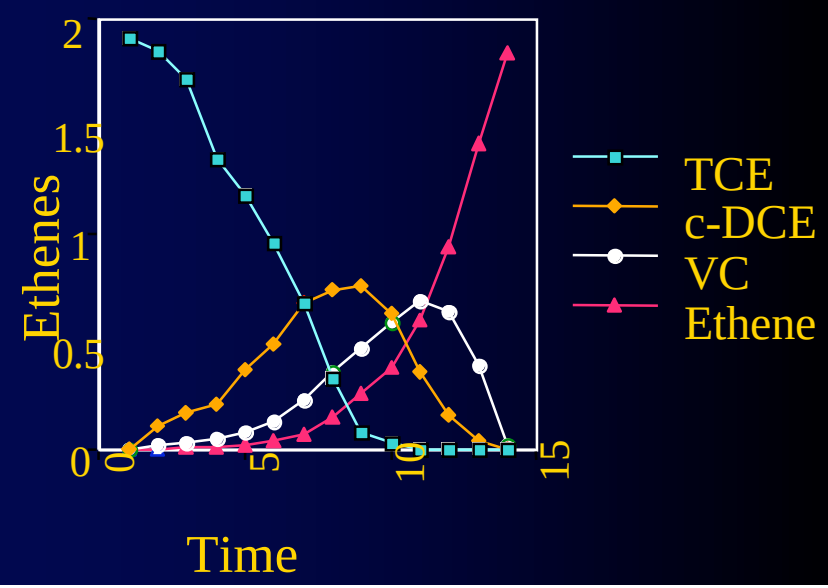
Paint Stripping

Dry Cleaning

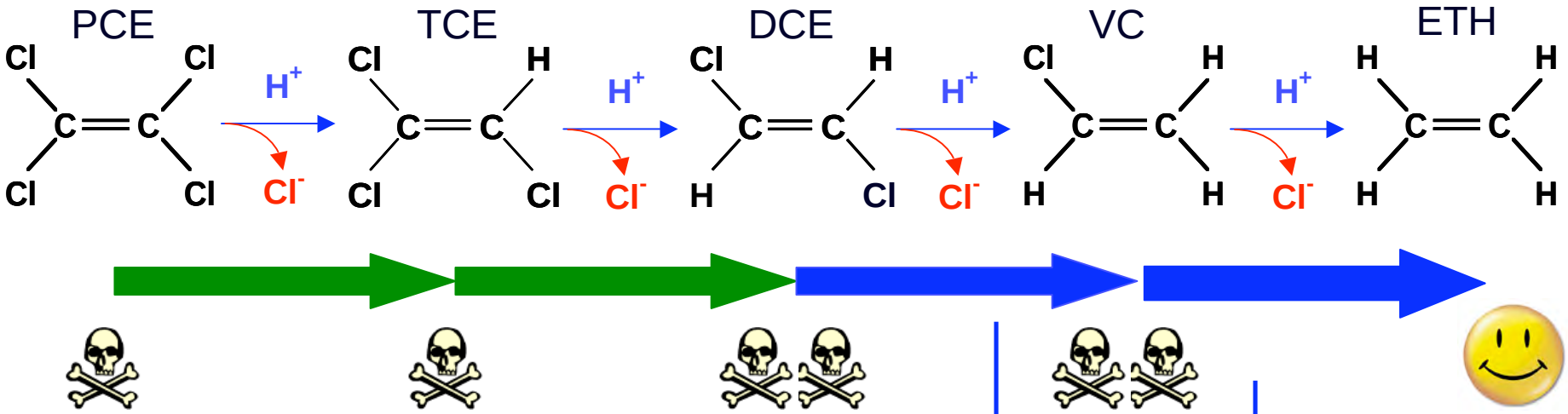
www.dexter.com

Reductive Dechlorination of TCE

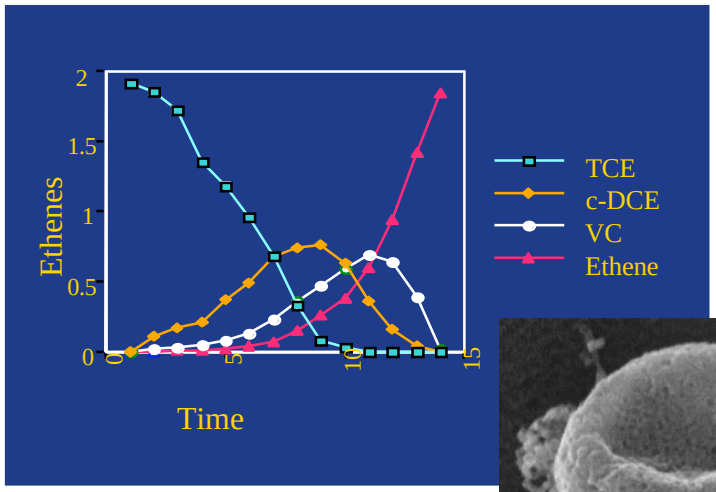


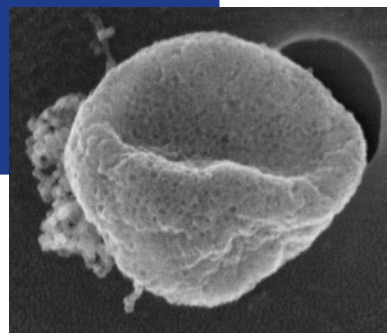


Anaerobic microbial reductive dechlorination



Dehalobacter, Dehalospirillum, Desulfitobacterium, Desulfomonile, etc.





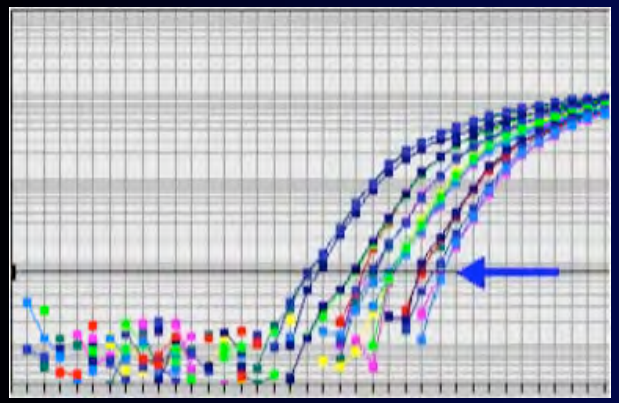
Dehalococcoides

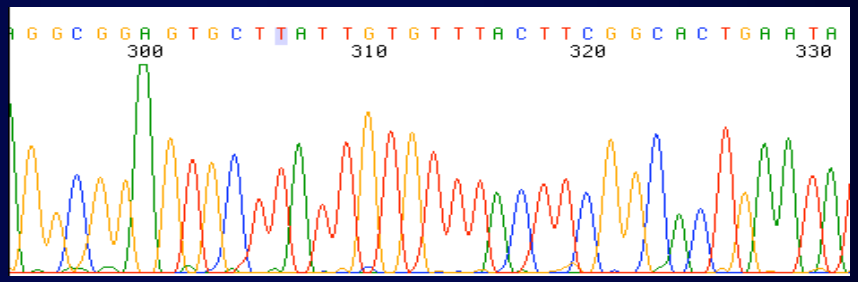
- Electron acceptors: chlorinated organics
- Electron donor: H_2
- Carbon source: acetate
- Some impt. RDase genes:
  - *pceA and tceA* from 195
  - *bvcA* from BAV1
  - *vcrA* from VS

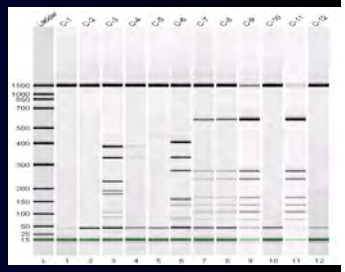
Microbial Communities Needed!

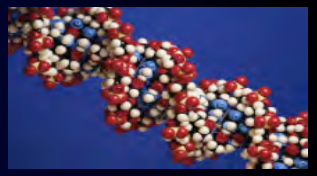
Background: *Dehalococcoides* spp. can bioremediate solvents - but can't live alone (need hydrogen, acetate, etc.)

Goal: Apply molecular approaches to characterize, understand and optimize chloroethene degrading microbial communities and their activity.

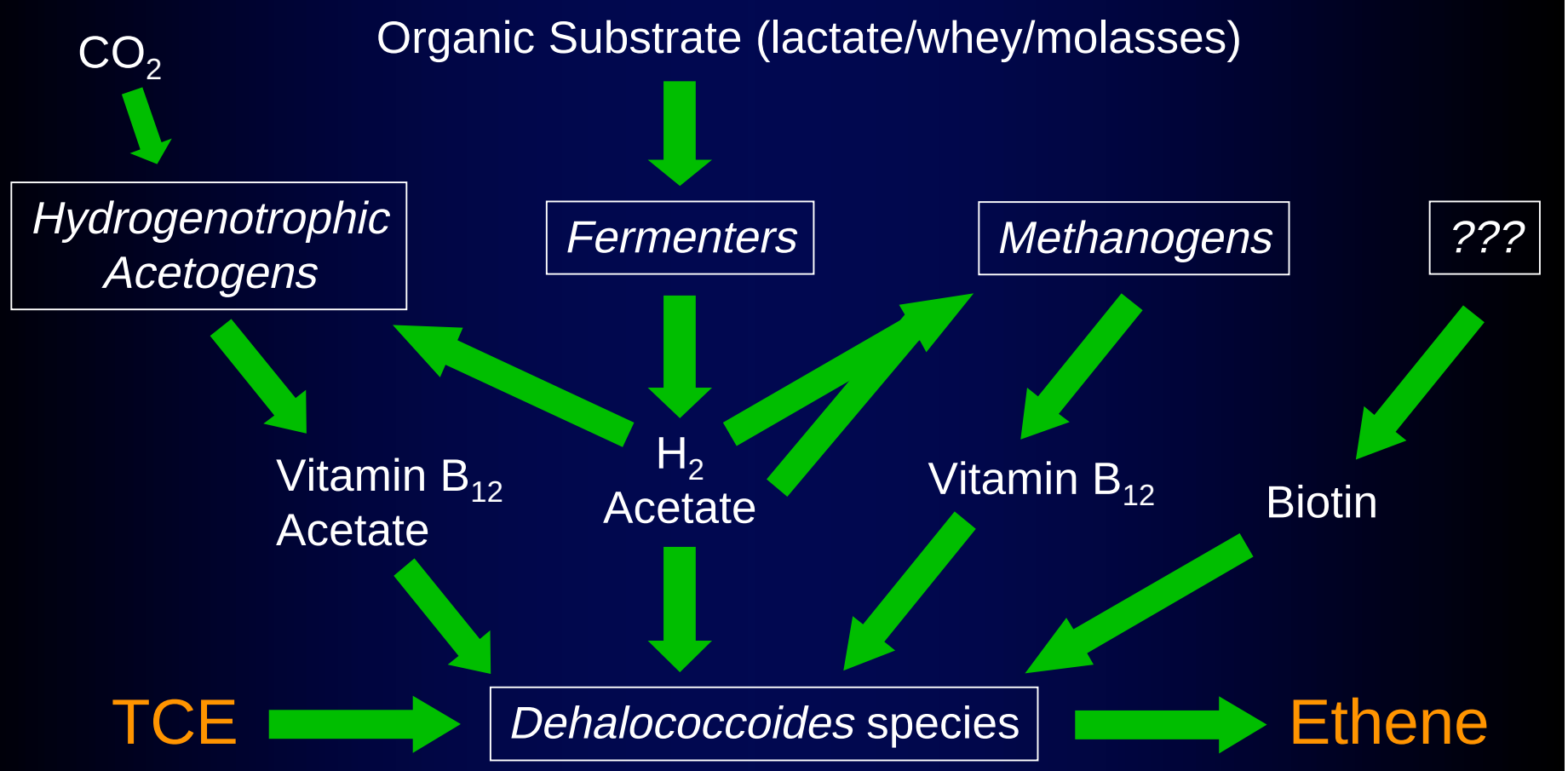






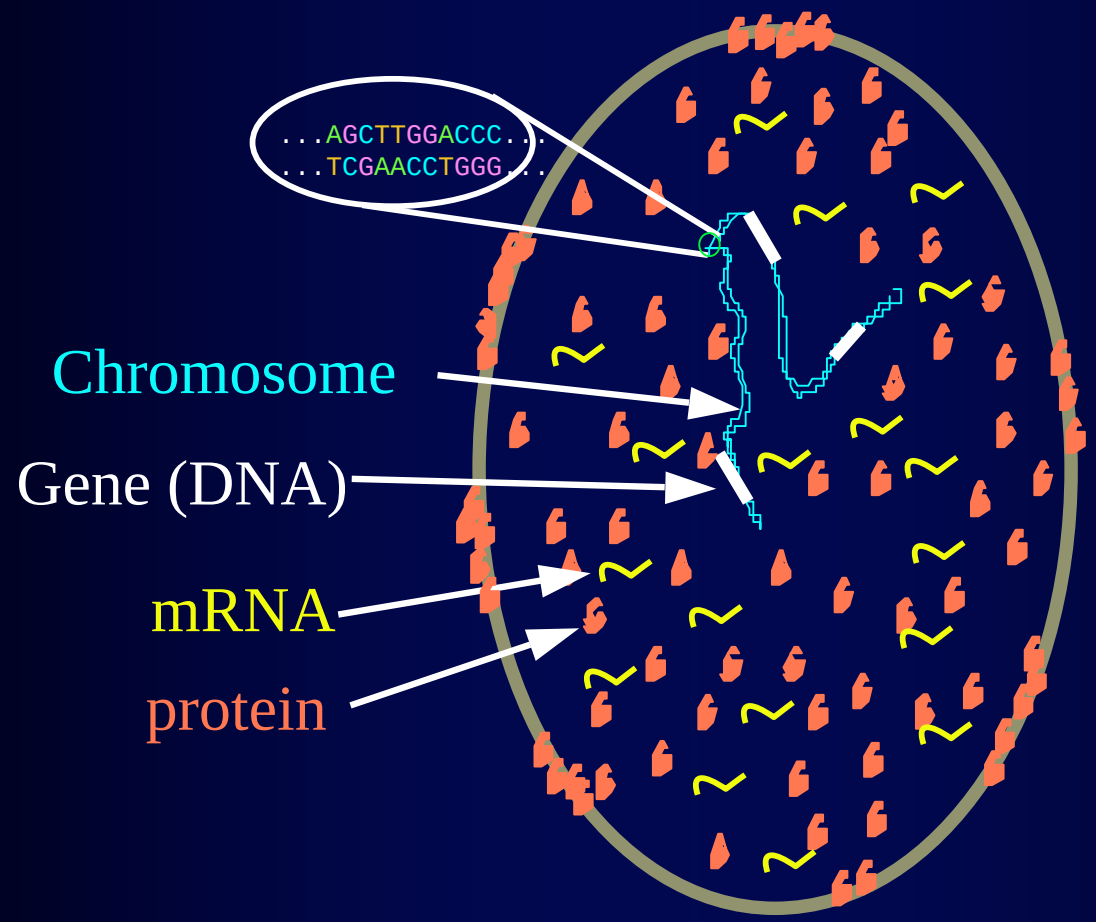


TCE degrading consortia



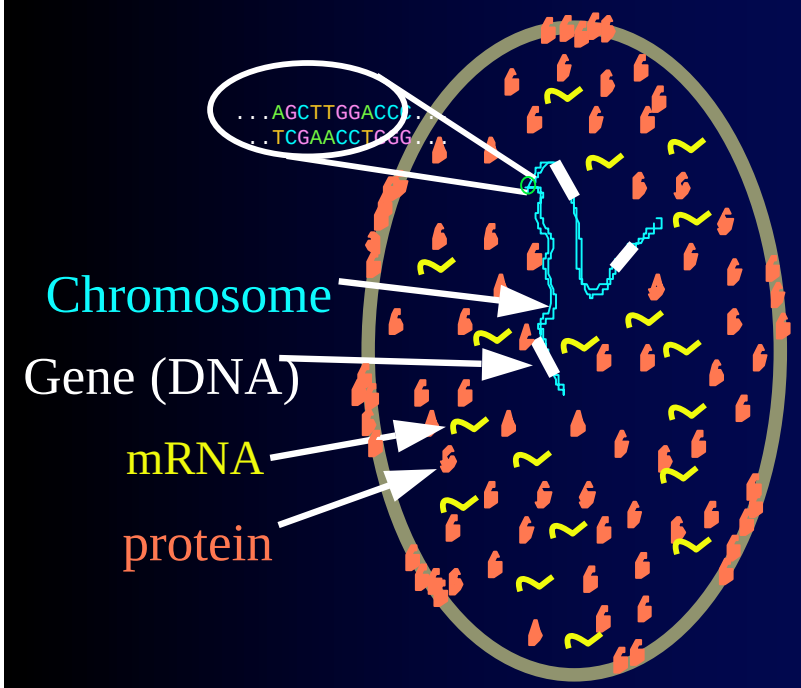
Microbial consortia ferment organic to hydrogen, providing electron donor required for *Dehalococcoides* to respire TCE

What biomarkers can track bioremediation?



