Thank you for signing in early

The webinar will begin promptly at
12:00 pm ET, 9:00 am PT

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SERDP and ESTCP Webinar Series

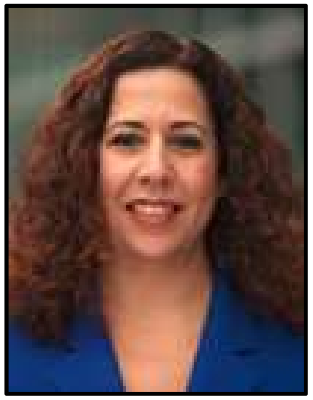
- The webinar will begin promptly at 12:00 pm ET, 9:00 am PT
- Two options for accessing the webinar audio
  - Listen to the broadcast audio if your computer is equipped with speakers
  - Call into the conference line
    - (669) 900-6833 or (929) 205-6099
    - Required webinar ID: 323-604-529
- For questions or technical issues, please email serdp-estcp@noblis.org or call 571-372-6565

The Use of Advanced Molecular Biological Tools in Groundwater Contaminated with Chlorinated Solvents

September 5, 2019

SERDP DOD • EPA • DOE | ESTCP

Welcome and Introductions



Rula A. Deeb, Ph.D.
Webinar Coordinator

SERDP DOD • EPA • DOE | ESTCP

Webinar Agenda

- **Webinar Logistics** (5 minutes)
  Dr. Rula Deeb, Geosyntec Consultants
- **Overview of SERDP and ESTCP** (5 minutes)
  Dr. Andrea Leeson, SERDP and ESTCP
- **Innovative Methods to Quantify Microorganisms Associated with the Biodegradation of Chlorinated Solvents and 1,4-Dioxane** (25 minutes + Q&A)
  Dr. Alison Cupples, Michigan State University
- **Validation of Advanced Molecular Biological Tools to Monitor Chlorinated Solvent Bioremediation and Estimate cVOC Degradation Rates** (25 minutes + Q&A)
  Dr. Mandy Michalsen, U.S. Army Engineer Research Development Center; **Dr. Kate Kucharzyk**, Battelle Memorial Institute
- **Final Q&A session**

In Case of Technical Difficulties

- Use a compatible browser (Firefox, IE or Edge)
- If material is not showing on your screen or if screen freezes
  - Key in Ctrl + F5 to do a hard refresh of your browser
- If connecting to computer audio
  - Click the arrow next to the "*Join Audio*" button
  - Select test "*Speaker and Microphone*"
  - Follow prompts
- If you continue to experience difficulties, call into the conference line
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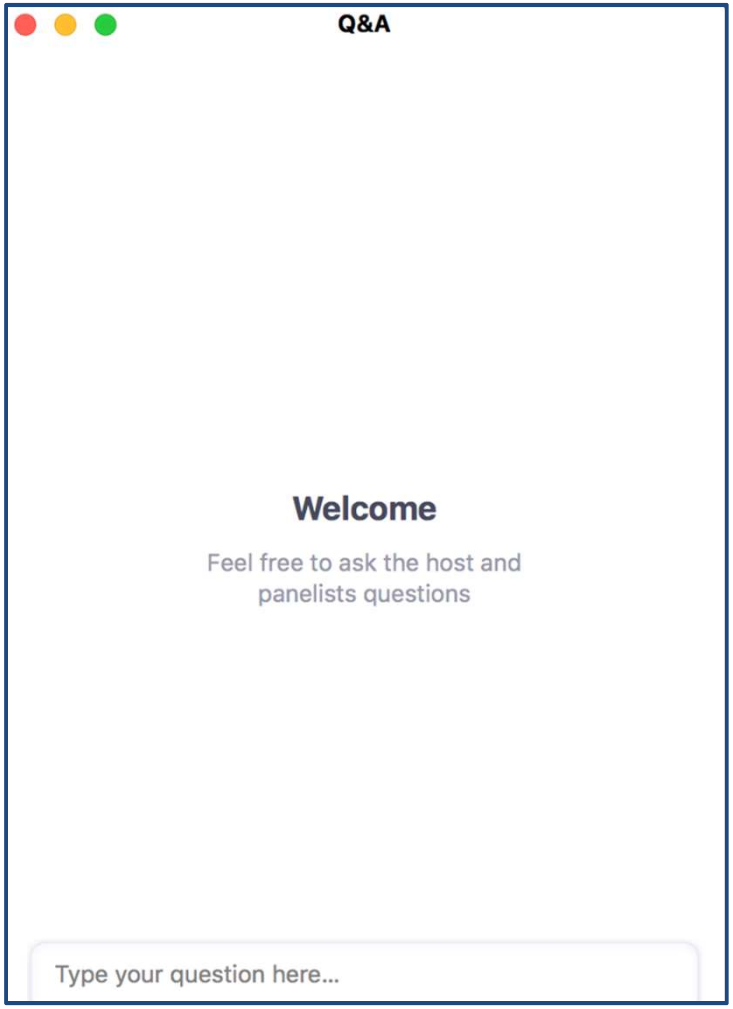
How to Ask Questions

