A-047, in: H.V. Rectanus and S.H. Rosansky (Chairs). *Remediation of Chlorinated and Recalcitrant Compounds—2014.* Ninth International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2014). ISBN 978-0-9819730-8-1, ©2014 Battelle Memorial Institute, Columbus, OH, **www.battelle.org/chlorcon**.

Next Generation qPCR: High-Throughput, Highly Parallel qPCR Arrays (QuantArrays) for Comprehensive Site Assessment

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Background/Objectives. Quantitative polymerase chain reaction (qPCR) is a robust, commonly applied environmental molecular diagnostic (EMD) to quantify gene targets in sediment and groundwater. *Dehalococcoides mccartyi* (*Dhc*) quantification has become an indispensable component of assessment, remedy selection, and performance monitoring at chlorinated ethene sites. Standard qPCR quantification, of a single gene target at a time, provides valuable but limited information on the microbial community involved in bioremediation processes. Specific reductase genes required for vinyl chloride and chloroform reductive dechlorination are not universally distributed among known halorespiring strains. Other organohalide respiring bacteria are directly responsible for biodegradation of common co-contaminants like chlorinated ethanes, while an array of microbial groups play supporting or antagonistic roles. Depending upon subsurface conditions, aerobic or cometabolic mechanisms may dictate contaminant destruction. This presentation builds upon the Interstate Technology & Regulatory (ITRC) EMD team's qPCR guidance documents, and describes newly identified functional genes and the development of high-throughput qPCR arrays (QuantArrays) to obtain more comprehensive quantification of microbial populations critical for site remediation.

Approach/Activities. With a nanoliter scale fluidics platform for aqueous reactions, qPCR arrays combine the highly parallel detection of DNA microarrays with the accurate quantification of qPCR. The plates are composed of 48 subarrays. With some through-holes used for QA/QC purposes, each subarray is capable of simultaneously performing 56 unique qPCR assays for a total of 2,688 reactions per array. In the current studies, QuantArrays included simultaneous quantification of: (1) six genera of dehalogenating bacteria; (2) reductase genes responsible for dechlorination of vinyl chloride, dichloropropane, and chloroform; (3) oxygenase genes encoding enzymes capable of co-oxidation of chlorinated ethenes; and (4) functional genes for competing electron accepting processes including sulfate reduction. QuantArray analysis was performed on groundwater samples from a chlorinated ethene/ethane site undergoing biostimulation. For comparison purposes DNA microarray and QuantArray analyses were conducted for a chlorinated ethene site where a carbon/organo-iron emulsion was used as a permeable reactive barrier (PRB). QuantArray analysis is ongoing at an urban creek where reductive dechlorination within the sediments appears mitigate impacts to the water column.

Results/Lessons Learned. At the chlorinated ethene/ethane site, the QuantArray demonstrated the initial stimulation of sulfate reducers, growth of fermenting bacteria, and ultimately increases in the abundance of organohalide respiring bacteria in response to electron donor addition. Moreover, assessing a wider array of target genes, troublesome

locations where bioaugmentation was required to foster vinyl chloride and chloroform reduction were identified. At the PRB site, a DNA microarray served as a valuable research tool, but the qPCR array provided more accurate quantification of selected gene targets. Analysis of creek sediments with the array tools are ongoing, and expected to reveal important differences in organohalide respiring populations and other relevant organisms in the microbial community.

Next Generation qPCR
High Through-put, Highly Parallel qPCR Arrays (QuantArrays) for Comprehensive Site Assessment

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Environmental Molecular Diagnostics (EMD)

"a collective term that describes a group of advanced and emerging techniques used to analyze biological and chemical characteristics of soils, sediments, groundwater, and surface water."

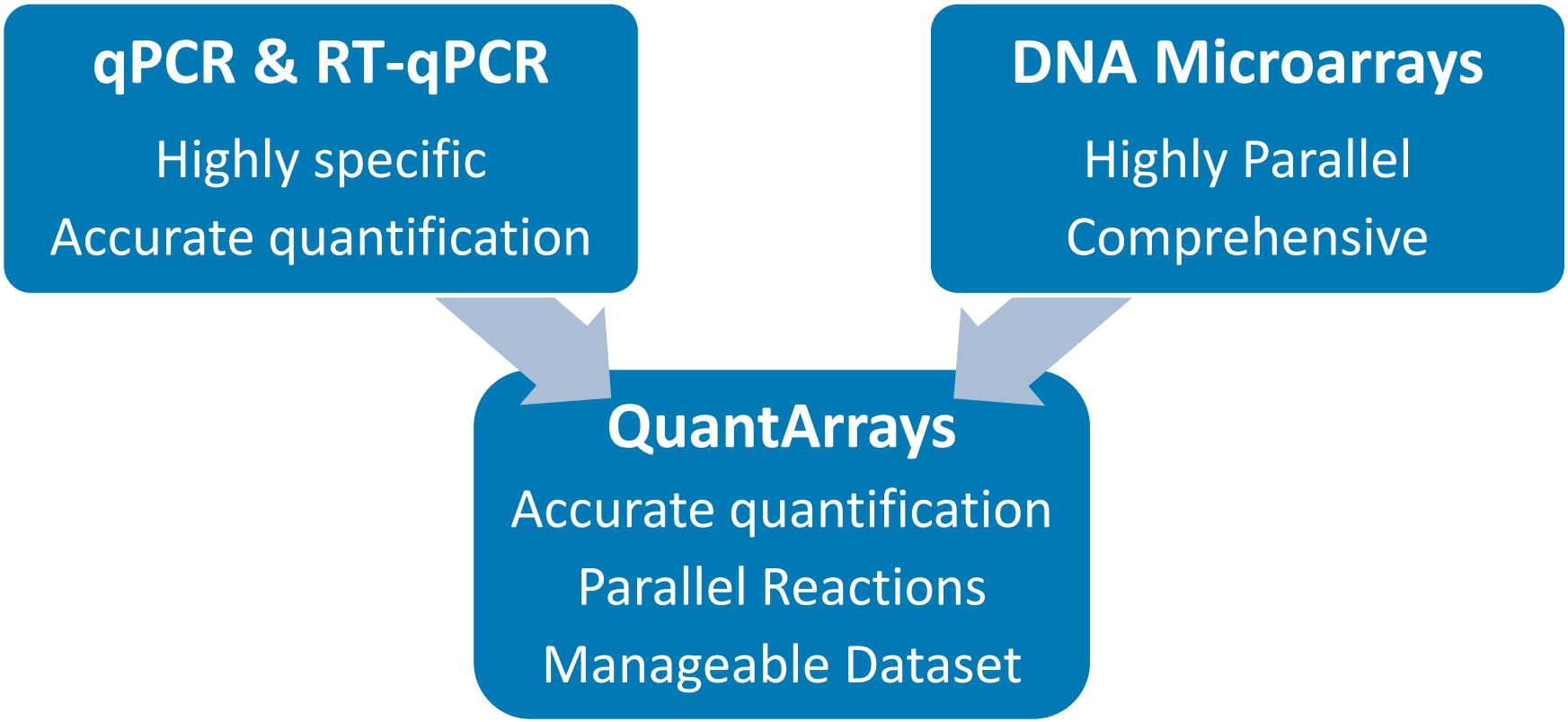
ITRC Fact Sheets and Guidance Documents

http://www.itrcweb.org/Team/Public?teamID=3

Environmental Molecular Diagnostics (EMD)

- Quantitative Polymerase Chain Reaction (qPCR)
- DNA Microarrays
- Stable Isotope Probing (SIP)
- Microbial Fingerprinting
- Enzyme Activity Probes
- Fluorescence In Situ Hybridiation (FISH)
- Compound Specific Isotope Analsyis (CSIA)

qPCR Arrays-The Best of Both Worlds



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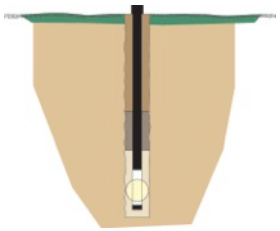
Solid Phase PCR

- Solid Phase DNA Amplification
  - Adessi et al. (2000) Nucleic Acids Res. 28(20): e87
- Nylon Membrane-Immobilized PCR
  - Onodera et al. (2002) BioTechniques 32:74-76
- Multiplex Microarray-Enhanced PCR*
  - Pemov et al. (2005) Nucleic Acids Res. 33(2): e11
- Megaplex PCR
  - Meuzelaar et al. (2007) Nature Methods 4(10): 835-837