Bacteria contain DNA, RNA & Proteins

What biomarkers can track bioremediation?



DNA: stable, easy to measure, easy to ID

RNA: labile, easy to ID, suggests activity

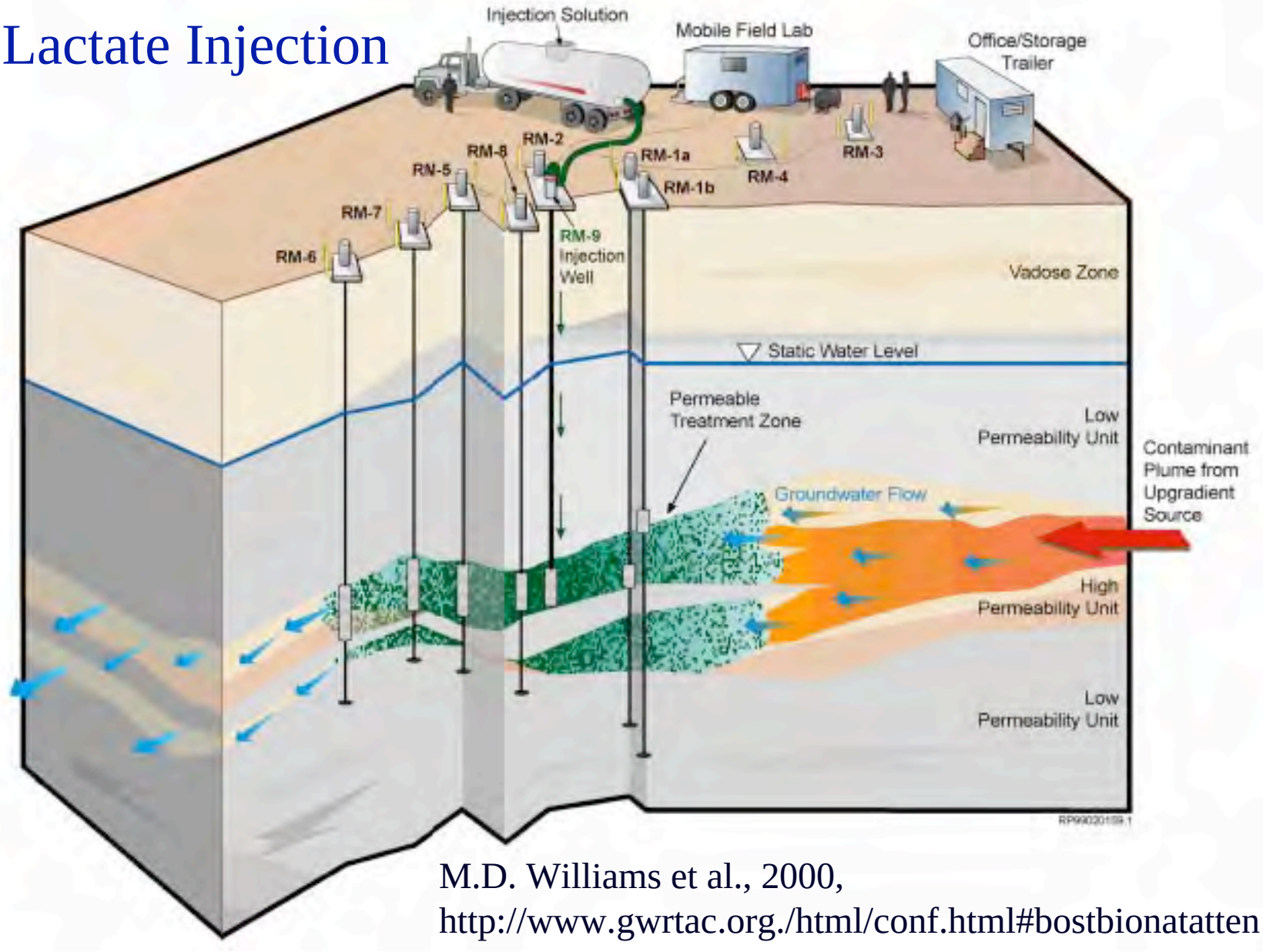
Proteins: stable, hard to quantify, hard to ID, confirms activity

Molecular tools for bioremediation

- Use biomarkers based on ribosomal genes (DNA) to measure *Dehalococcoides* number at sites
- Quantify functional genes (DNA) as biomarkers to distinguish between useful strains
- Use reverse transcription with quantitative PCR to quantify cell mRNA expression
- Use microarrays to identify important conditions, biomarkers and communities to optimize growth

In Situ Bioremediation of TCE

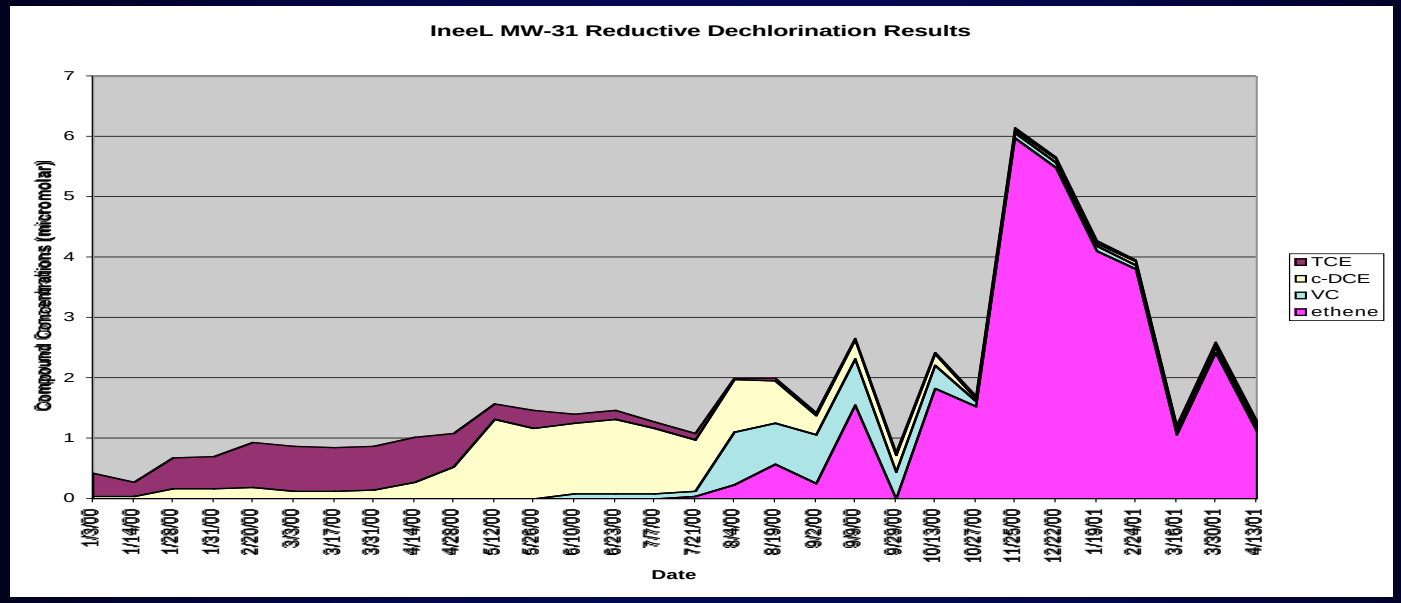
Lactate Injection

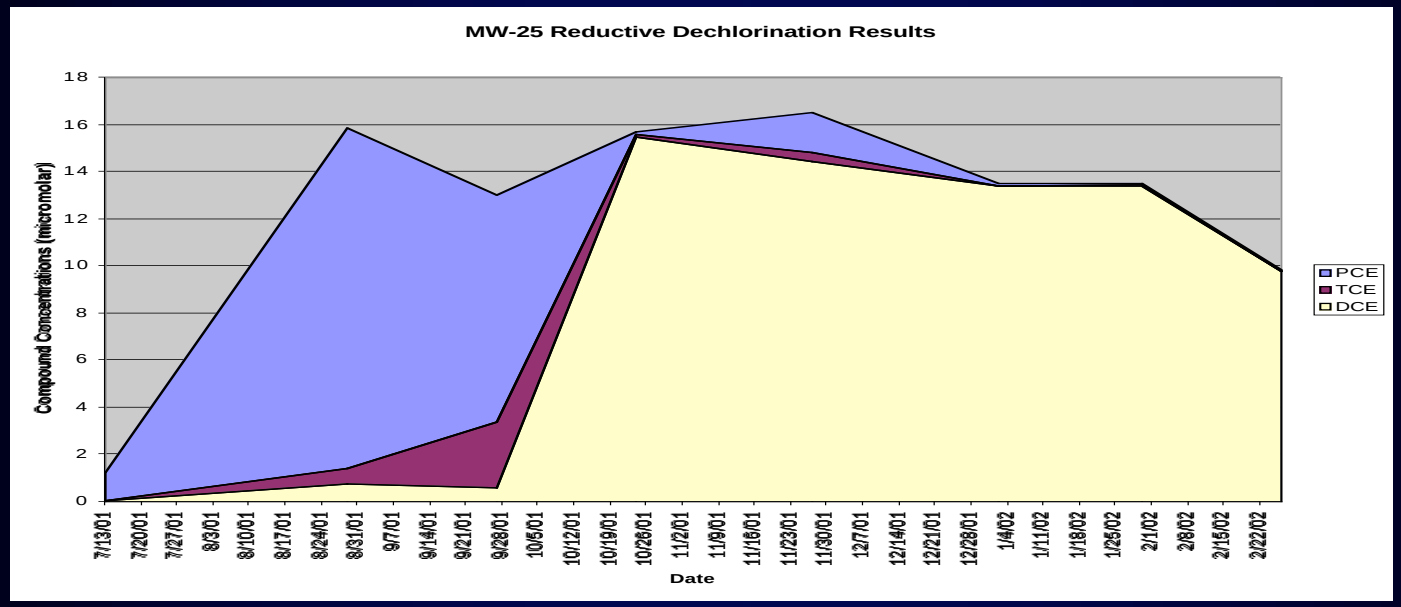


M.D. Williams et al., 2000,
http://www.gwrtac.org./html/conf.html#bostbionatatten