- Find the Q&A button on your control bar and type in your question(s)
- Make sure to add your organization name at the end of your question so that we can identify you during the Q&A sessions



SERDP and ESTCP Overview

Andrea Leeson, Ph.D.
SERDP and ESTCP

DoD's Environmental Technology Programs

Science and Technology

- Statutory program established 1991
- DoD, DOE, EPA partnership
  - Advanced technology development to address near-term needs
  - Fundamental research to impact real world environmental management

Demonstration and Validation

- Demonstrate innovative cost-effective environmental and energy technologies
  - Transition technology out of the lab
  - Establish cost and performance
  - Partner with end user and regulator
  - Technology transfer
    - Accelerate commercialization or broader adoption
    - Direct technology insertion

Environmental Drivers

Sustaining Ranges, Facilities and Operations

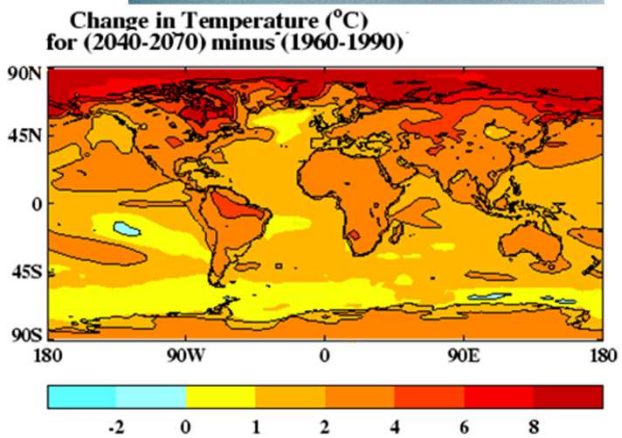
Maritime Sustainability
Threatened and Endangered Species