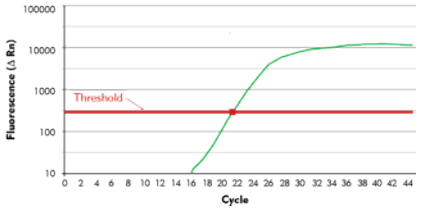
* Gel element array

qPCR Approach

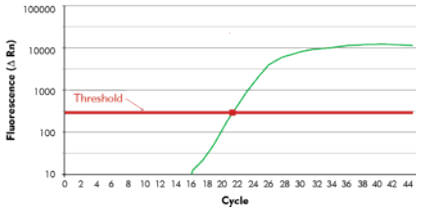
Sample Collection



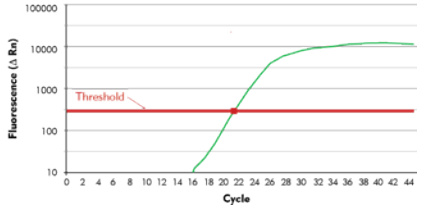
Extraction



DHC



DHB



DHG

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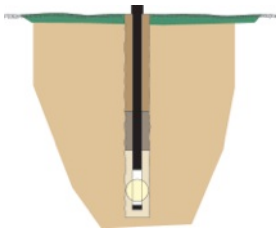
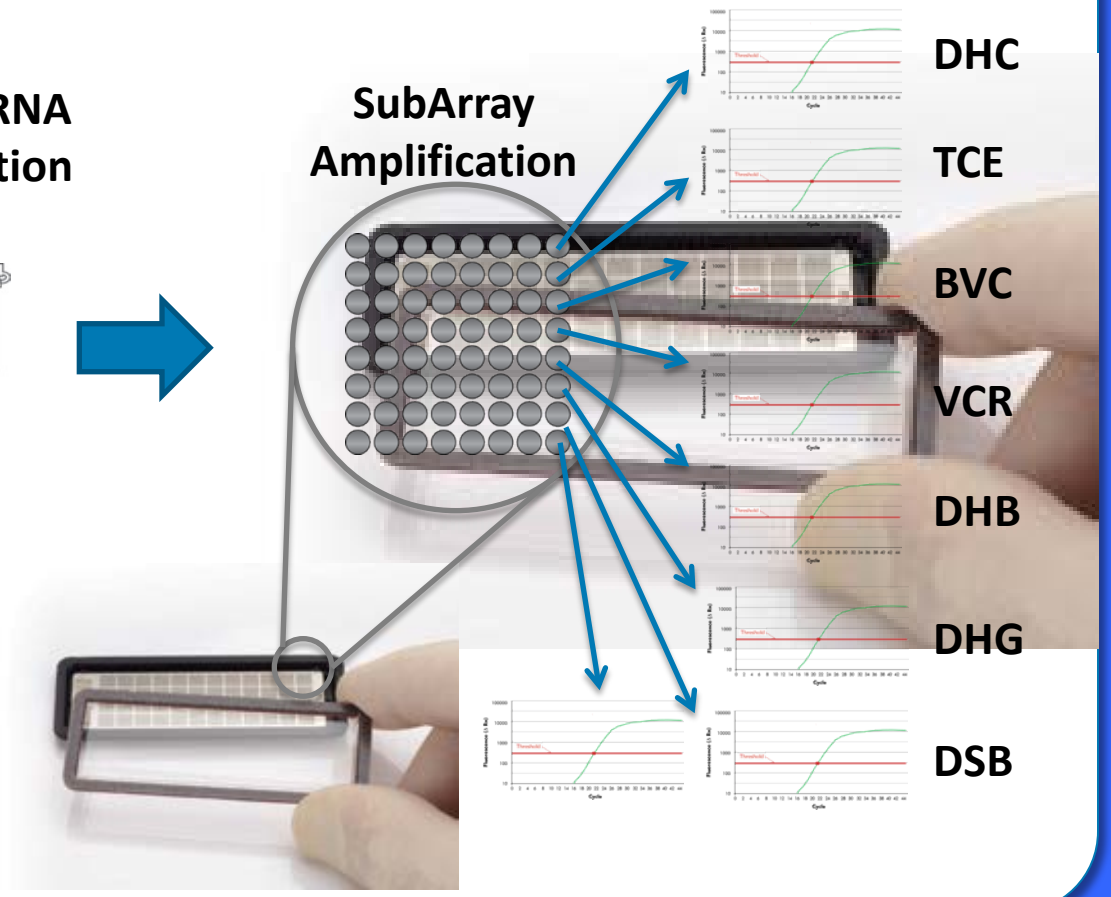
qPCR Array Approach

Sample Collection



DNA/RNA Extraction

SubArray Amplification



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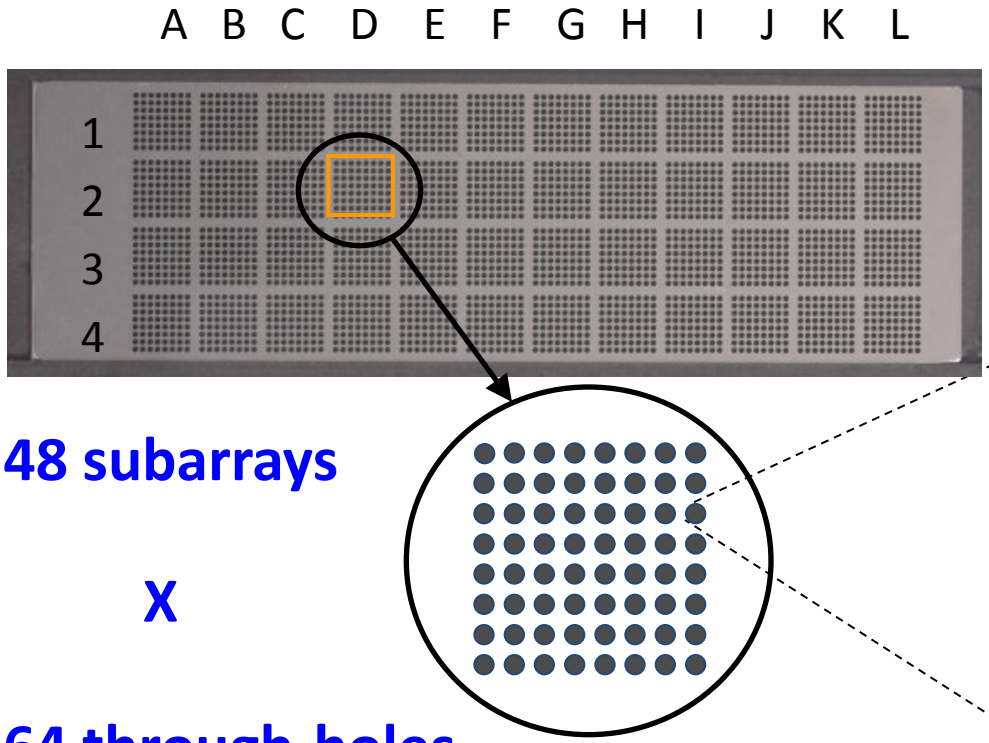
qPCR Array Plate

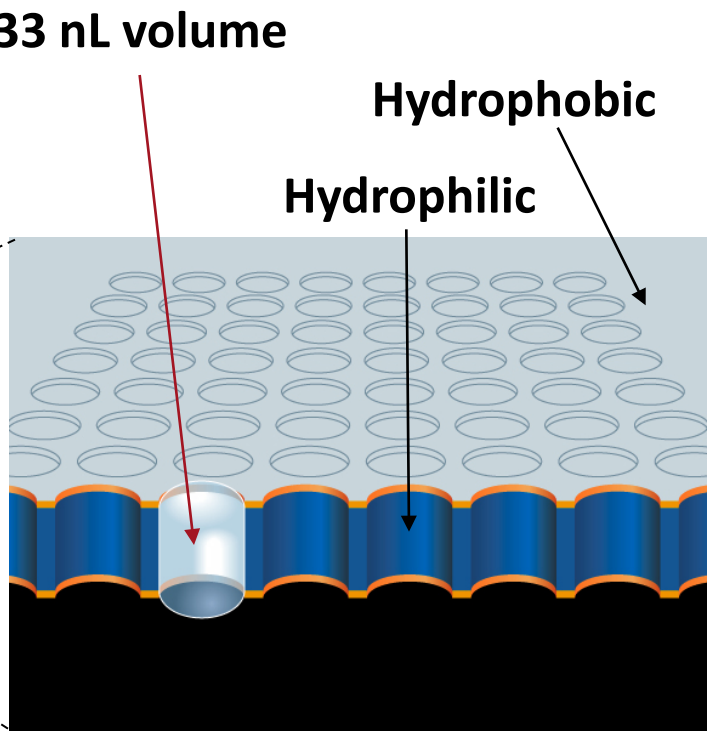


48 subarrays

X

64 through-holes

3072 through-holes per array



Hydrophilic and hydrophobic coatings enable reagents to stay in the bottomless through-holes via capillary action.