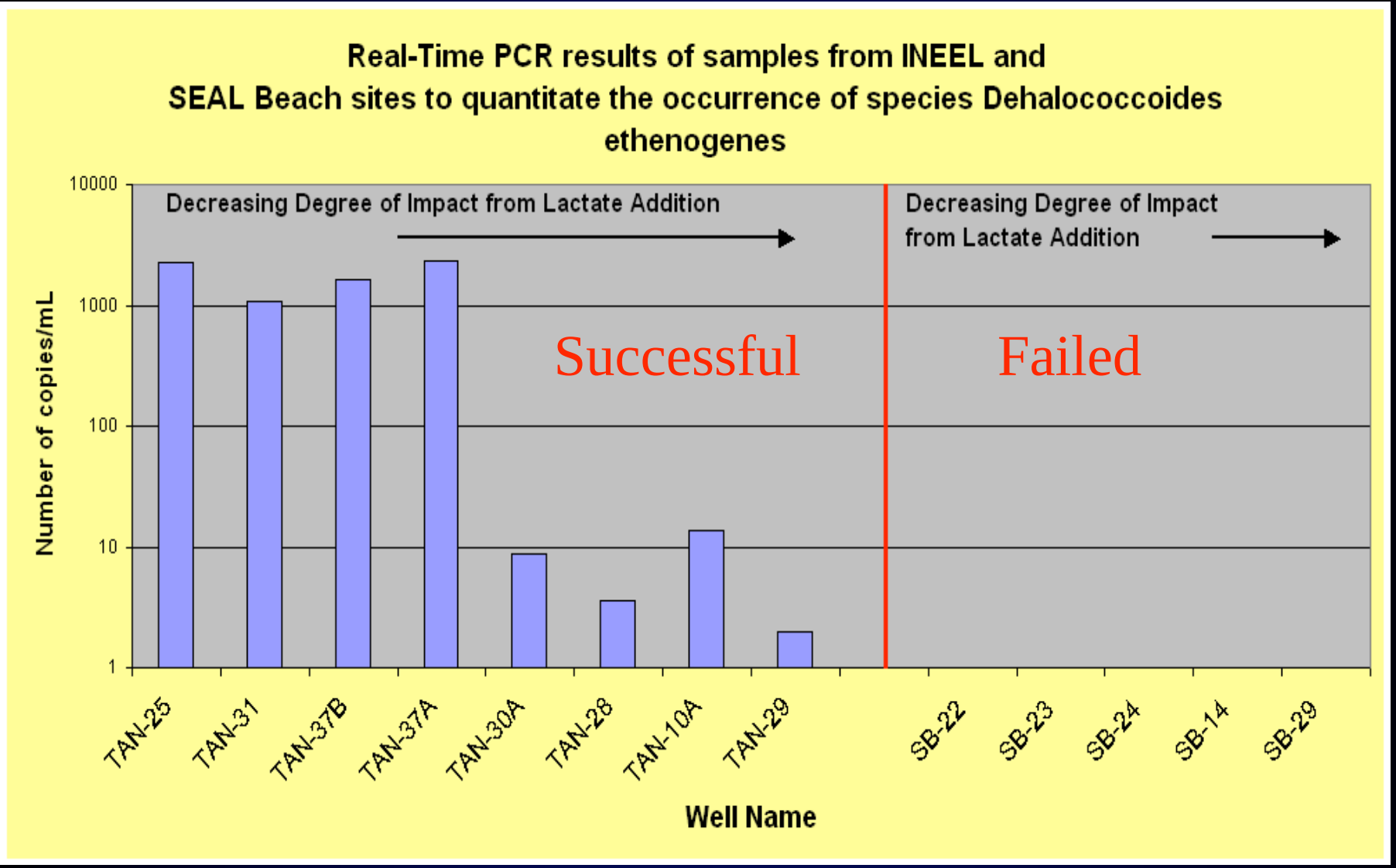
A TALE OF TWO SITES

Solvents at 2 Sites w/ Lactate Injection





Quantifying 16S genes for ID

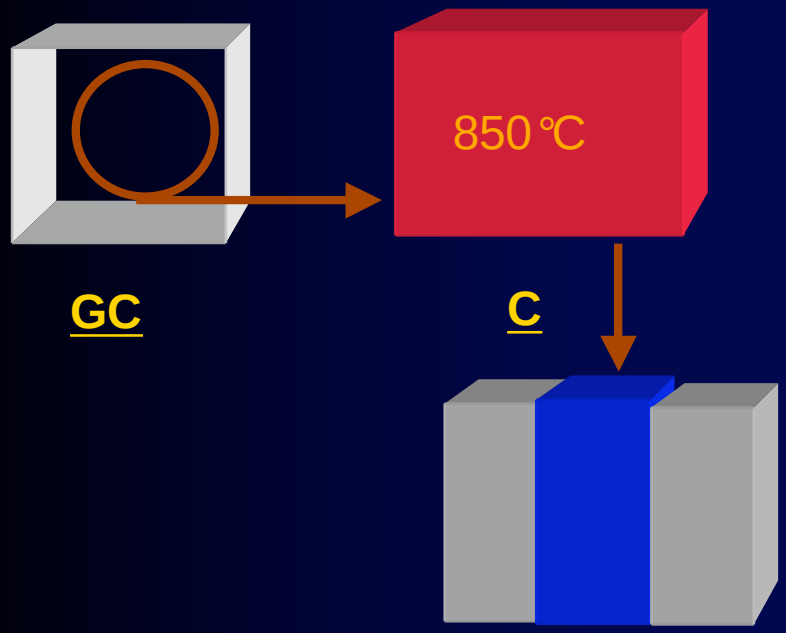


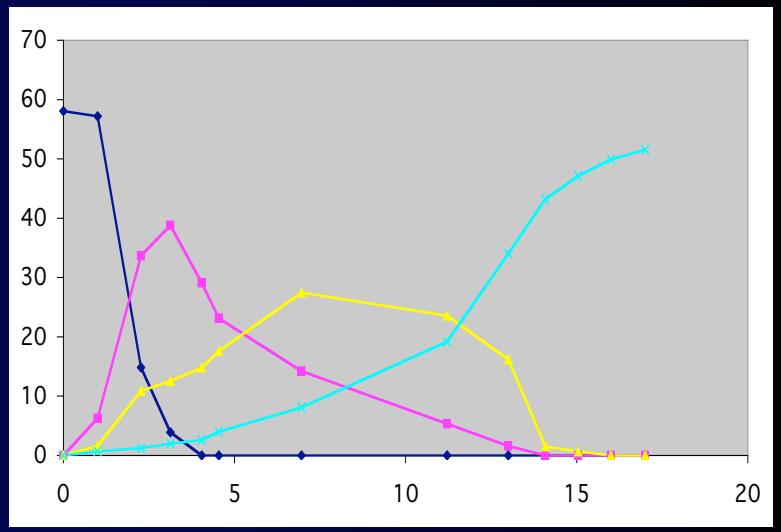
Stable Isotopic Fractionation of Ethenes

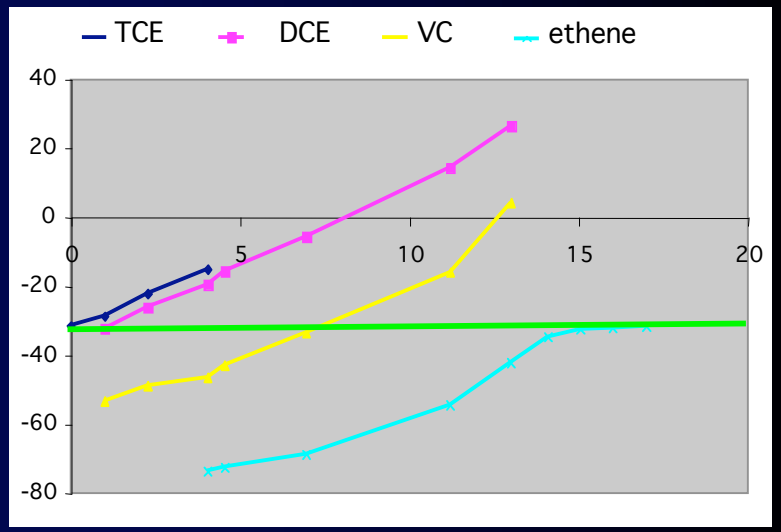
^{12}C 98.89%

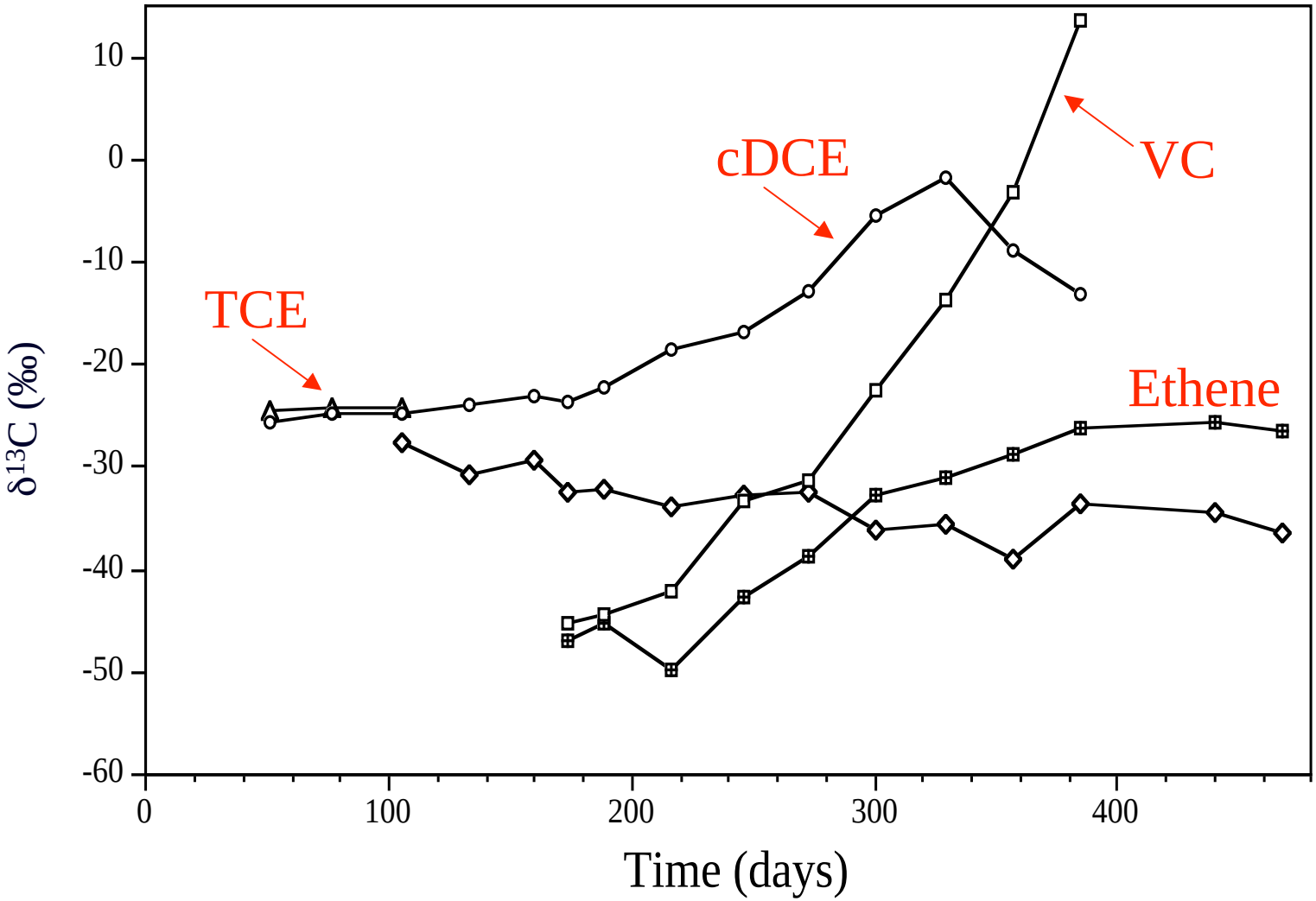
^{13}C 1.11%

Compound specific isotope analysis








10
0
-10
-20
-30
-40
-50
-60
δ13C (‰)
0
100
200
300
400
Time (days)
TCE
cDCE
VC
Ethene

Identified Dehalococcoides reductases

- *D. ethenogenes* **195**
- *Dehalococcoides* **Bav1**
- *Dehalococcoides* **VS**
- *Dehalococcoides* **FL2**
- *Dehalococcoides* **CBDB1**

pceA **tceA**

bvcA

vcrA

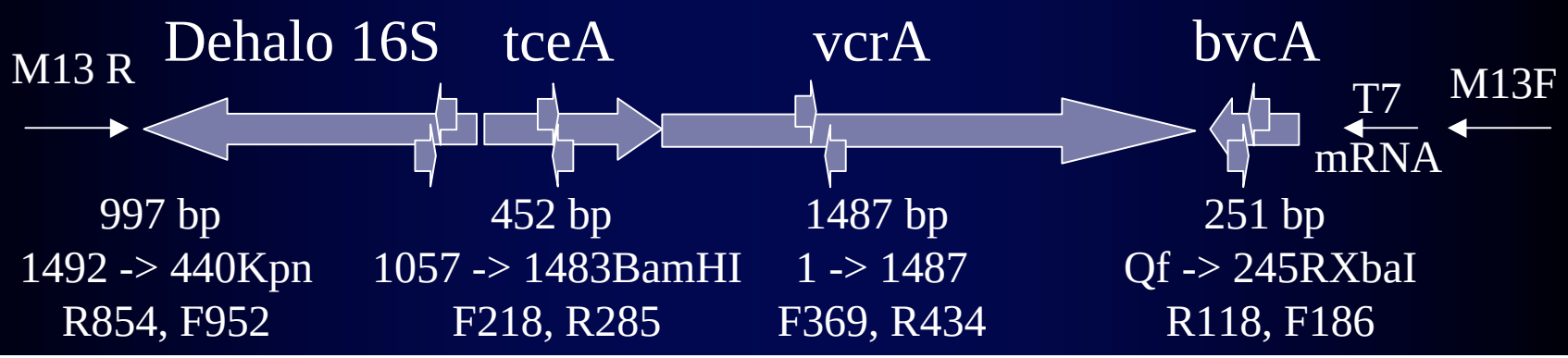
TCE DCE VC ethene

Quantification of Functional Genes

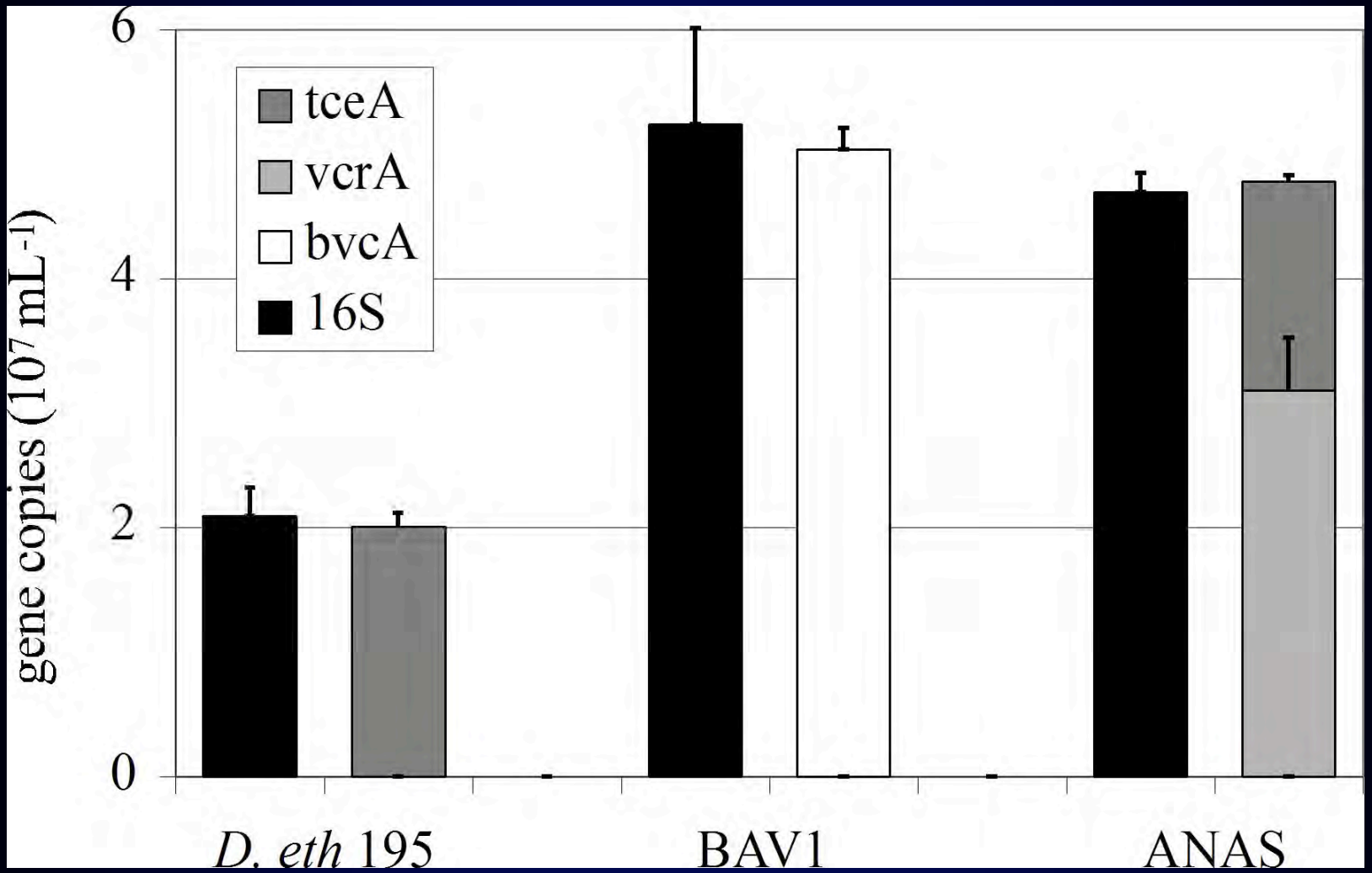
- Problem: even if *Dehalococcoides* is present, are the needed capabilities present?
- *Dehalococcoides* strains have variety of functions.
- 16S of strains highly conserved.
- How to distinguish if useful strains are present?

  e.g. CBDB1, 195, BAV1, VS, FL2 etc.