Toxic Air Emissions and Dust

UXO and Munitions Constituents

Noise

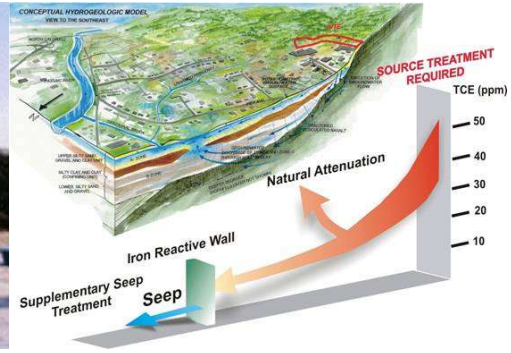
Sustainable FOB



Environmental Drivers

Reducing Current and Future Liability

Contamination from Past Practices

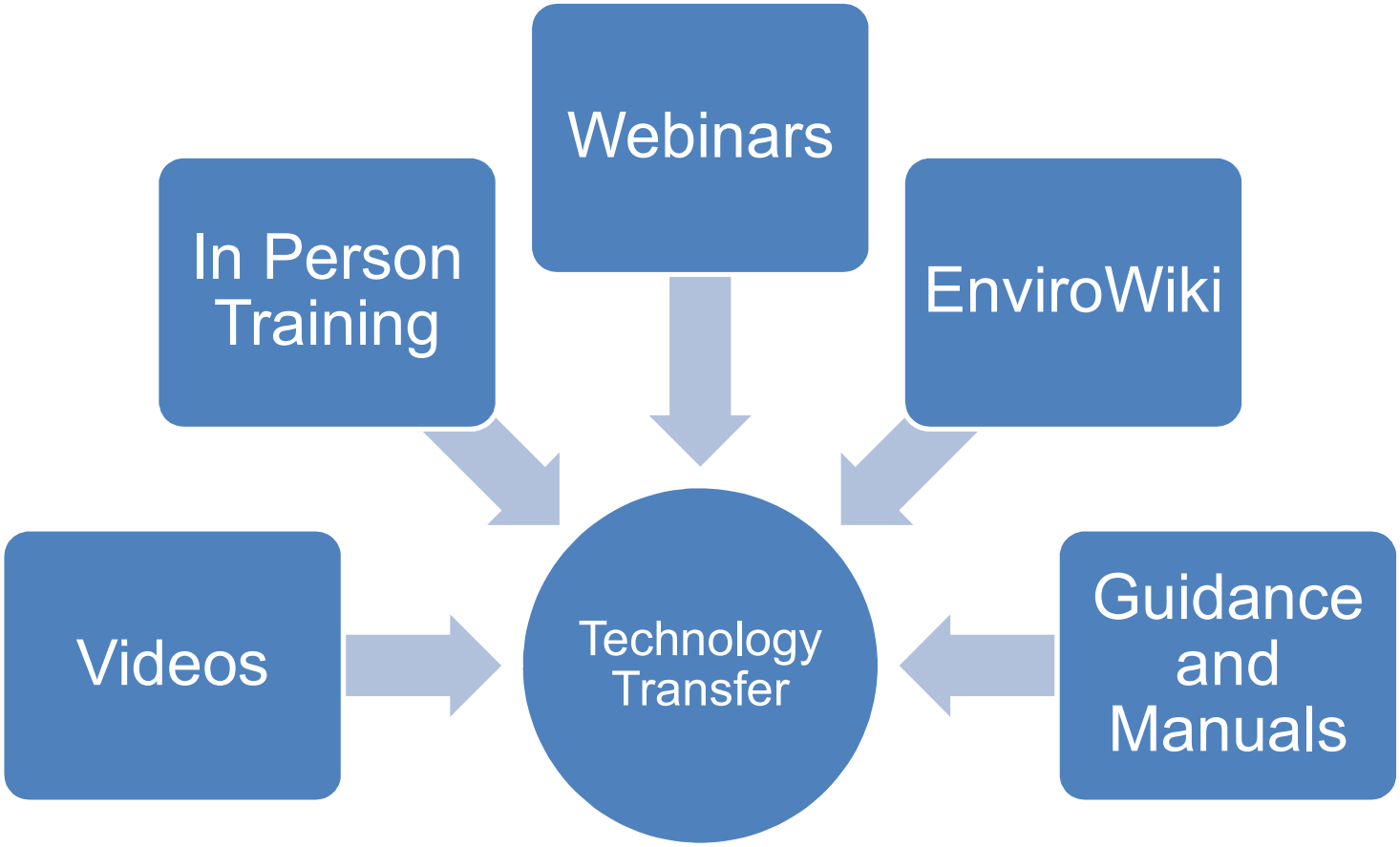


- Groundwater, soils and sediments
- Large UXO liability
- Emerging contaminants

Pollution Prevention to Control Life Cycle Costs

- Elimination of pollutants and hazardous materials in manufacturing, maintenance, and operations
- Achieve compliance through pollution prevention

www.serdp-estcp.org



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Webinars

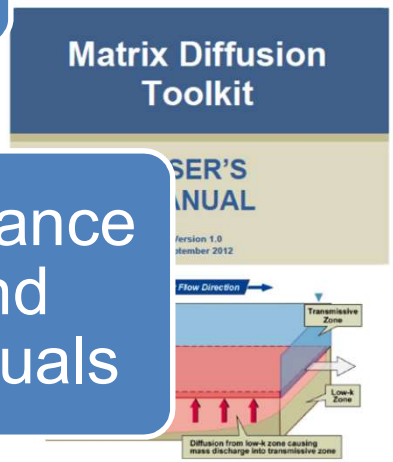
In Person Training

EnviroWiki

Videos

Technology Transfer

Guidance and Manuals



SERDP and ESTCP Webinar Series

Date	Topic
September 19, 2019	Life Cycle Assessment and Developmental Environment Safety and Occupational Health Evaluation: Tools for Sustainment and Health
October 3, 2019	Variation in Phenological Shifts: How do Annual Cycles and Genetic Diversity Constrain or Enable Responses to Climate Change?
October 17, 2019	Managing Aqueous Film Forming Foam (AFFF) Impacts to Subsurface Environments and Assessment of Commercially Available Fluorine-Free Foams
October 24, 2019	Advances in Managing Contaminated Groundwater Using High Resolution Site Characterization and Contaminant Mass Flux Reduction
October 31, 2019	Developing and Demonstrating Non-Toxic and Sustainable Coating Systems for Military Platforms

For upcoming webinars, please visit

http://serdp-estcp.org/Tools-and-Training/Webinar-Series

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Save the Date

SERDP • ESTCP
SYMPOSIUM

A three-day symposium showcasing the latest technologies that enhance DoD's mission through improved environmental and energy performance

December 3-5, 2019

Washington Marriott Wardman Park

Registration is open!