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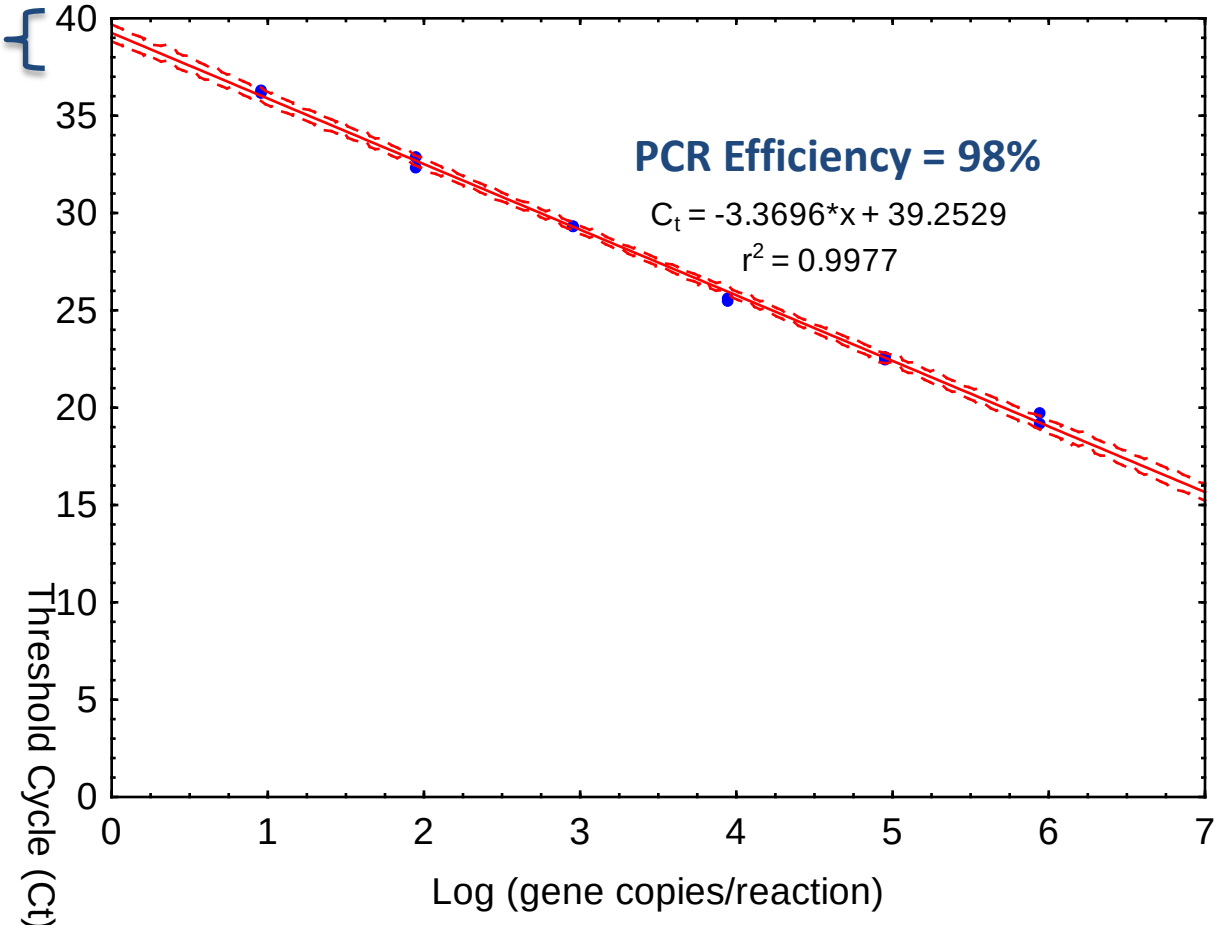
Standard Curve

Y-intercept (38 – 40)



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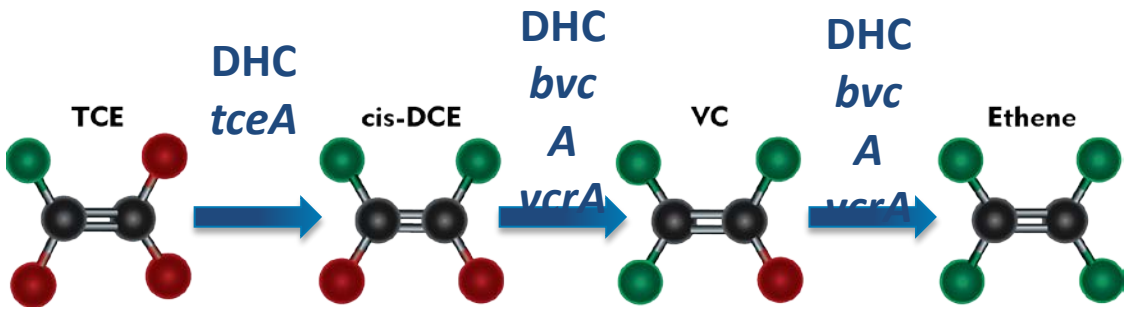
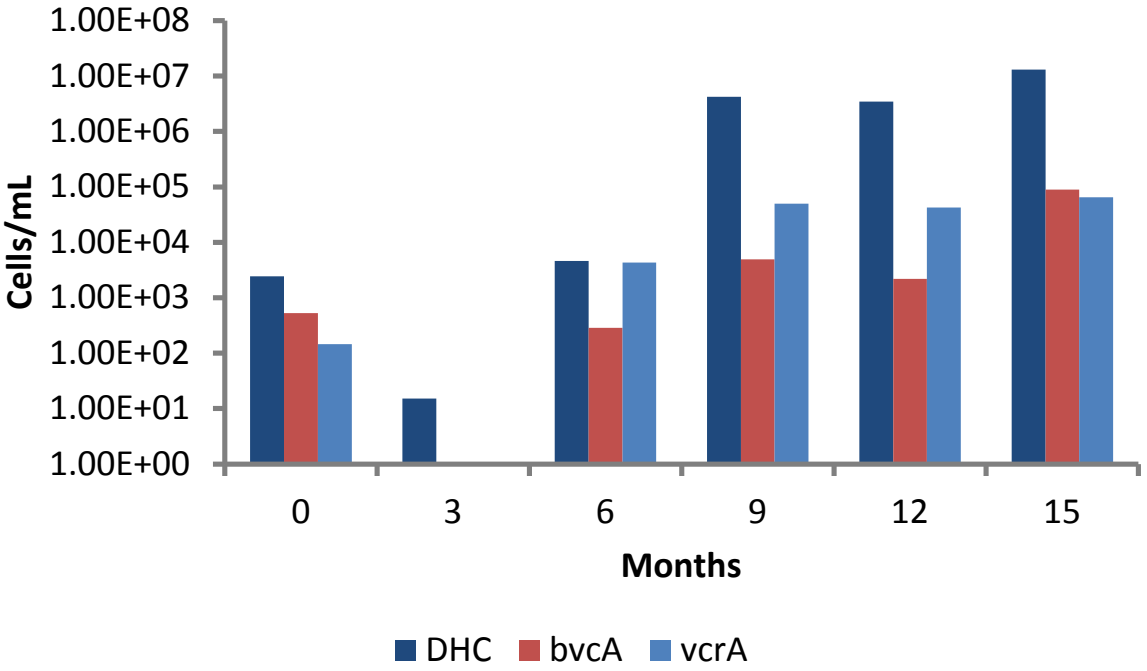
Sensitivity - Limit of Detection

"…lowest concentration at which 95% of the positive samples are detected." Bustin et al. (2009)

- Conventional qPCR ~ 3-10 gene copies/rxn
- Digital PCR (dPCR) - 0.1 to 10 gene copies/rxn

“Simple” Site





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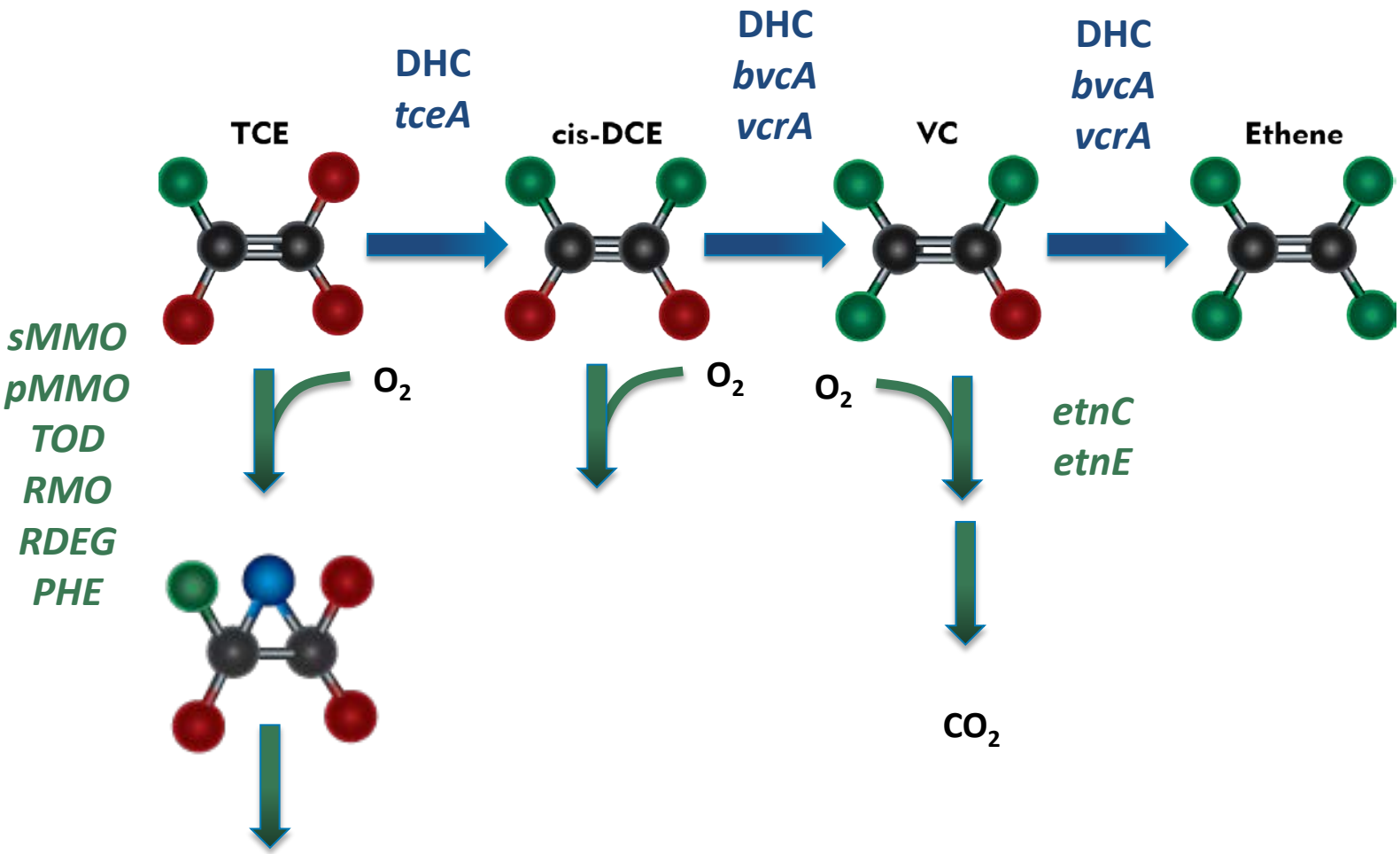
"Simple" Site