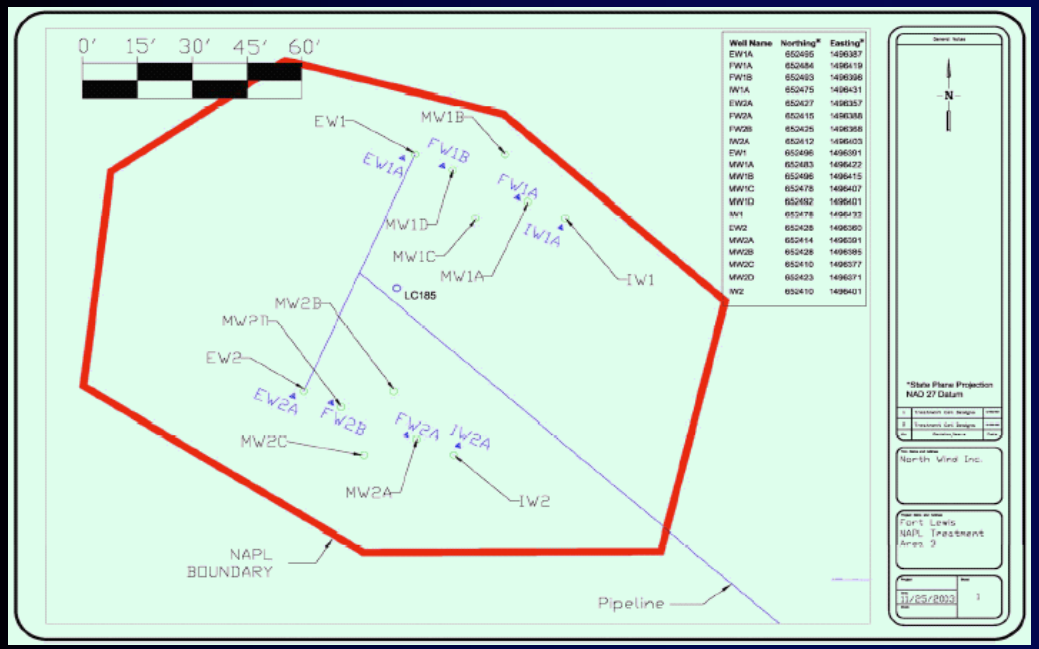
Four-gene qPCR standard plasmid

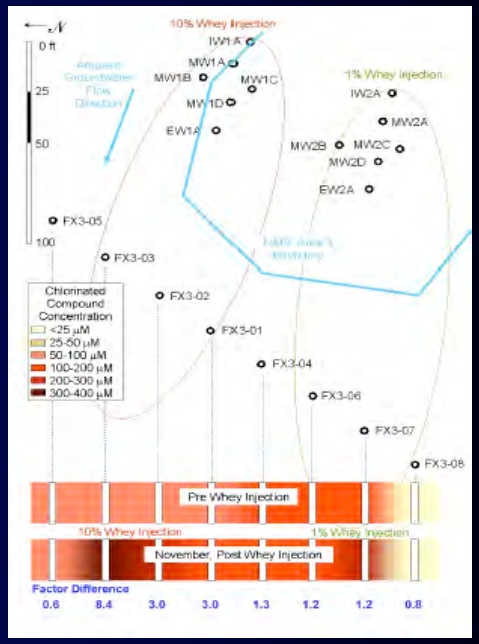


Sum of Functional and 16S genes of *Dehalococcoides* in lab



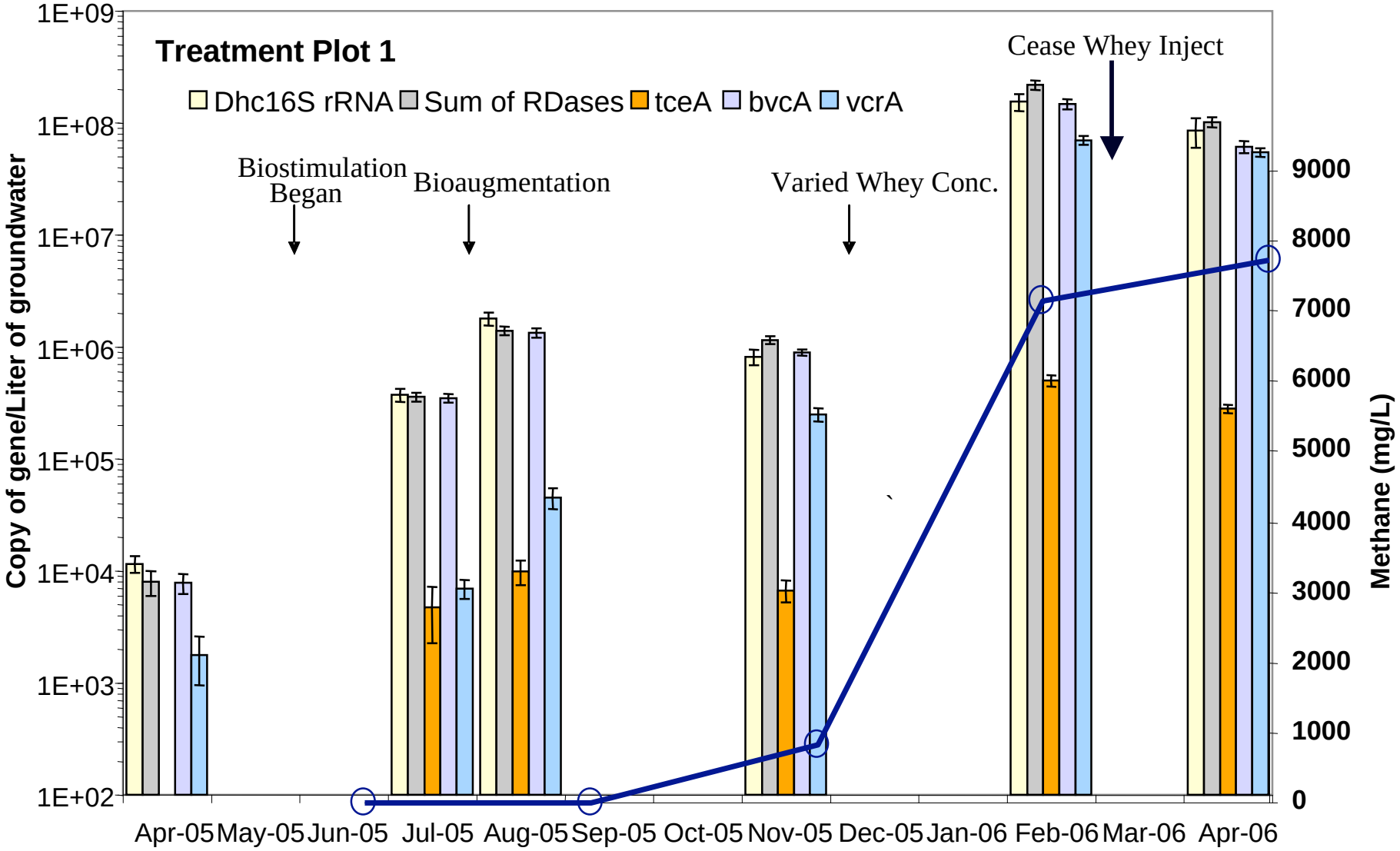
Ft. Lewis treatment site



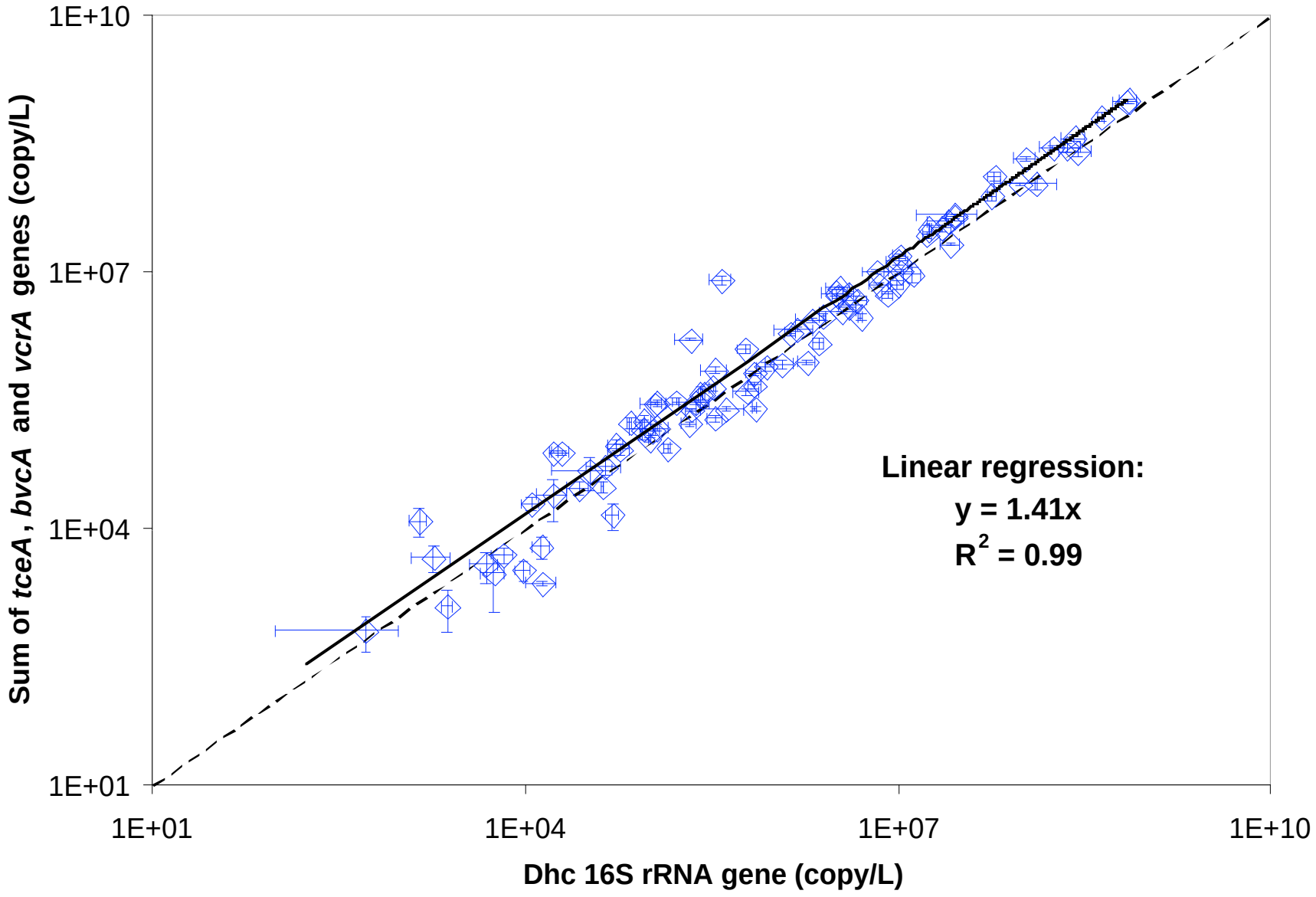


Figures courtesy of North Wind Inc.

Biostimulation & bioaugmentation



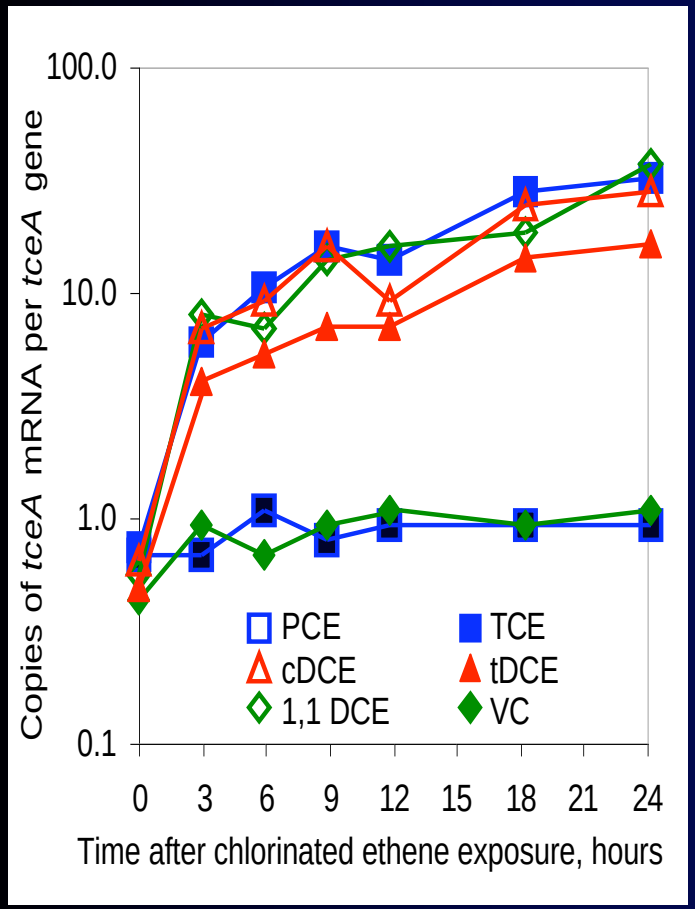
Sum of RDases and cells in field samples



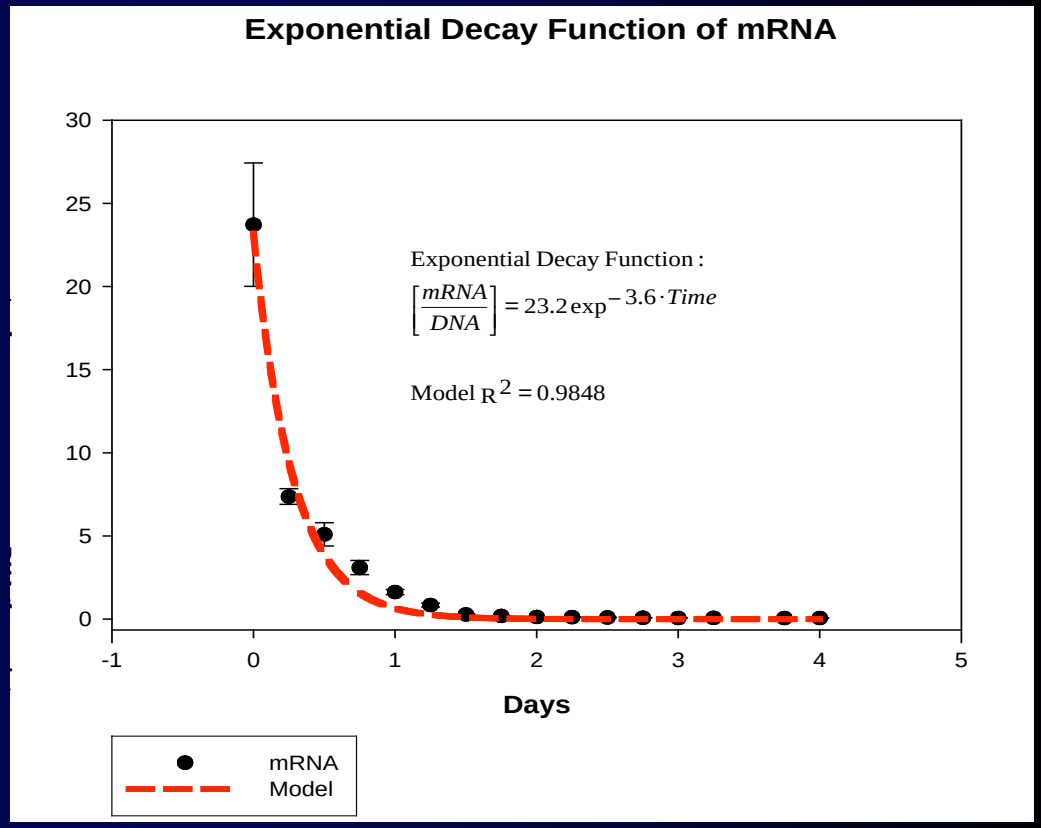
Species and genes aren't enough!

- How do we show that the genes of interest are not only present, but also expressed?

- Quantify after RT-qPCR of active samples (turn mRNA into DNA).