Innovative Methods to Quantify Microorganisms Associated with the Biodegradation of Chlorinated Solvents and 1,4-Dioxane

Alison M. Cupples, Ph.D.
Michigan State University

SERDP DOD • EPA • DOE | ESTCP

Agenda

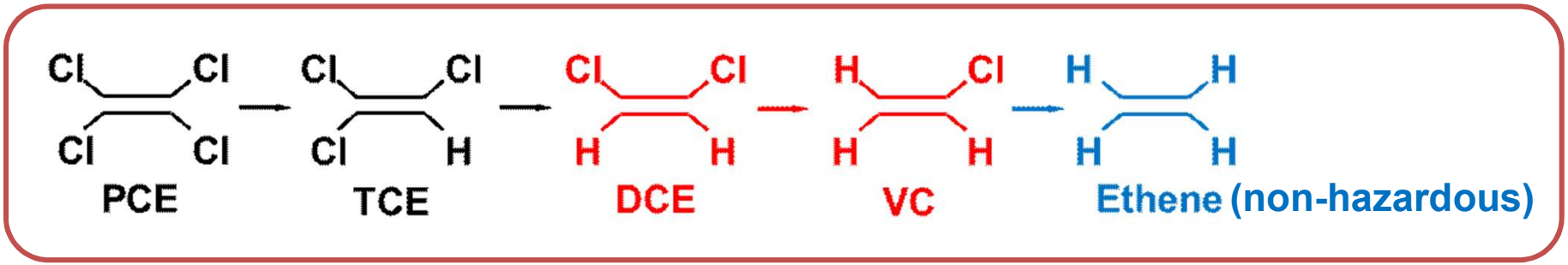
- Overall objective
- LAMP assay development and validation
  - Primer design
  - Effect of humic acid
  - Comparison of LAMP to qPCR
  - Visual based LAMP assay (no DNA extraction)
- Shotgun sequencing to characterize groundwater communities
- Key points and conclusions

***LAMP**: Loop mediated isothermal amplification; **qPCR**: Quantitative polymerase chain reaction*

Technical Objective

- To develop innovative methods to detect key microorganisms associated with contaminant biodegradation in groundwater
  - Develop and validate LAMP assays
  - Explore quantification without DNA extraction
  - Develop potentially field deployable methods
  - Determine the applicability of shotgun sequencing to detect key genes in groundwater

Background



Occurrence of *Dehalococcoides mccartyi* is determined using molecular biological tools, targeting functional genes

vcrA and *bvcA*: Encode for vinyl chloride reductive dehalogenases

tceA: Encode for TCE reductive dehalogenase

Gene Z Analyzer: Developed by Dr. Syed Hashsham's group for the parallel detection of genes using quantitative isothermal amplification

Advantages of LAMP

HIGH AMPLIFICATION EFFICIENCY,
DNA amplified 10^9-10^{10} times in 15 - 60 min

- Amplification at one temp., thermal cycler is NOT NEEDED
- Analysis without DNA extraction IS POSSIBLE
- LESS SENSITIVE to substances that inhibit PCR
- Six primers makes LAMP HIGHLY SPECIFIC