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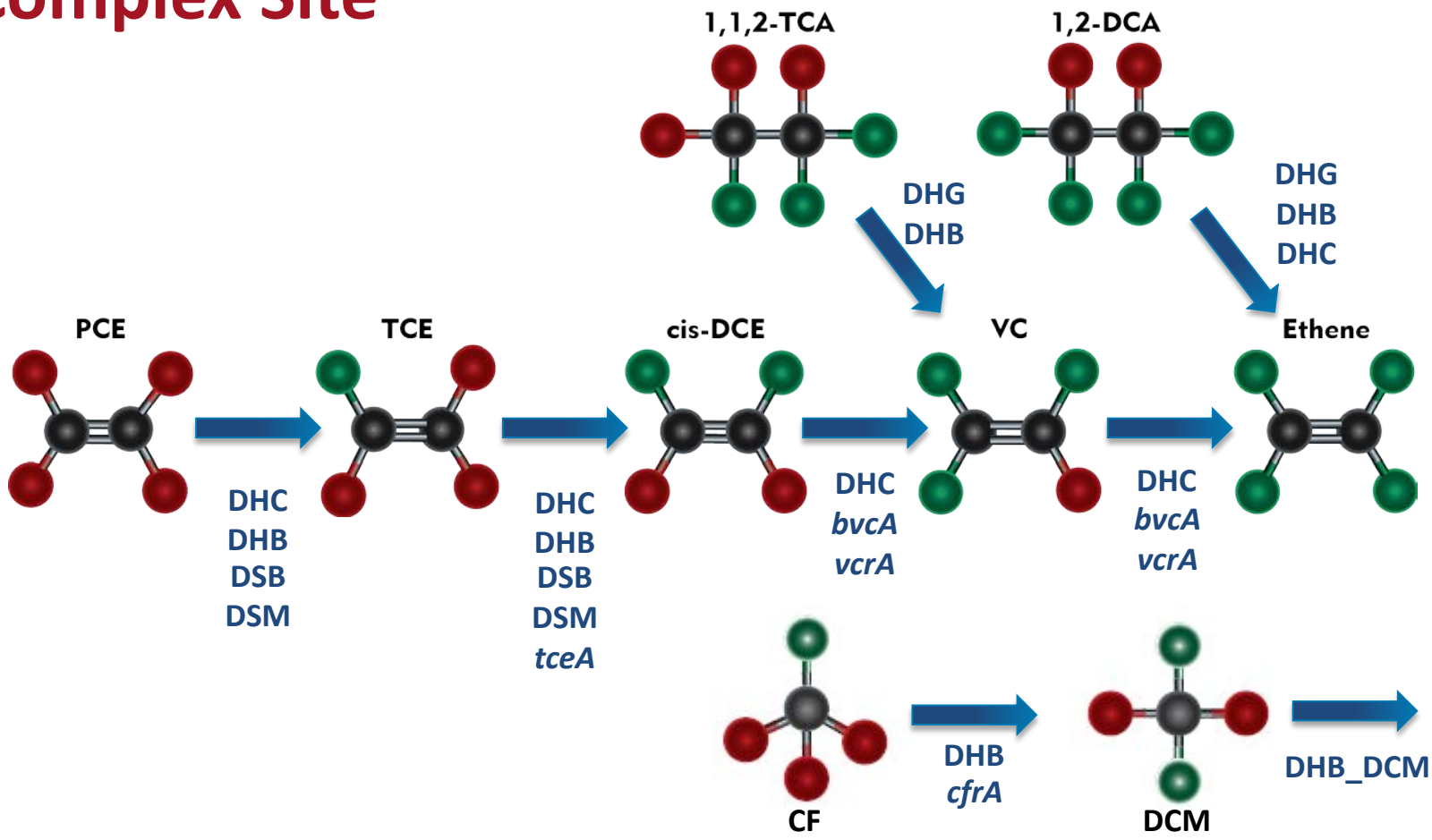
Complex Site



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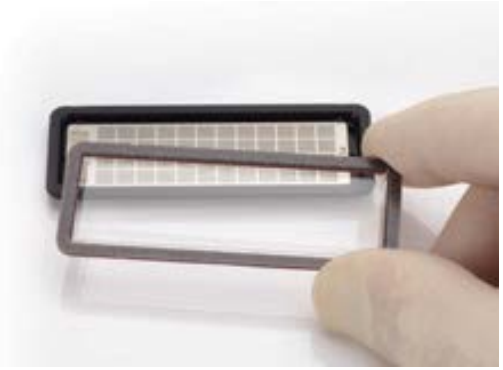
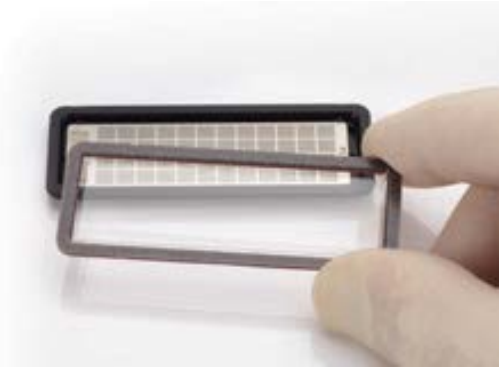
QuantArray



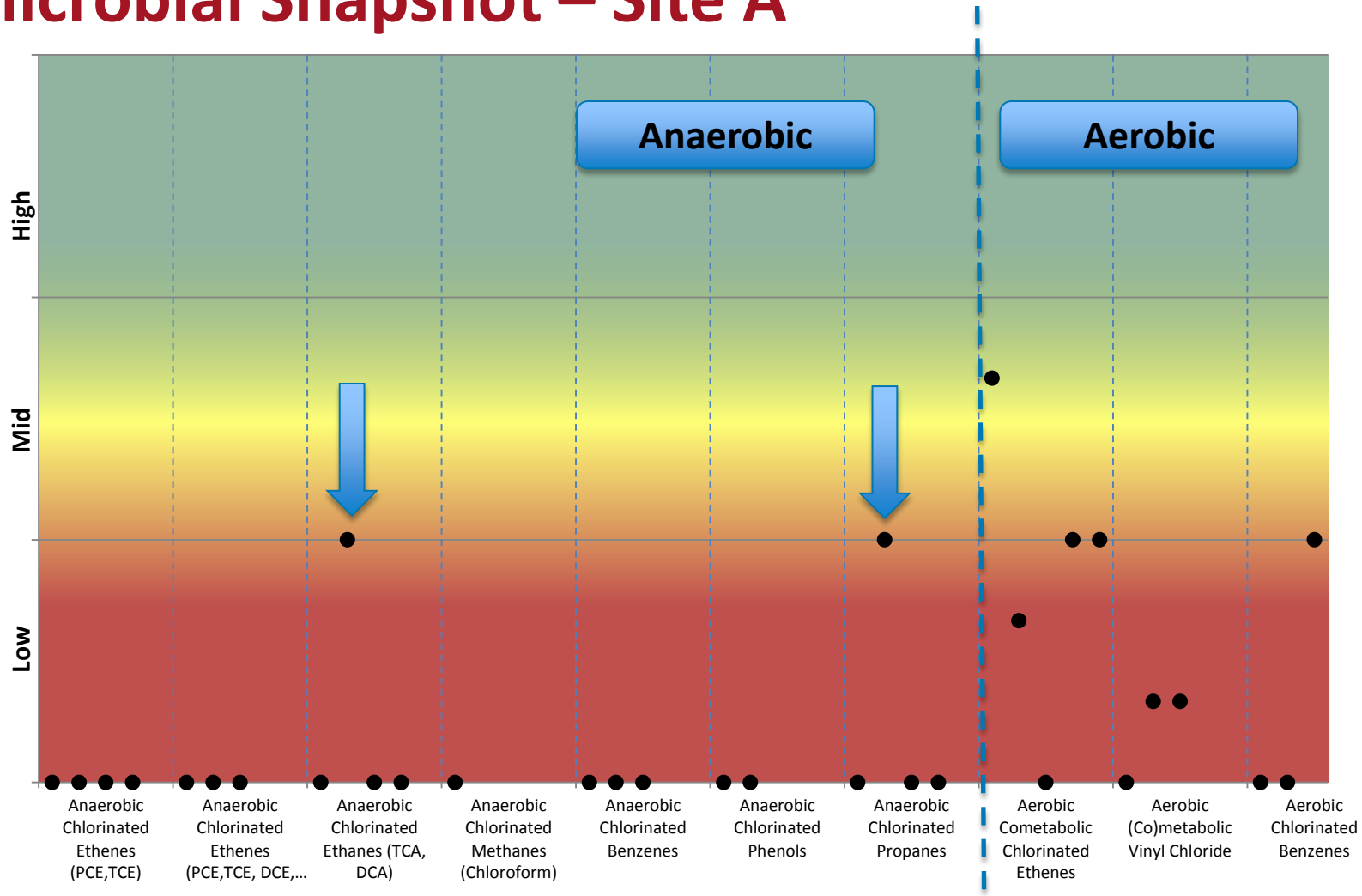
Reductive Dechlorination	
Dehalococcoides	PCE, TCE, DCE, VC, CPs, CBs, PCBs
TCE Reductase	TCE
BAV1 Vinyl Chloride Reductase	DCE, VC
Vinyl Chloride Reductase	DCE, VC
Dehalobacter spp.	PCE, TCE, TCAs, DCAs
chloroform reductase	CF
Dehalogenimonas spp.	TeCA, 1,1,2,2-TCA, 1,2-DCA, DCP
Desulfitobacterium spp.	PCE, TCE, DCA*, CPs
"Dehalobium chlorocoercia"	PCBs, CBs
Desulfuromonas spp.	PCE, TCE
Aerobic (Co)Metabolic	
Soluble Methane Monooxygenase	TCE, DCE, VC, CF, 1,2-DCA
Particulate Methane Monooxygenase	TCE, DCE, VC
Toluene Dioxygenase	TCE
Phenol Hydroxylase	TCE
Trichlorobenzene Dioxygenase	TCBs
Toluene Monooxygenase 2	TCE
Toluene Monooyxgenase	TCE, 1,2-DCEs, 1,1-DCE, VC, CF
Ethene Monooxygenase	VC
Epoxyalkane transferase	VC
General	
Total Eubacteria	Total
Sulfate Reducing Bacteria	Competitors
Methanogens	Competitors