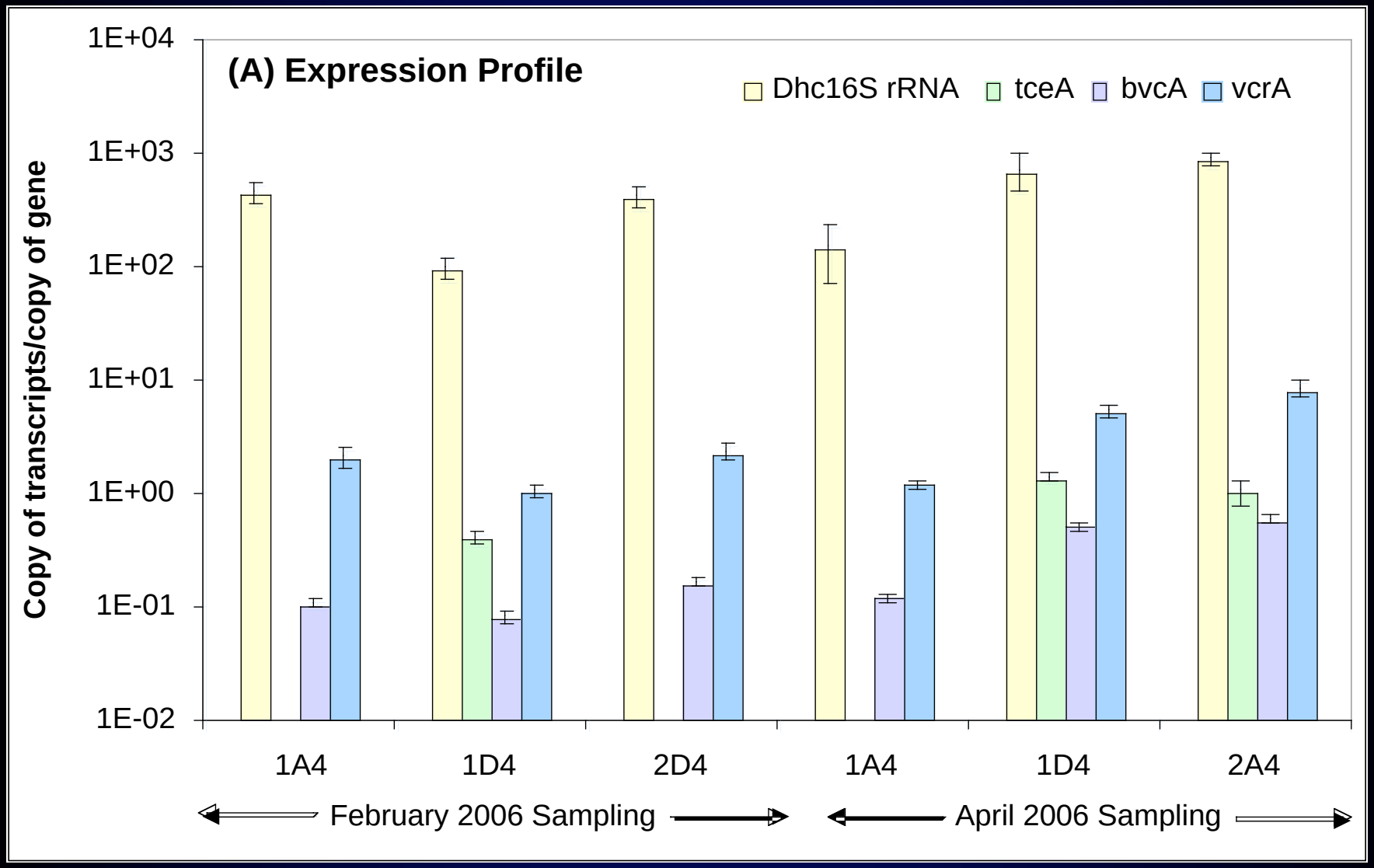
mRNA expression of tceA gene after solvent exposure



mRNA decay after solvent removal

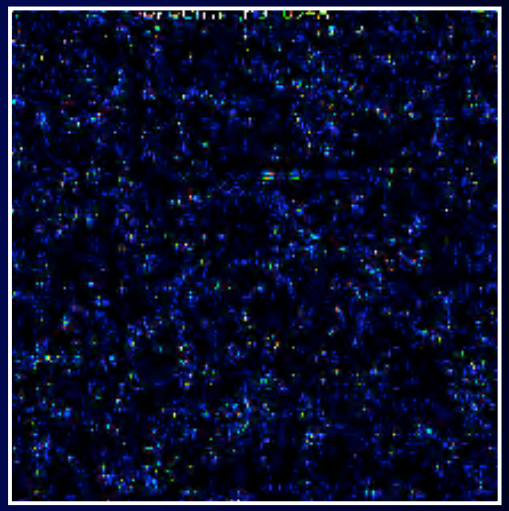
Johnson, D.R et al., 2005. Appl. Environ. Microbiol.

RT-qPCR Profile of RDases and 16S rRNA transcripts

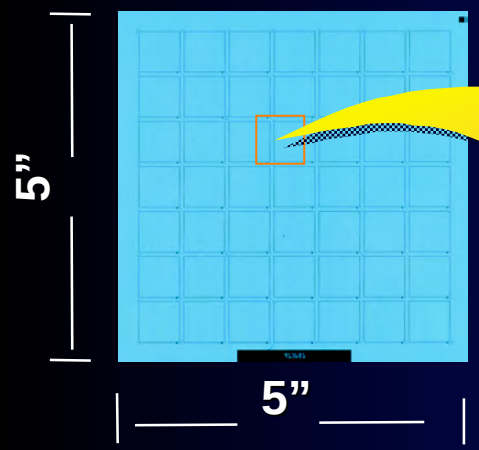


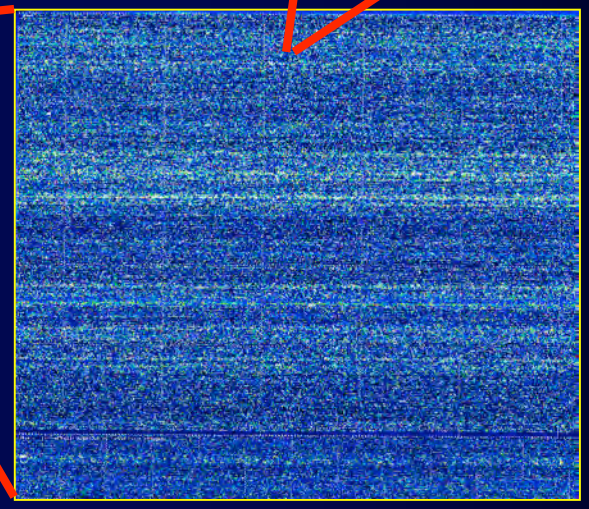
Microarrays to ID Community

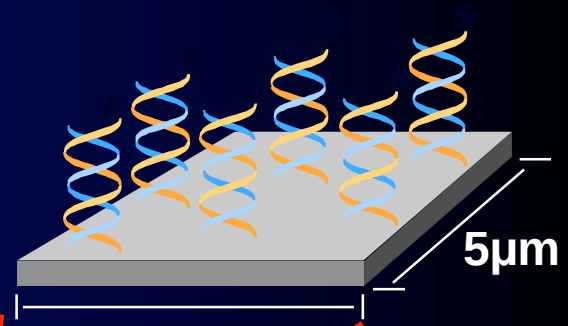
- *Dehalococcoides* spp. need other Bacteria/Archaea
- How do we know if they are present?
- Use a microarray that detects ≈ 9,000 species in single test (16S phyloarray)



The Dimensions of a GeneChip







Millions of identical probes/feature

Up to ~6,500,000 features/chip

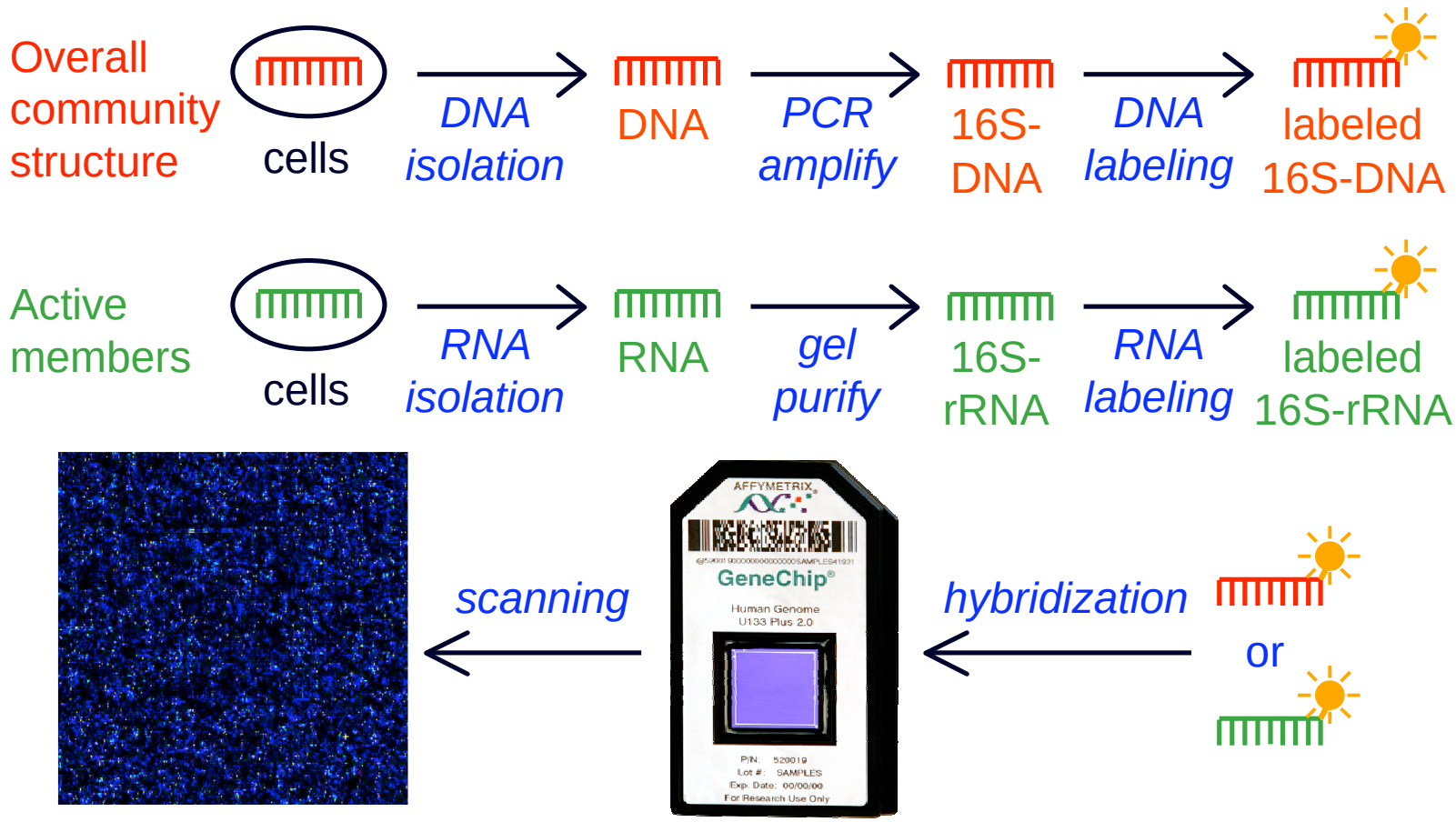
1.28cm

1.28cm

PhyloChip

- One probe set for each cluster
- Average 24 probe-pairs (25-mers) in each probe set
- Multiple taxa can be identified simultaneously by targeting unique regions or combinations of sequence.
- 8,741 clusters (OTUs) with corresponding probe sets.
- *Desirable*: No cross-hybridization with other clusters
  - Central 17-mer not found in other clusters
  - At least 2 non-continuous loci are probed

Phylogenetic Microarrays for 16S ID

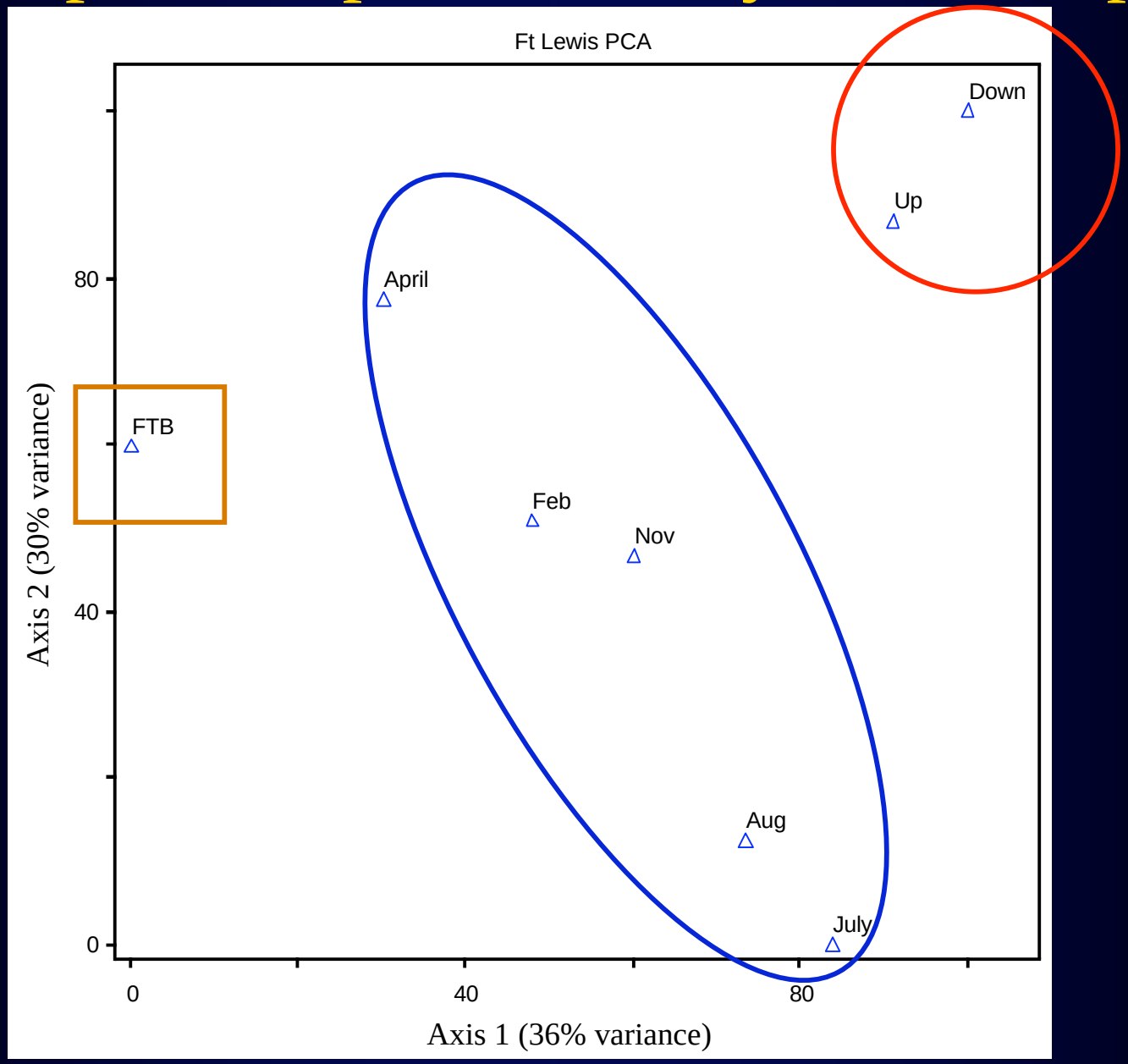


Richness in groundwater samples

	Samples	# of OTUs Present	
		DNA	RNA
	Bioaugmentation Enrichment	1334	n/a
	Up -stream	1520	
	Down -stream	1448	
Treatment Plot	July	1402	
	Aug	1445	
	Nov	1432	
	Feb	1458	583
	April	1340	631

Ref: Lee et al, manuscript in prep

Principal Component Analysis of samples



What can we learn from the entire genome - microarrays?