Löffler Lab



Target						
Bacteria	BAC1					
Methanogens	ARCH1					
Geobacteraceiae	GEOGC					
Anaeromyxobacter	ADEH					
Dehalobacter	DHB	DFORM	DHB	DHB2	DHB3	DHB4
Dehalococcoides	DHC1-K	DHC2-J	DHC3-H			
Dehalogenimonas	DHGM					
Desulfuromonas	DSM1	DSM2				
Dehalobium chlorocoercia	DF1					
Acidaminobacter	ACMB					
Nitrospira	NTSP					
Spirochaetes	SPIRO1	SPIRO2				
Desulfovibrio	DSV					
Sphingobacteria	SPG					
Methanospirillum	METSP1	METSP2				
VadinBC27	VAD27					
pceA	GEO	195	CBDB	SFP	DF	
tceA	DHCFL2					
mbrA	MBRA1	MBRA2				
vcrA	VCRA1	VCRA2				
bvcA	BVCA1	BVCA2				
cbrA	CBRA					
dcpA	DCPA					
cbiZ	CBIZ1	CBIZ2				
cobU	COBU					
etnE	ETNE	ETNE2				
etnC	ETNC1					
fdhA	FHCA					
vhuA	VHUA					
hycE	HYCE					
Fe hymC	HYMC1	HYMC2				
hupL	HUPL					
ADEH 2CPC	ADEH CYTC1					

Microbial Snapshot – Site A



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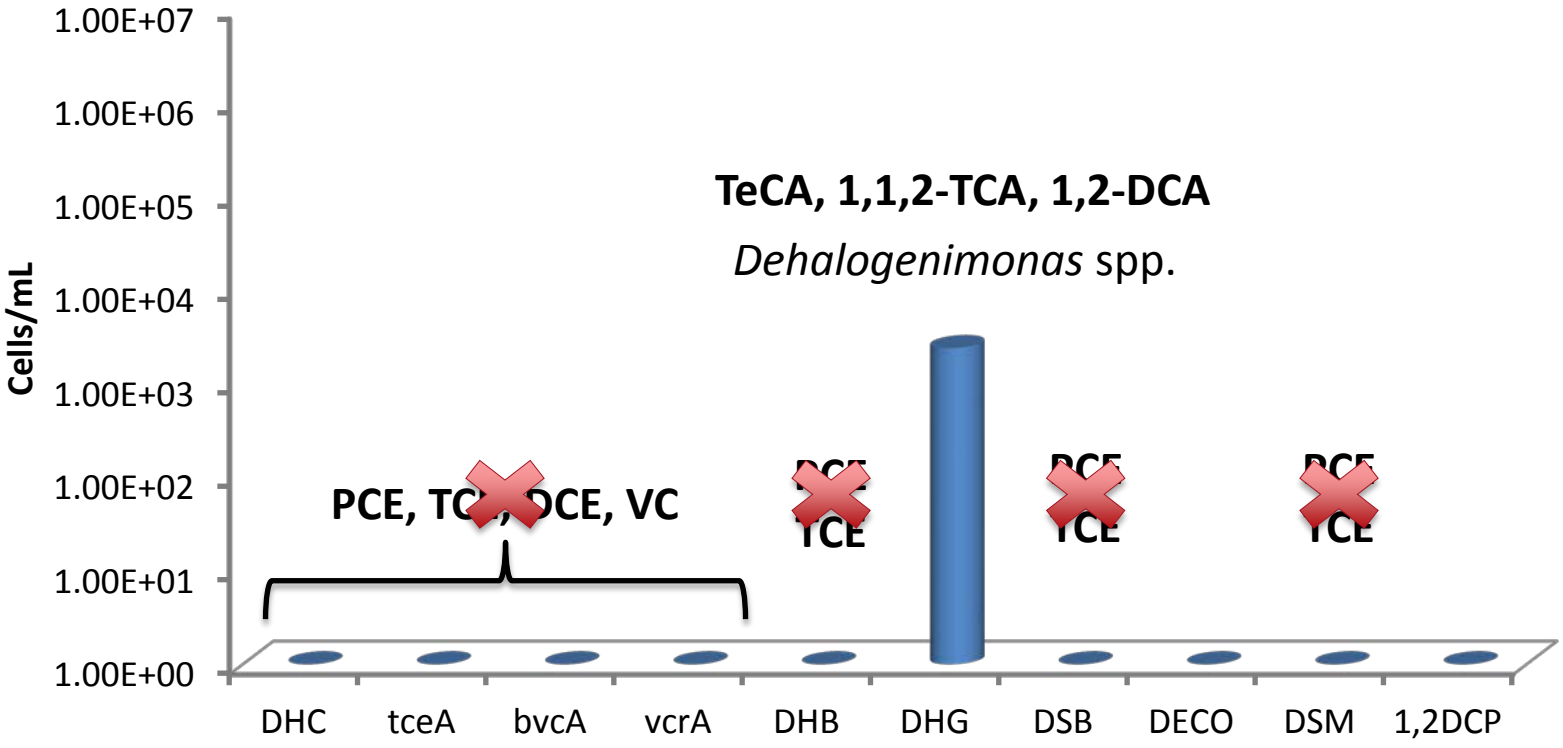
Reductive Dechlorination – Site A



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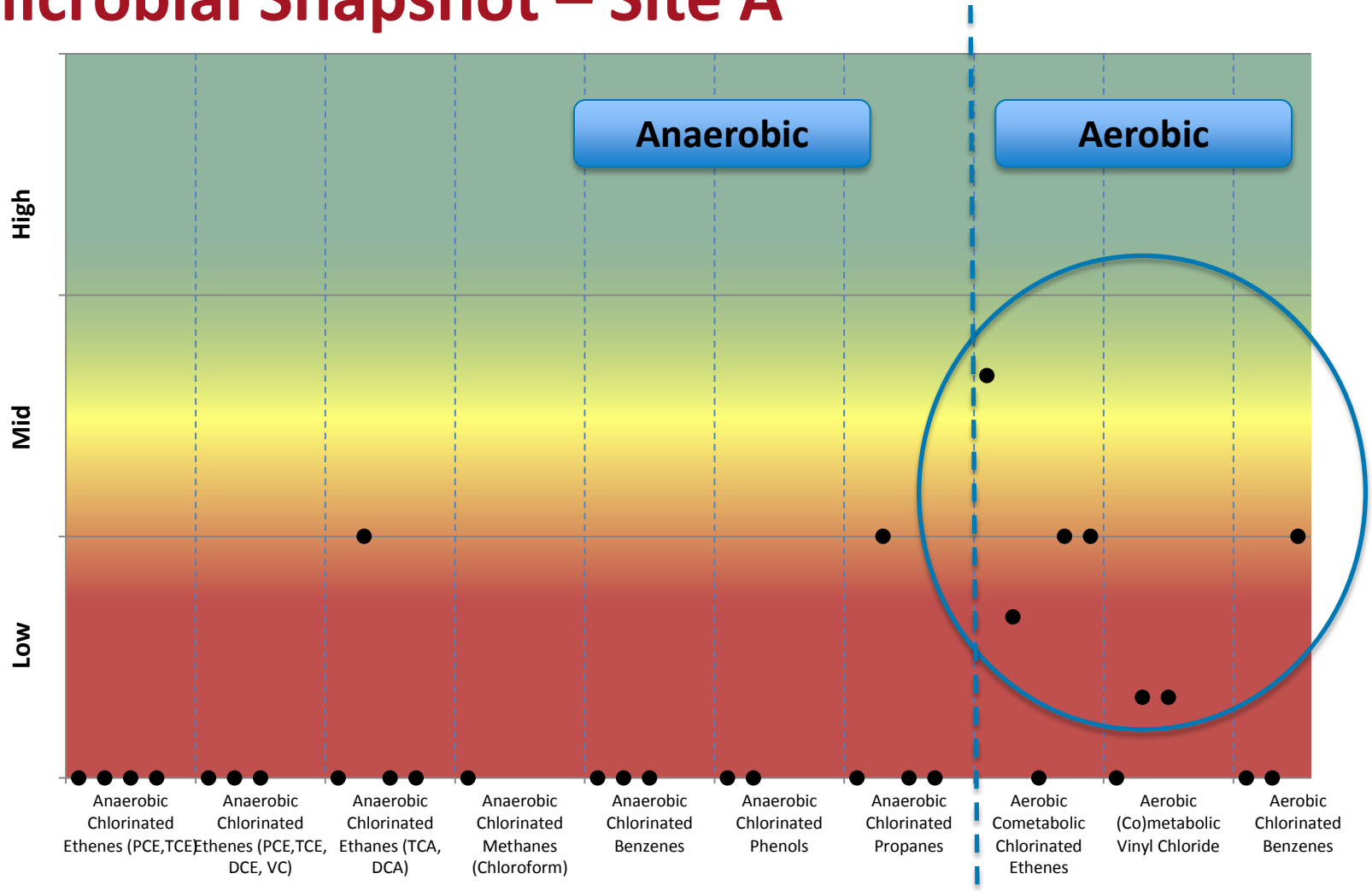
Microbial Snapshot – Site A



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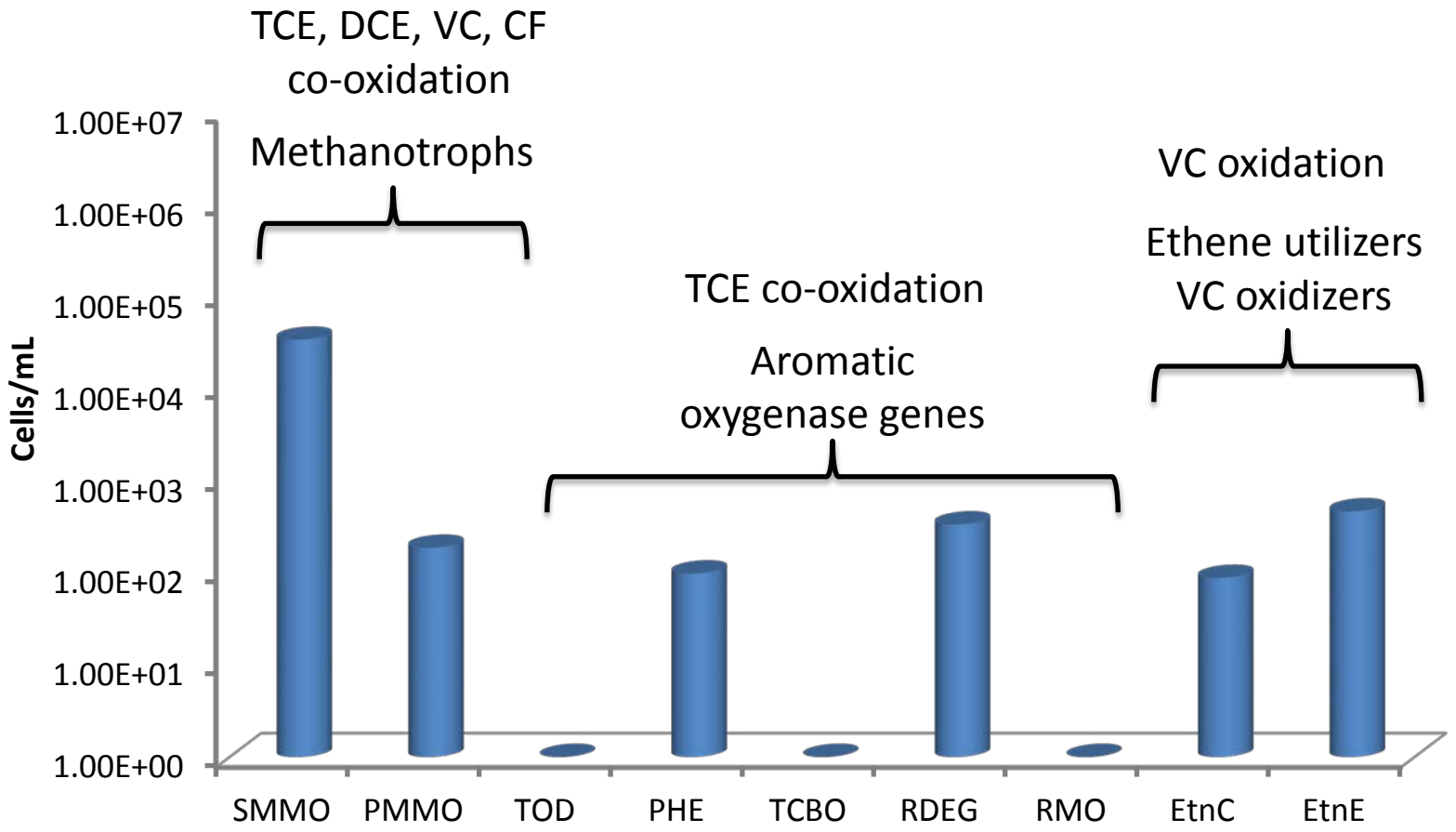
Aerobic (Co)metabolism – Site A



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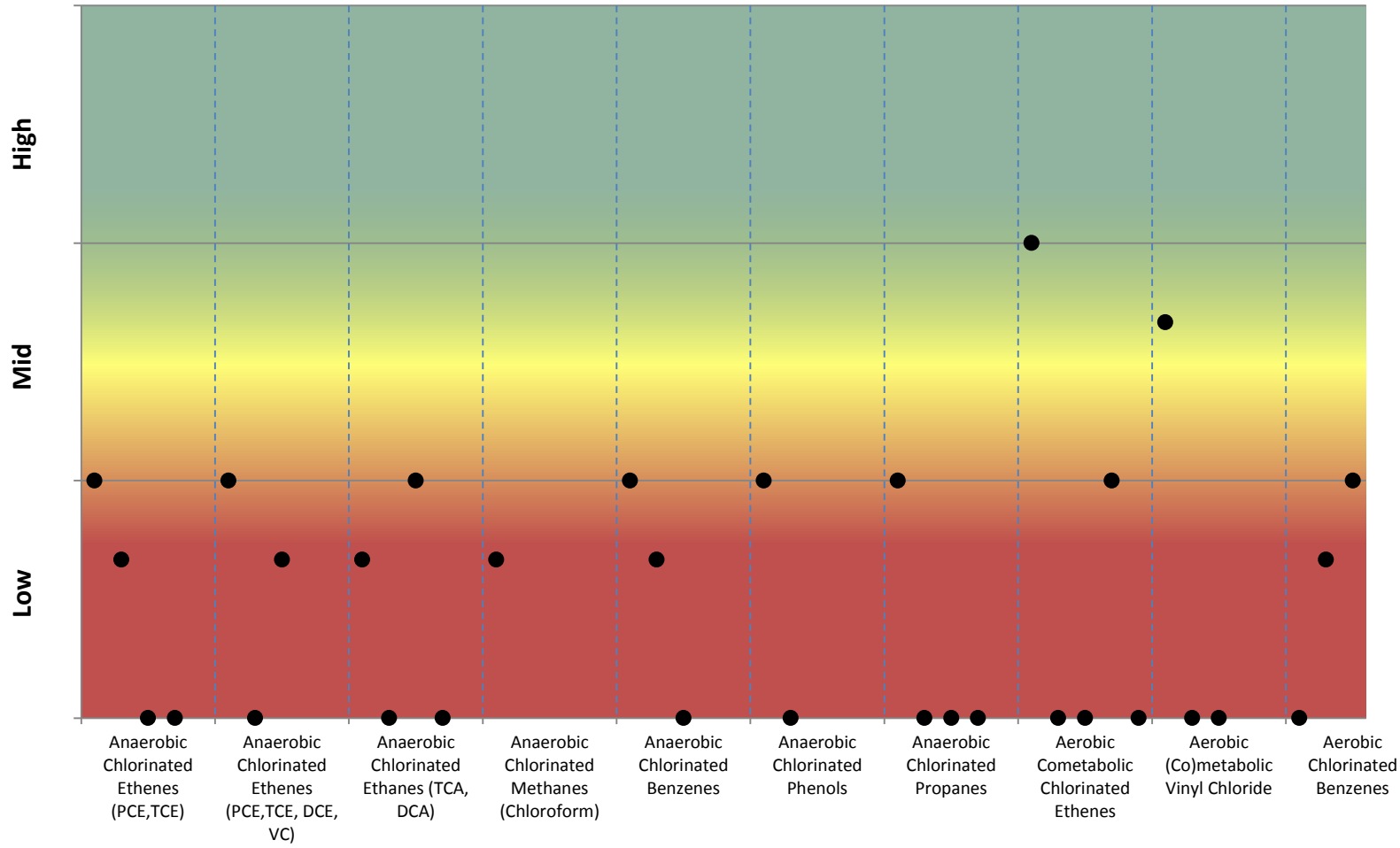
Microbial Snapshot – Site B