- Compare the entire genome of individual species with other isolates and more importantly, communities

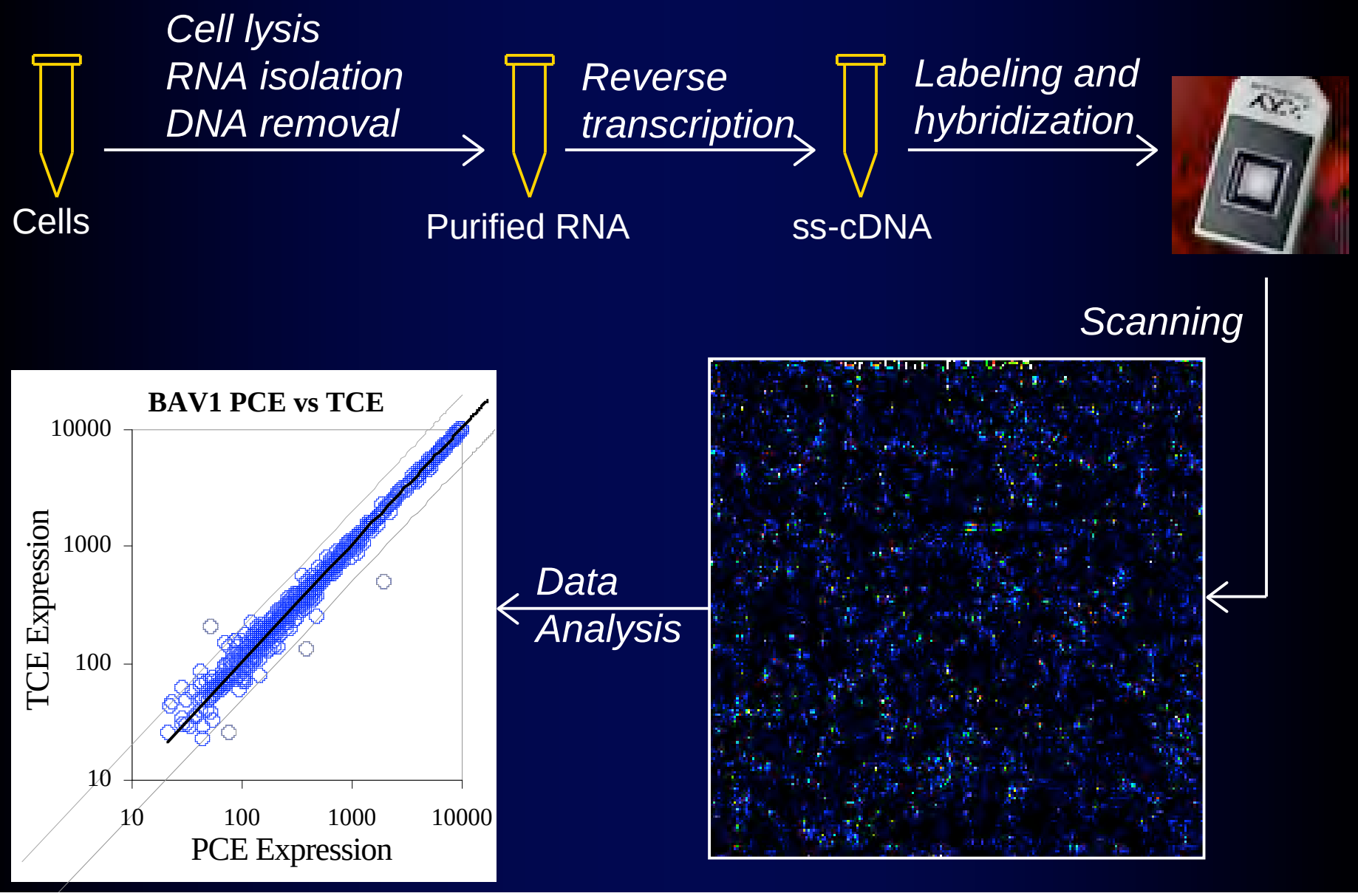
- Evaluate transcriptome under different conditions and for different cultures

Dehalococcoides ethenogenes 195 whole-genome microarrays

- Microarrays designed to include all ORFs in *D. ethenogenes* 195 genome

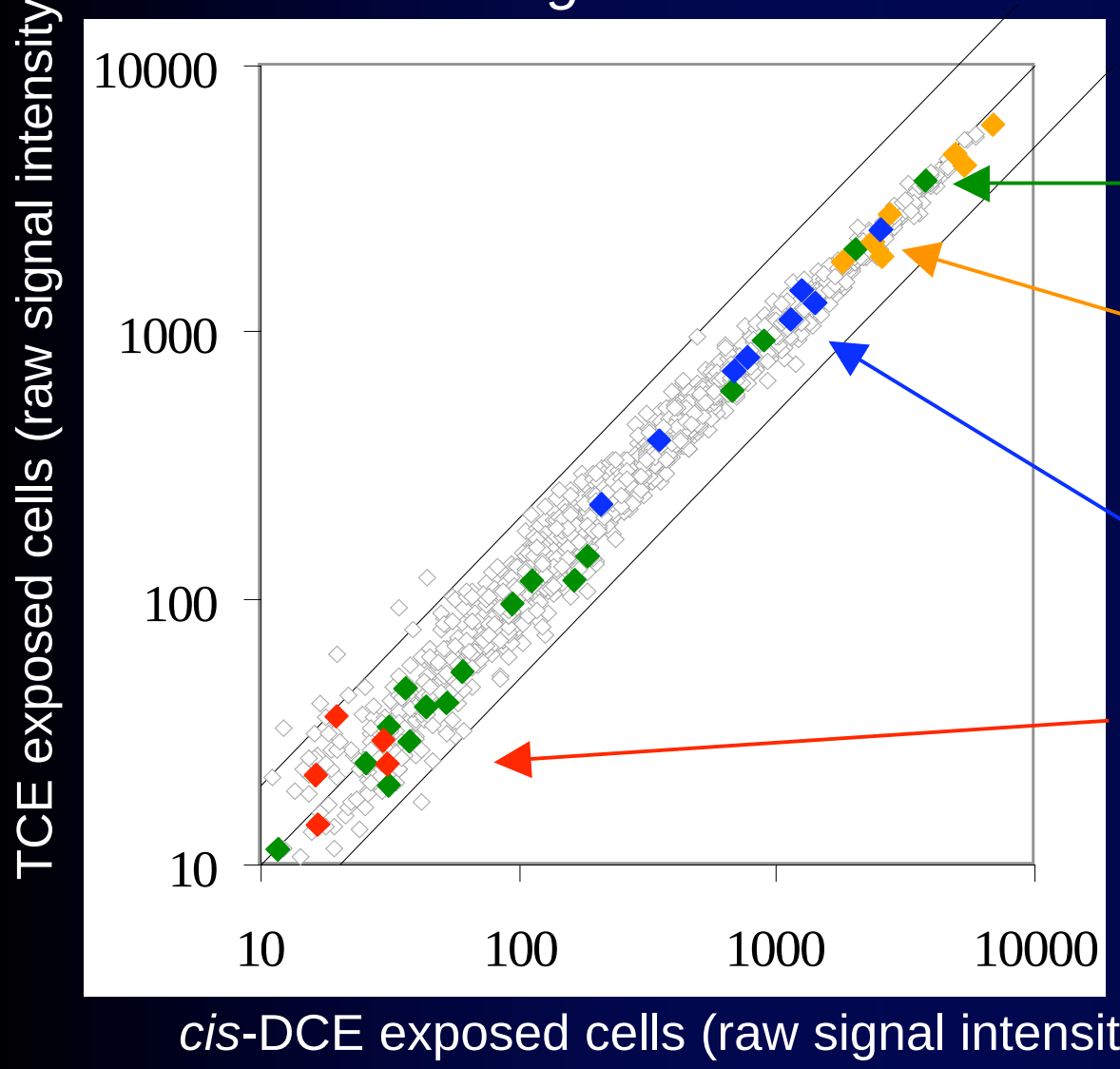
- Probes
  - ≈ 36,000 probes - 25mers
  - 1,579 DHC 195 genes + 45 control genes
  - 22 probes per gene
    - 11 match probes per gene, 11 mismatch probes
  - Extremely high specificity

Microarray whole-genome expression analysis



Gene Expression with TCE or DCE

D. ethenogenes strain 195

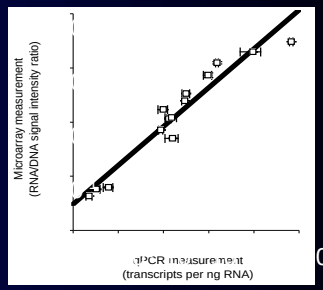


1) putative RDases
tceA RDase

2) corrinoid uptake and recycling

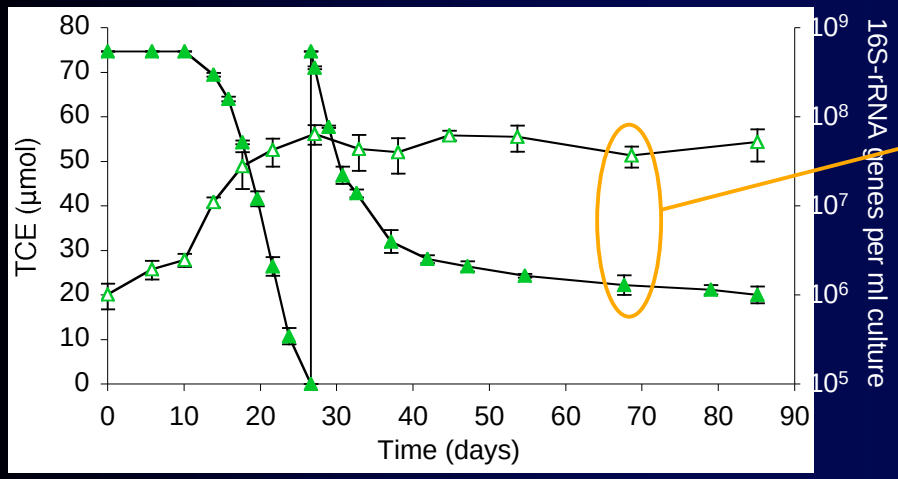
3) carbon fixation (Wood/Ljungdahl)

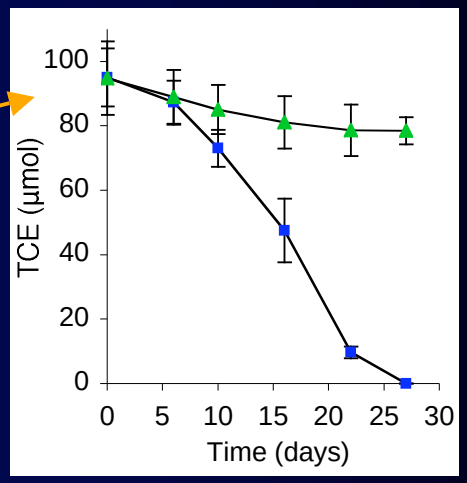
4) nitrogen fixation (*nif*)