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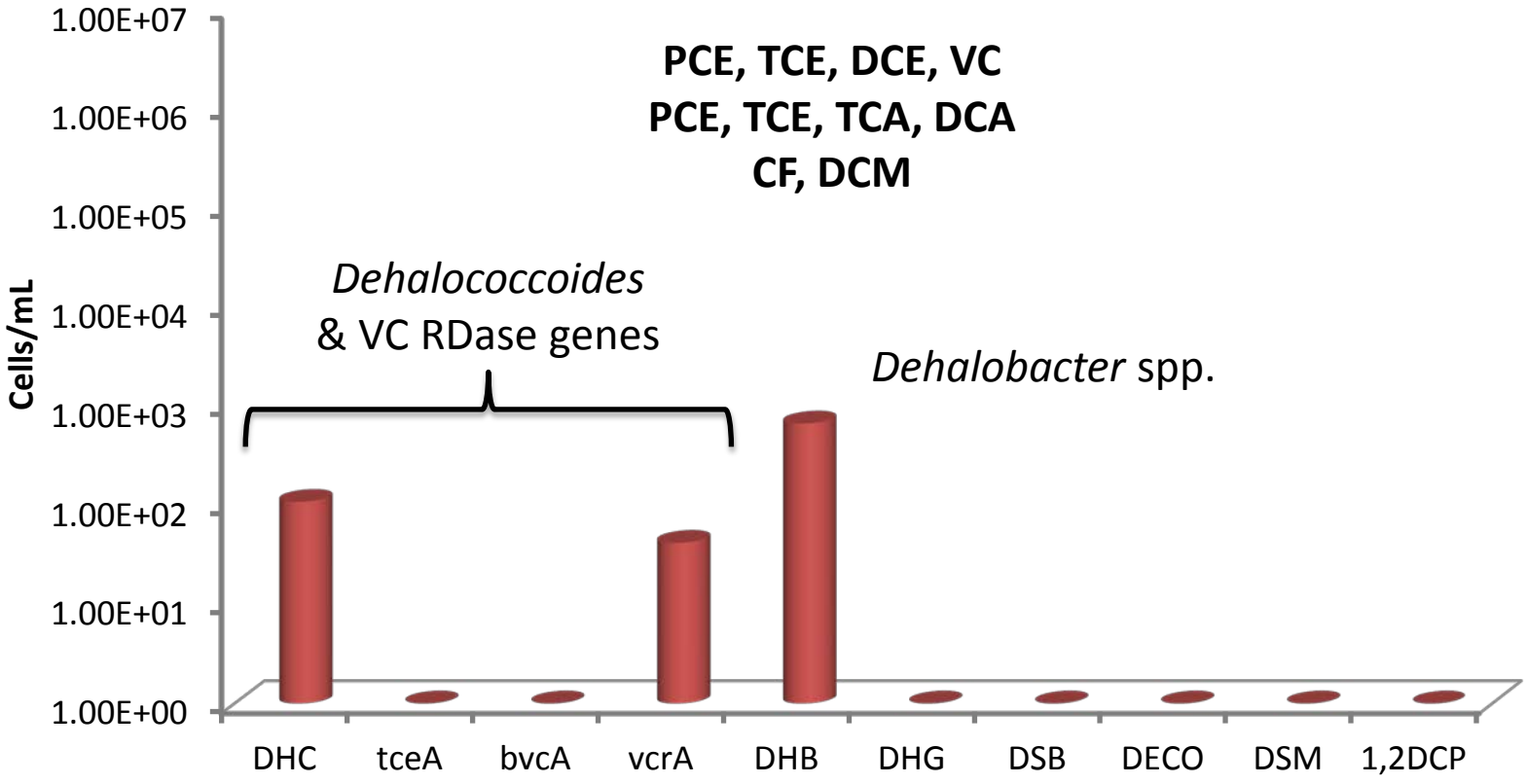
Reductive Dechlorination – Site B



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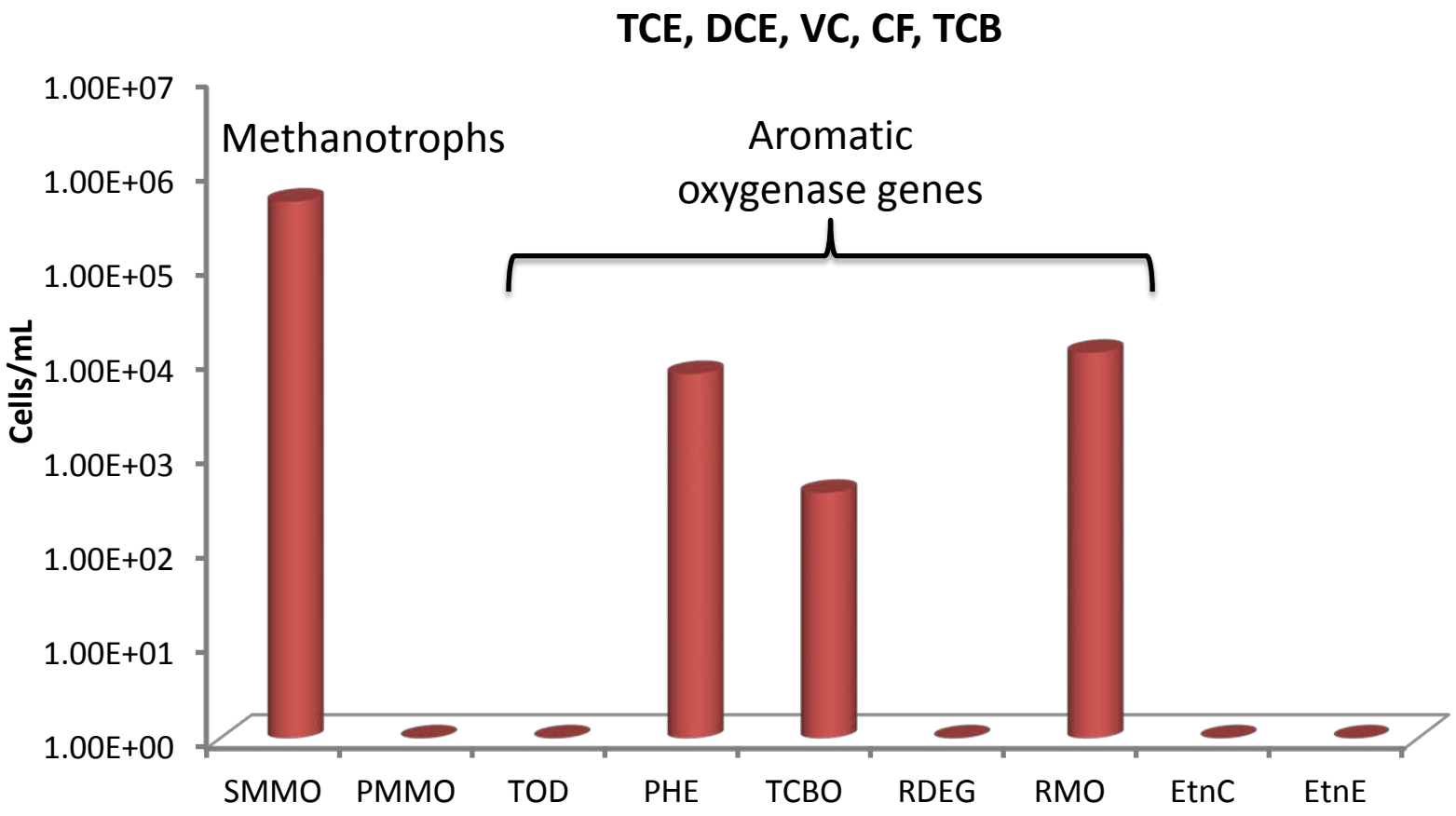
Aerobic (Co)metabolism – Site B



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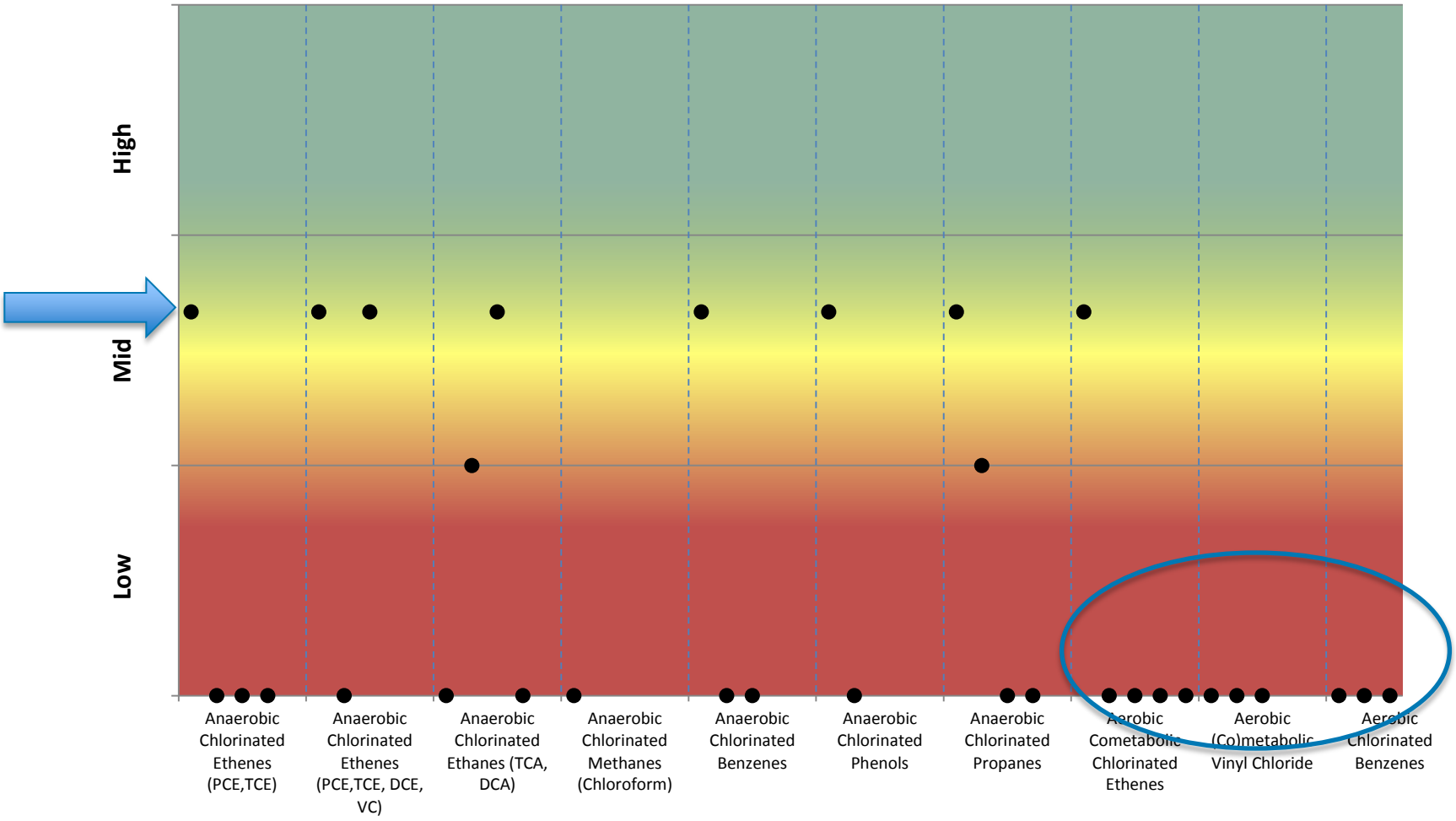
Microbial Snapshot – Site C