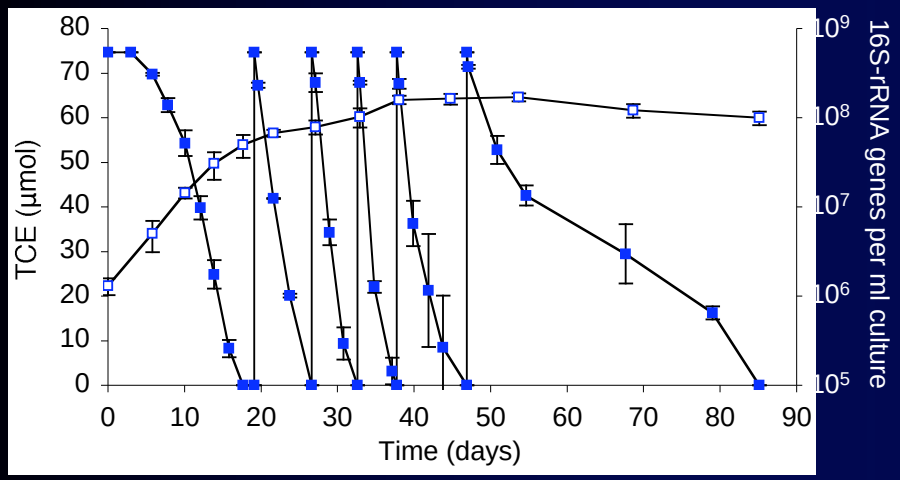
B12 Effects on Dehalococcoides

t_d=4.1 days; X_{max}=6x10^7 ml^{-1}





t_d=3.3 days; X_{max}=2x10^8 ml^{-1}

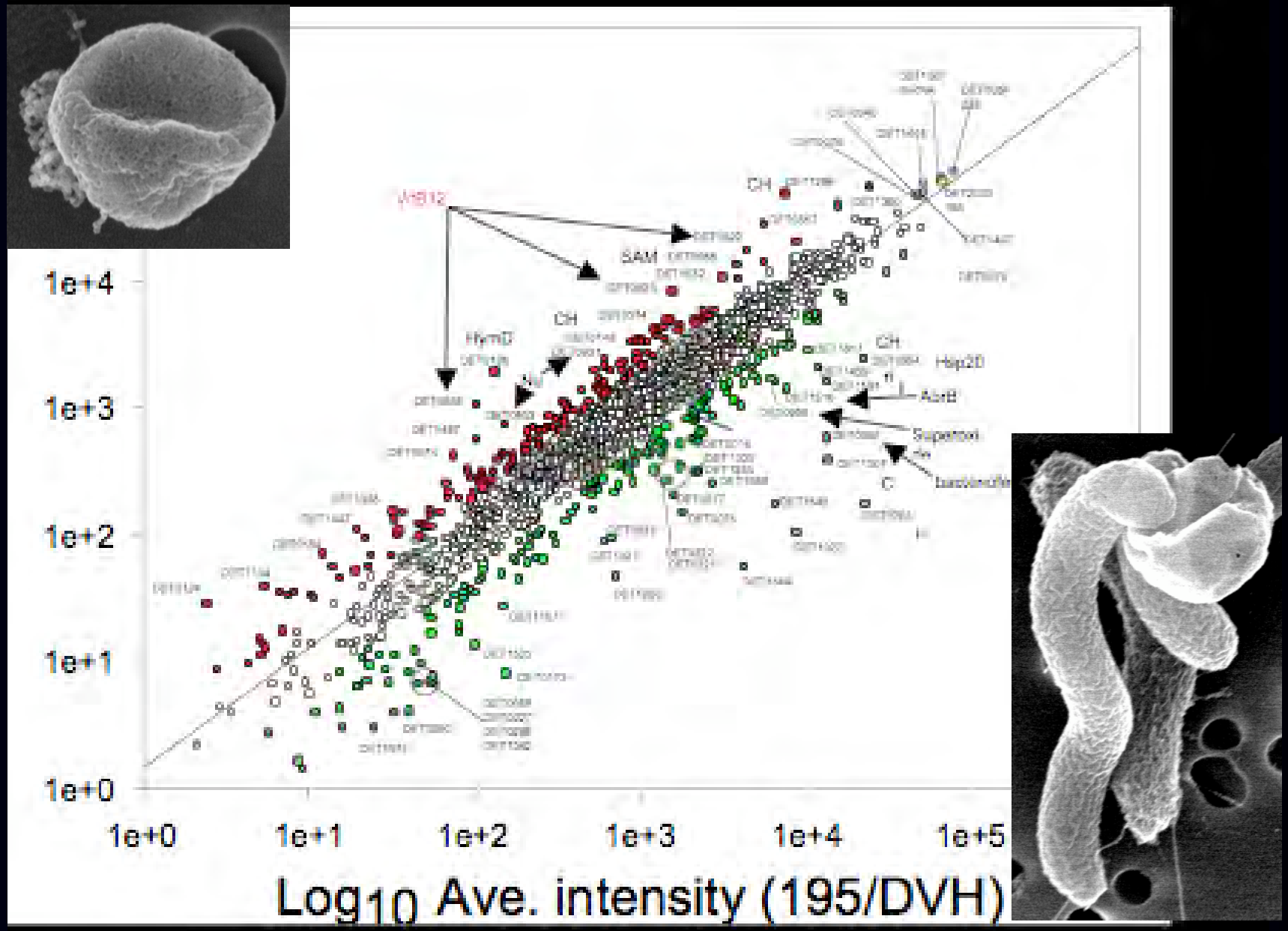


Closed symbols: TCE
Open symbols: 16S-rRNA genes

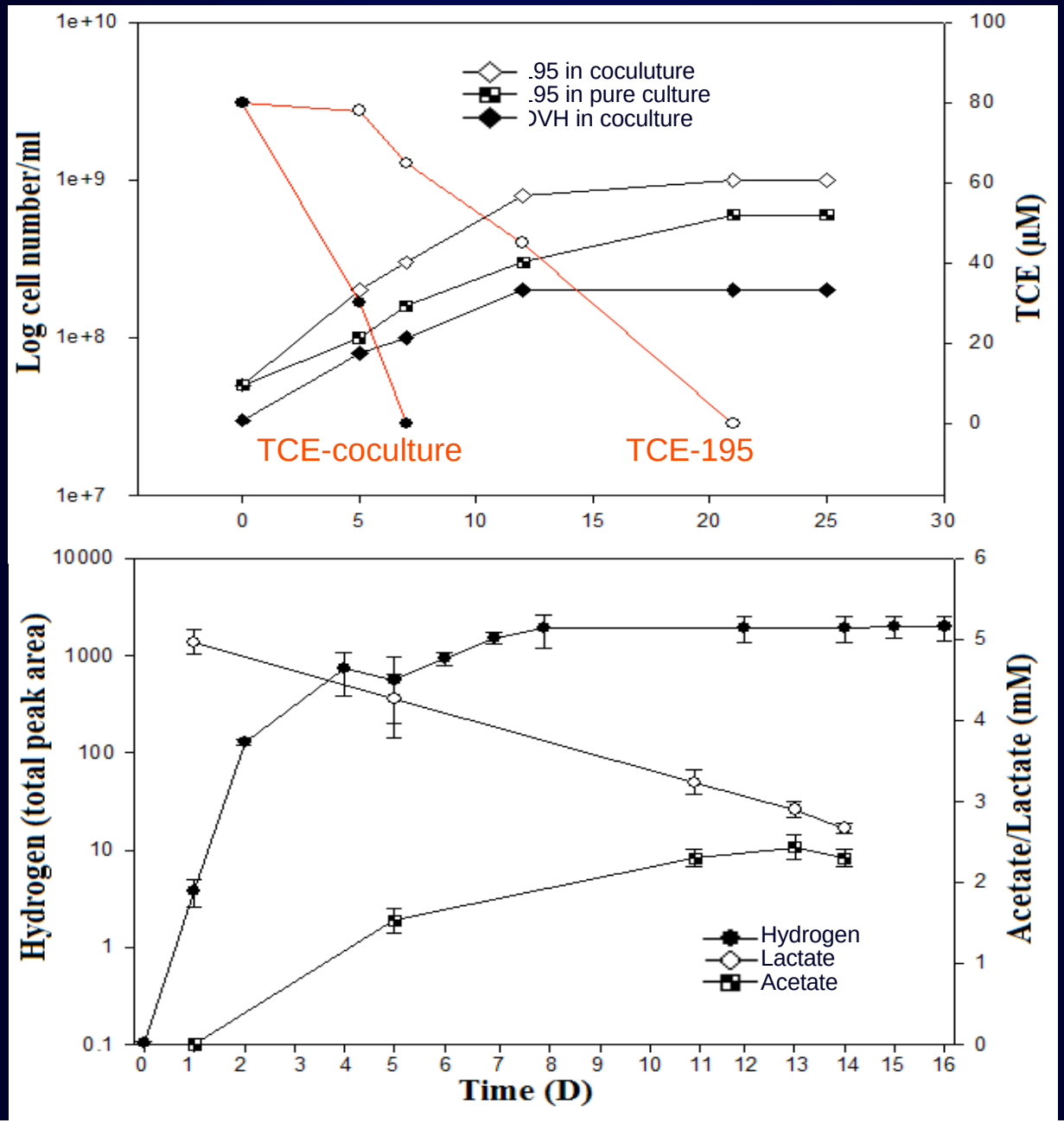
Green: 1 µg/L vitamin B_{12}
Blue: 100 µg/L vitamin B_{12}

Johnson, Feil, DeSantis, Andersen, Zinder, Alvarez-Cohen. *In preparation*

Global expression in isolate and co-culture



Kinetic comparison of isolate and co-culture



What can we learn about bioremediation with molecular tools?

- When will biostimulation work?
  - (count ribosomal genes: qPCR 16S - Who's there, how much?)
- When is bioaugmentation necessary?
  - (count functional genes: qPCR RDases etc. What can they do?)
- How to optimize growth of *Dehalococcoides* in complex communities?
  - (measure mRNA: RT-qPCR - Are they actually doing it?)
- How does this unusual bacteria exist in such diverse environments - and how do we use this?
  - (fundamental knowledge! microarrays, proteomics etc. How can we learn more?)

Acknowledgments

- UCB: Patrick Lee, David Johnson, Victor Holmes, Weiqin Huang, Brian Rahm, Kimberlee West, Shan Yi, Helene Feil, Jianzhong He
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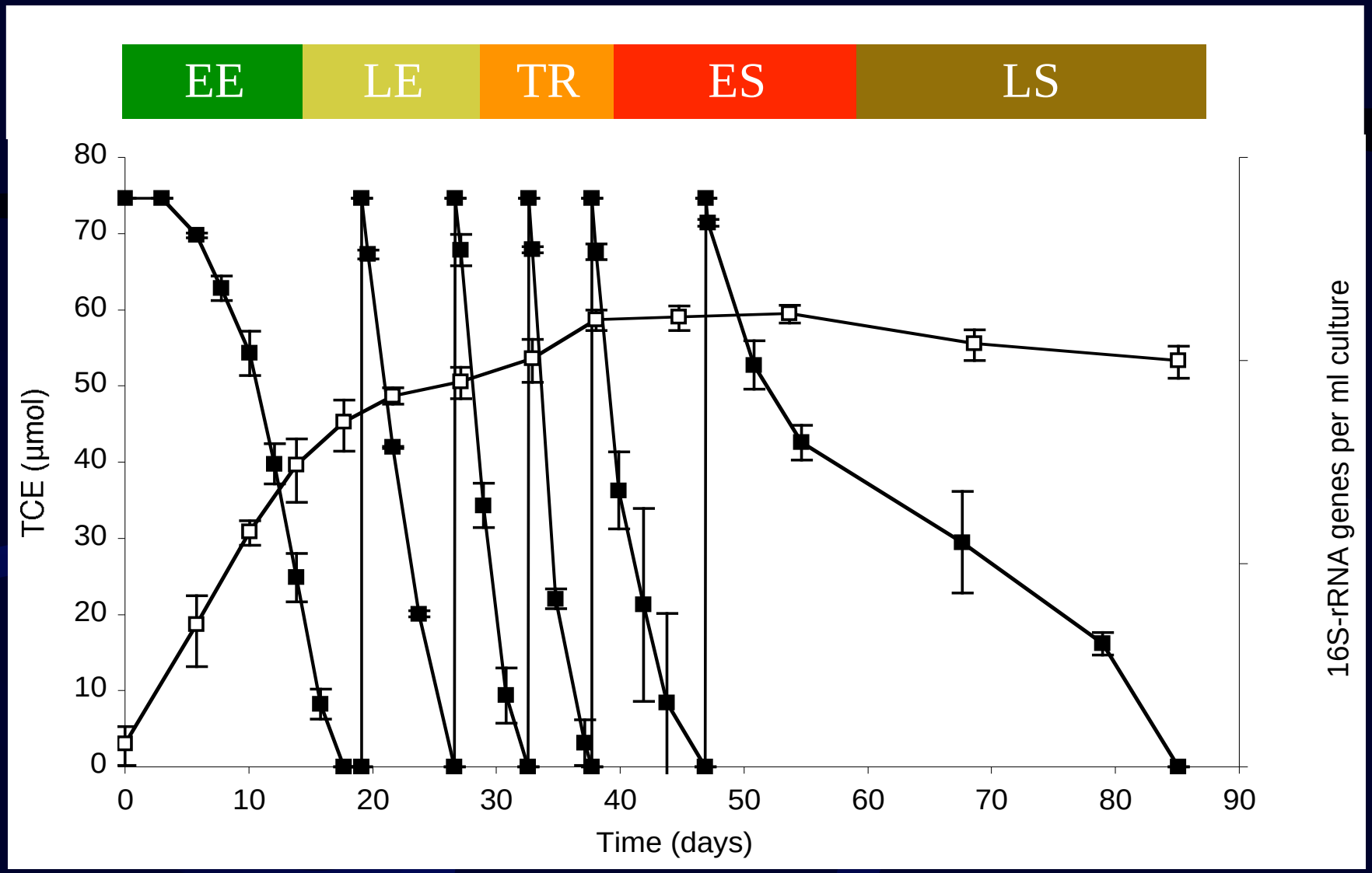
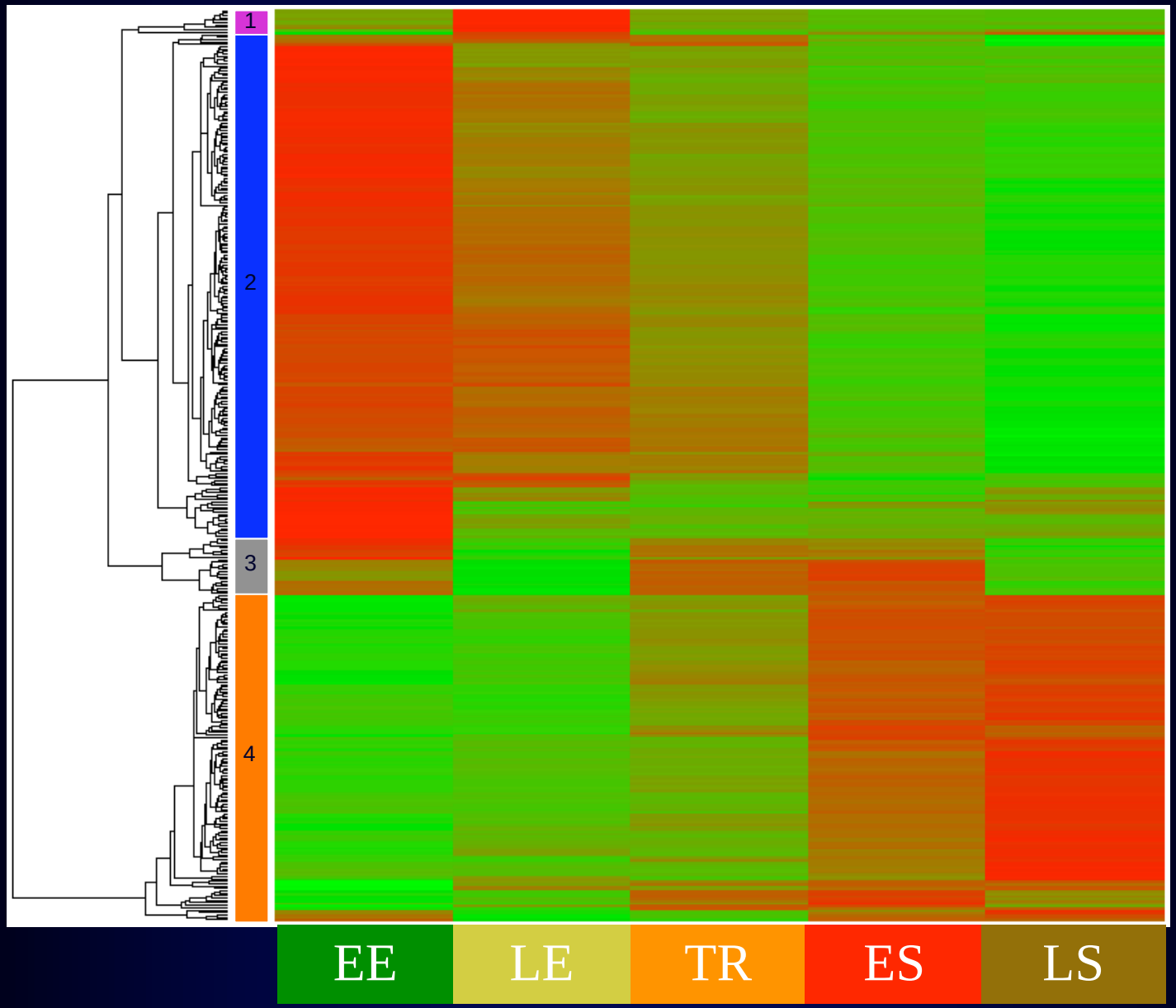
- LBNL: Gary Andersen, Eoin Brodie, Ping Hu, Terry Hazen,
- INEEL: Kent Sorenson, Tamzen Wood
- Malcolm Pirnie: Mike Kavanaugh, Rula Deeb