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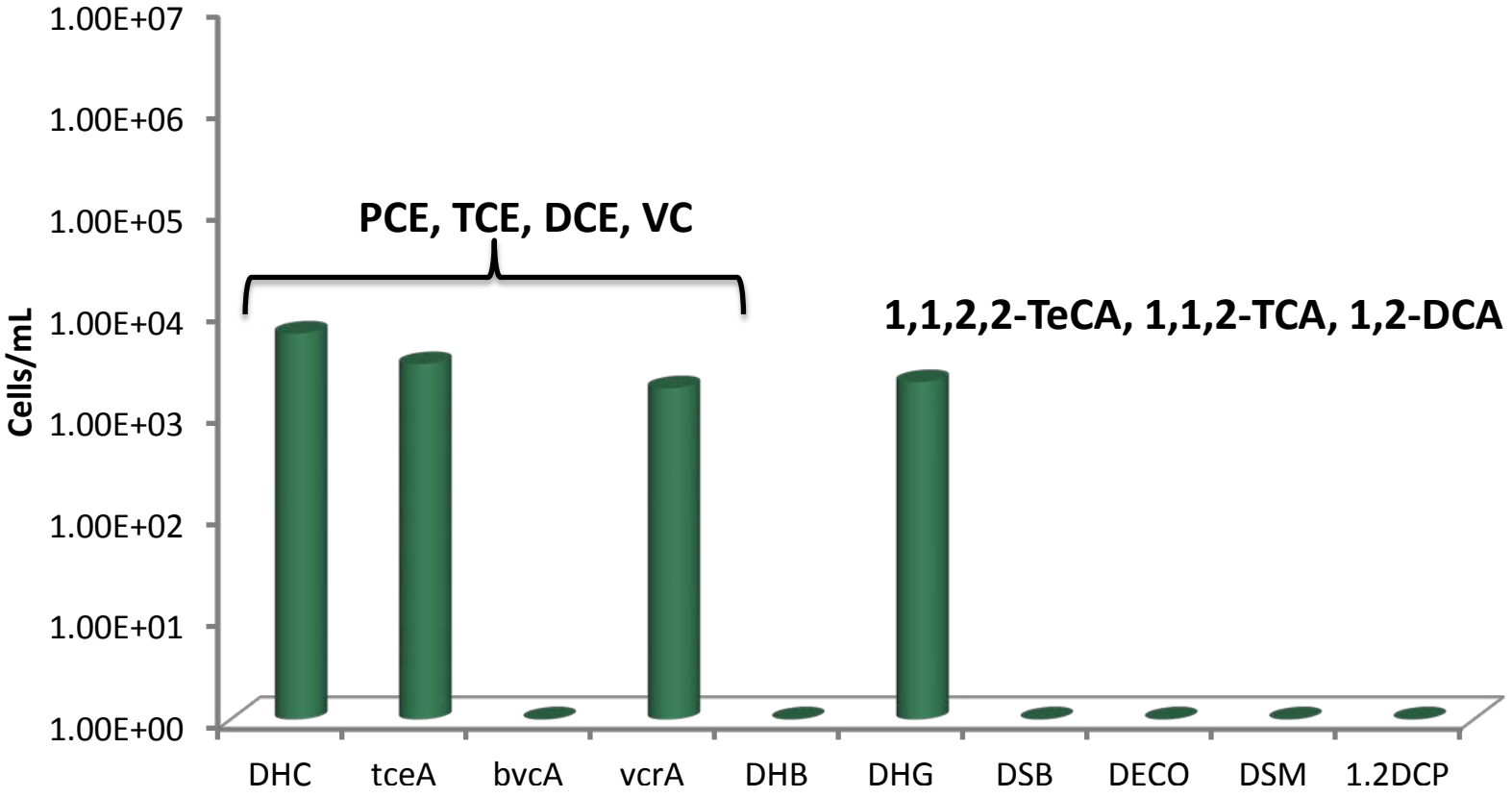
Reductive Dechlorination – Site C



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Site Tailored Monitoring Tools-qPCR

qPCR

- "Simple" Sites
  - One or two parent compounds (e.g. TCE)
  - Remedy selected or corrective action in place
  - Limited potential for competing electron accepting processes
- Specific questions
  - Will reductive dechlorination "stall" at cDCE?
  - How can it be overcome?
  - Will these tools help us?

Site Tailored Monitoring Tools-QuantArray

QuantArray

- Site Characterization/Remedy Selection
  - Simultaneous assessment of aerobic and anaerobic pathways
- Multiple contaminants
  - Petroleum hydrocarbons
  - Mixtures of chlorinated ethenes, ethanes, benzenes, etc.
  - Potential for competing electron accepting processes

Why use QuantArray

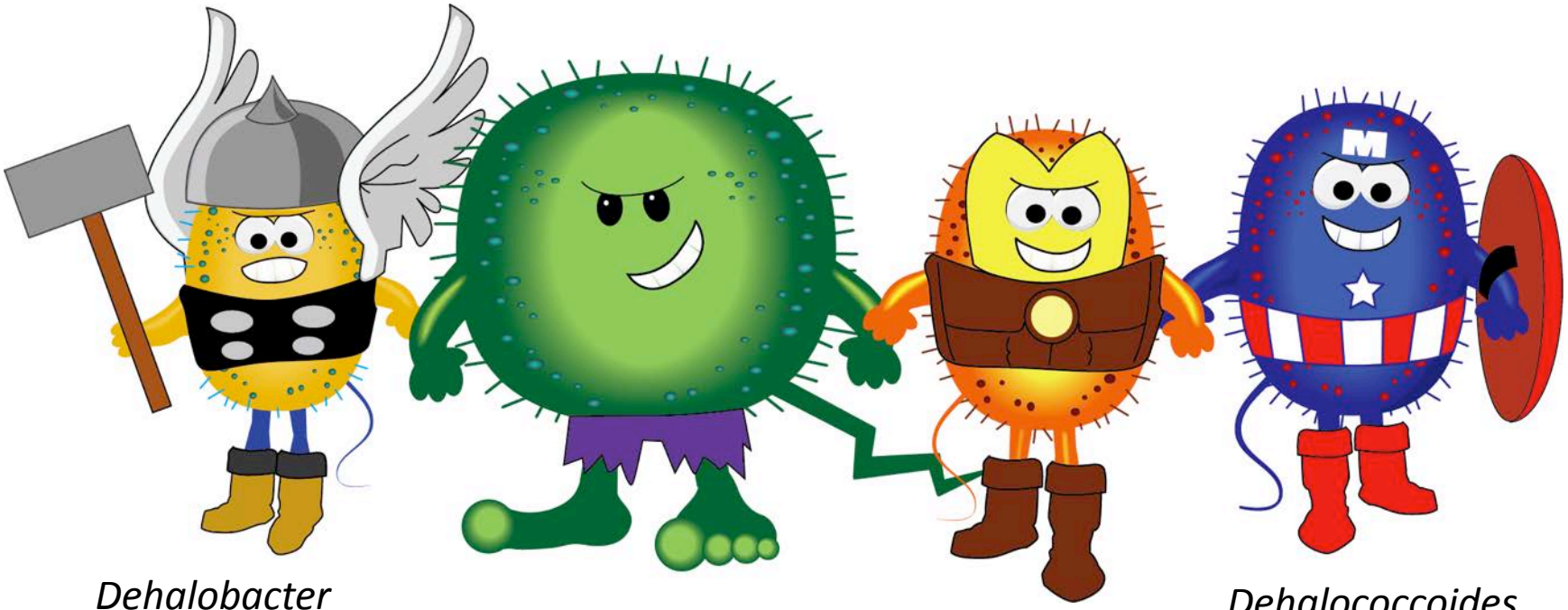
- Up to 224 targets can be analyzed
- Multiple samples processed in parallel
- More complete picture of relevant microbial processes for efficient site management

Acknowledgements

- Jun Yan and Burcu Simsir

- Kate Clark and Charles Slater

Questions?



Dehalobacter

Dehalogenimonas

Desulfitobacterium

Dehalococcoides

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