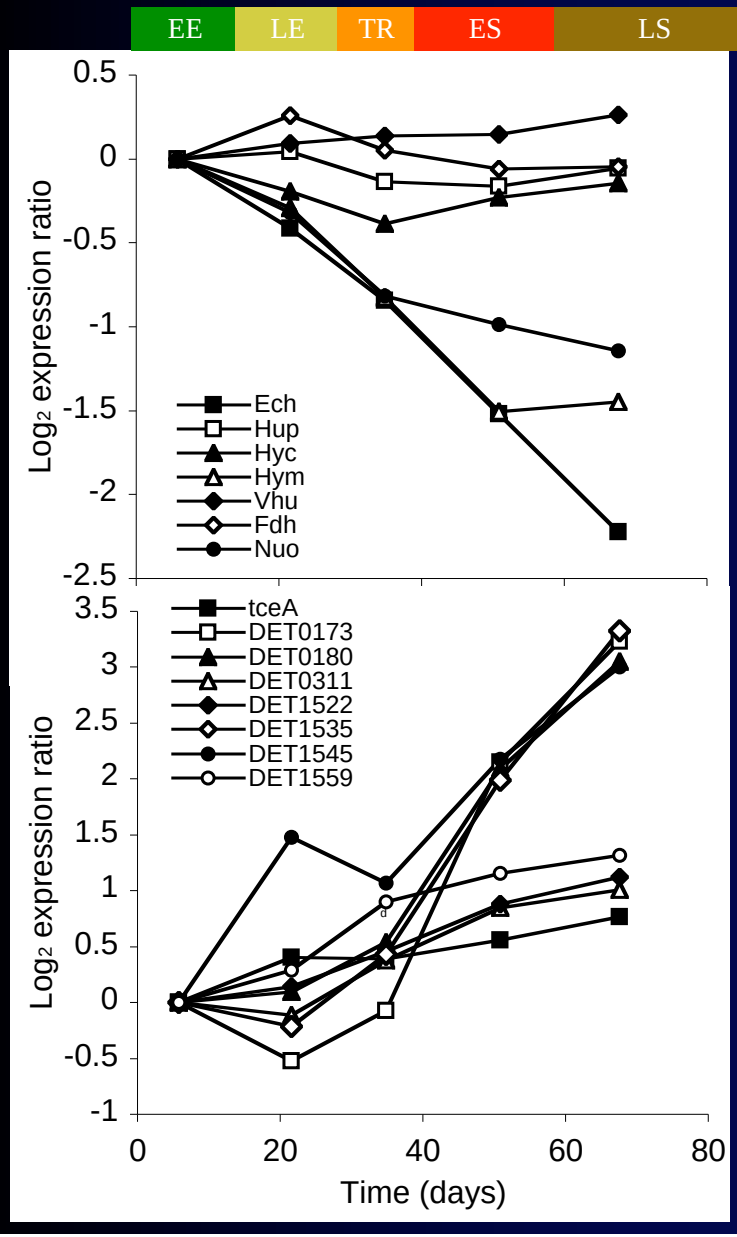
Growth Cycle of 195



Transcriptome Changes in 195

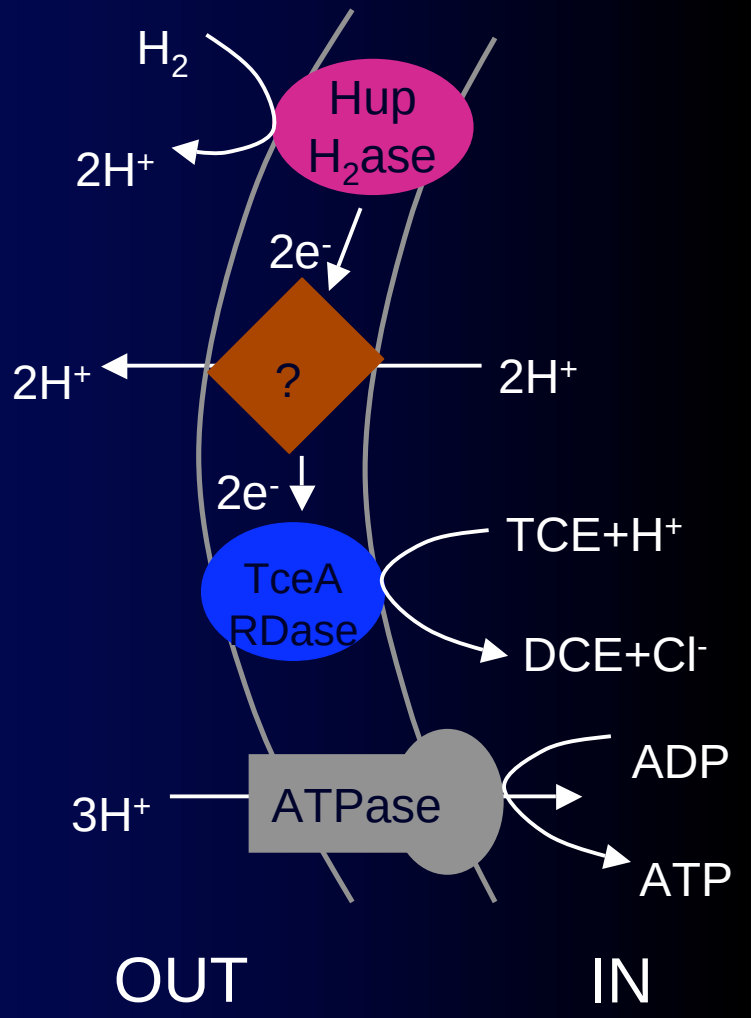


Biomarker Expression Cycle

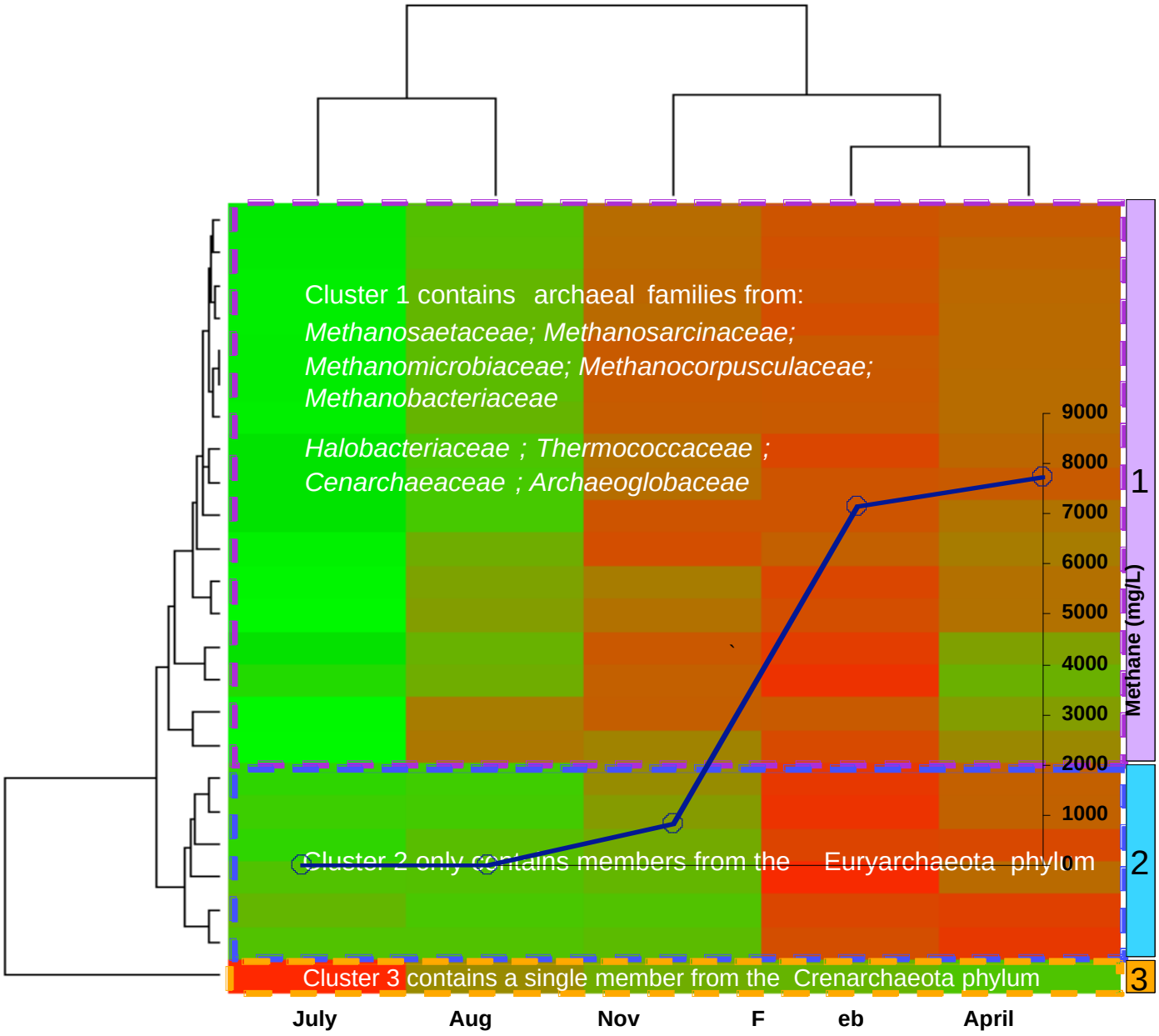


Hydro-genases

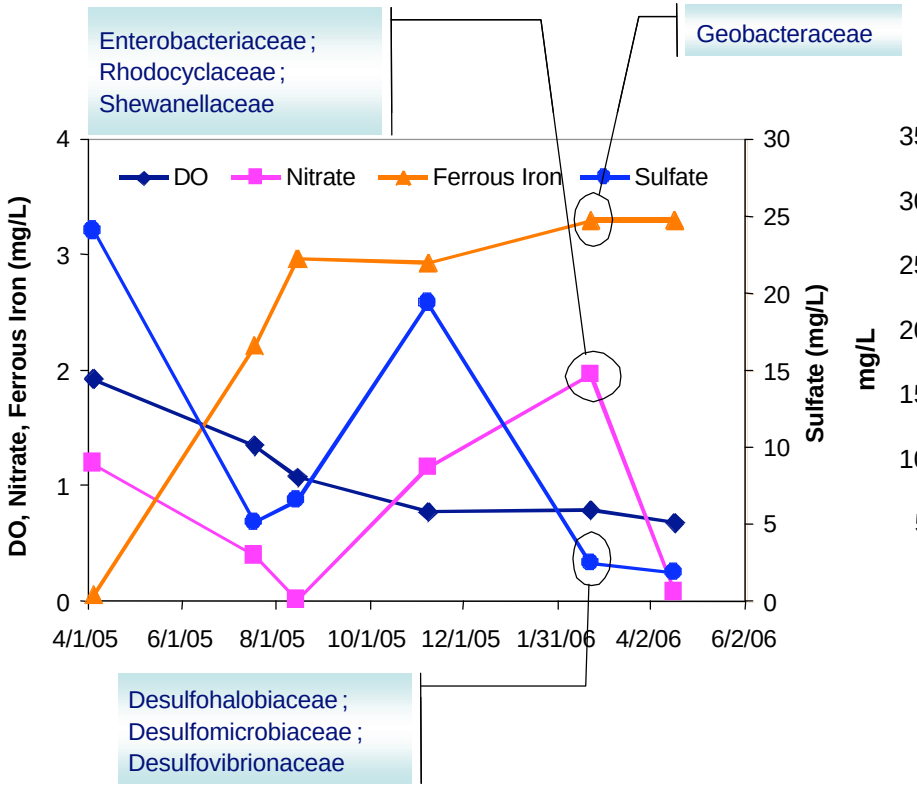
RDases

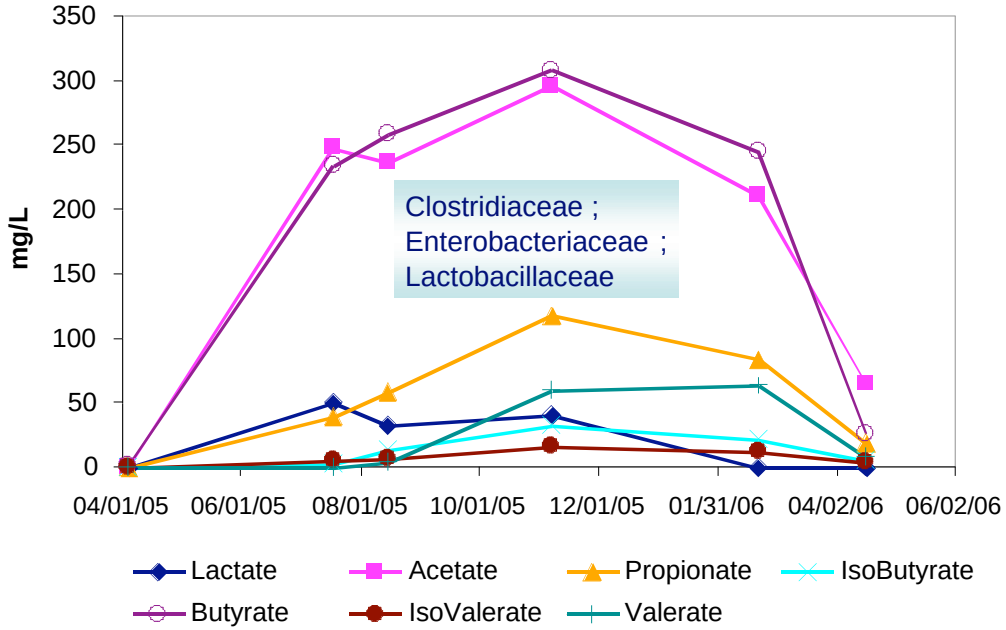


Dynamics of archaeal populations



Linking active bacterial families & geochemistry





Follow-up clone library of 16S rRNA
• candidate bacterial divisions

*Based on RNA hybridization

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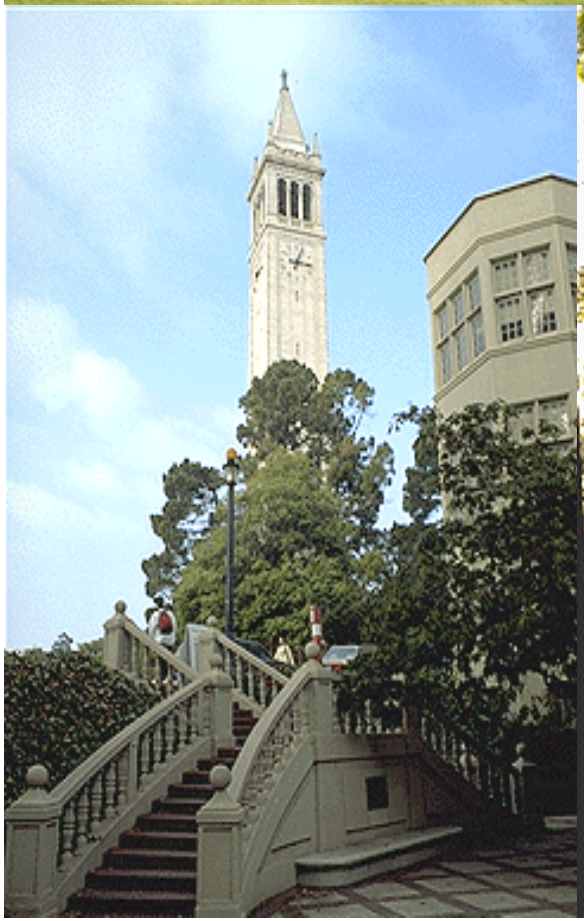

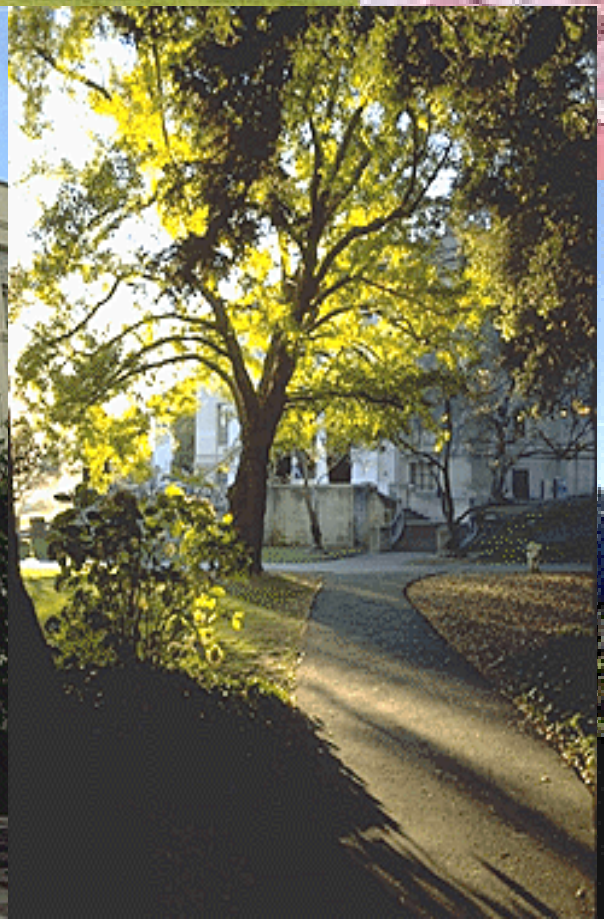

Go Bears!