LAMP Primer Design

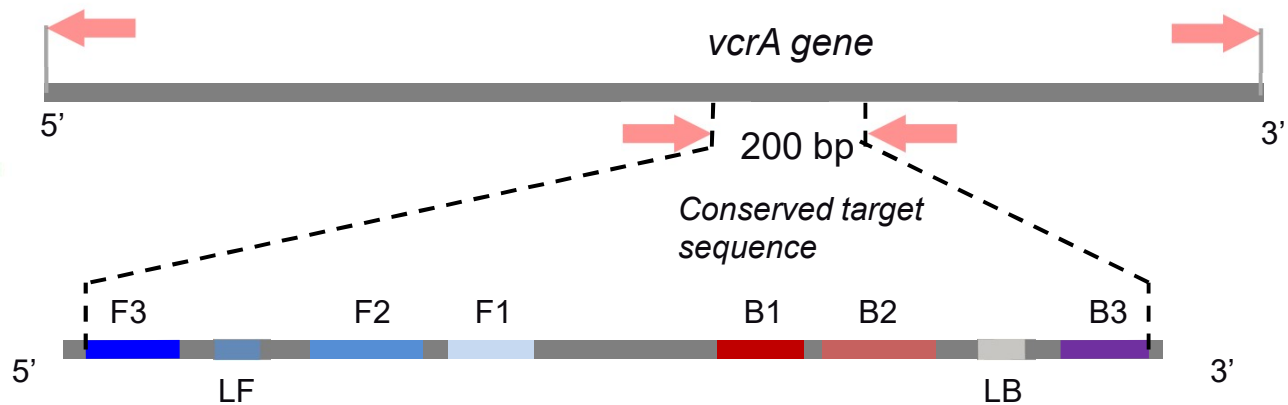
Design LAMP primer sets for each target gene (*bvcA, vcrA, tceA*)

Amplify DNA from mixed cultures using primer sets

Select primers w/ consistent threshold times (signal above background) at low dilutions

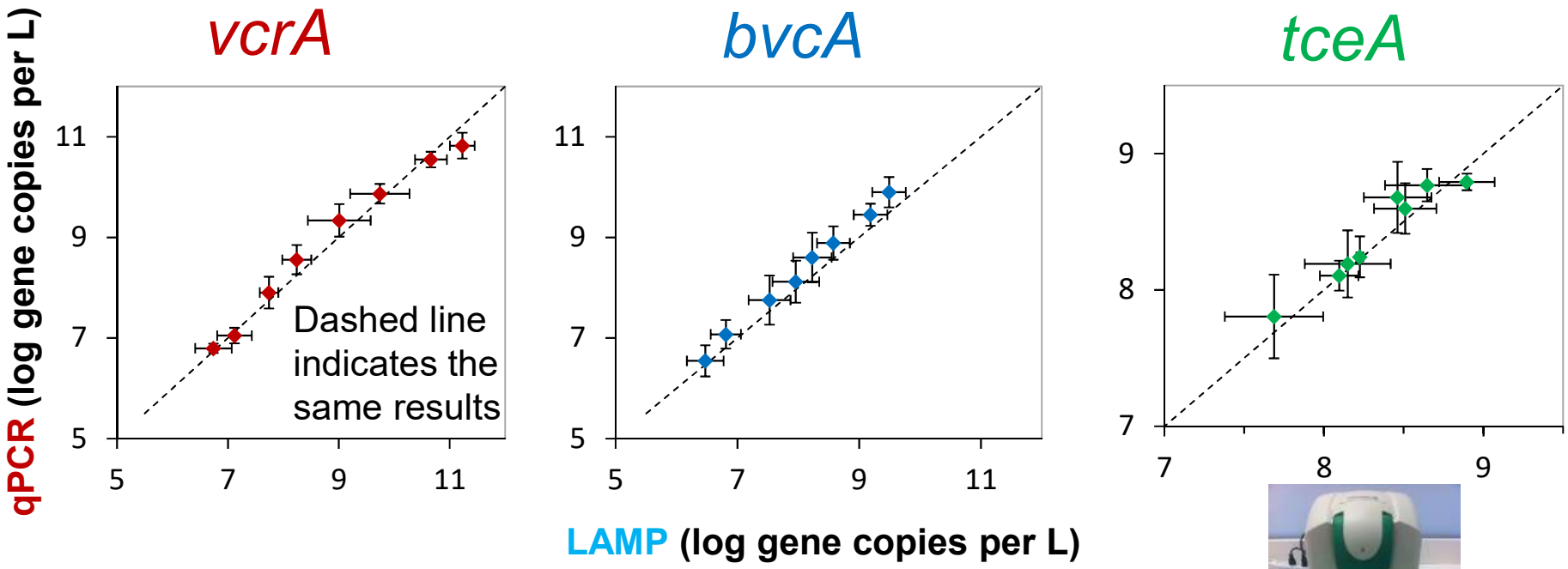
Primer sets were tested with bioaugmentation cultures, SDC-9 (APTIM) and KB-1 (SiREM), containing *Dehalococcoides mccartyi*

LAMP primer designing software
PrimerExplorer
https://primerexplorer.jp/e/



LAMP Primer Validation

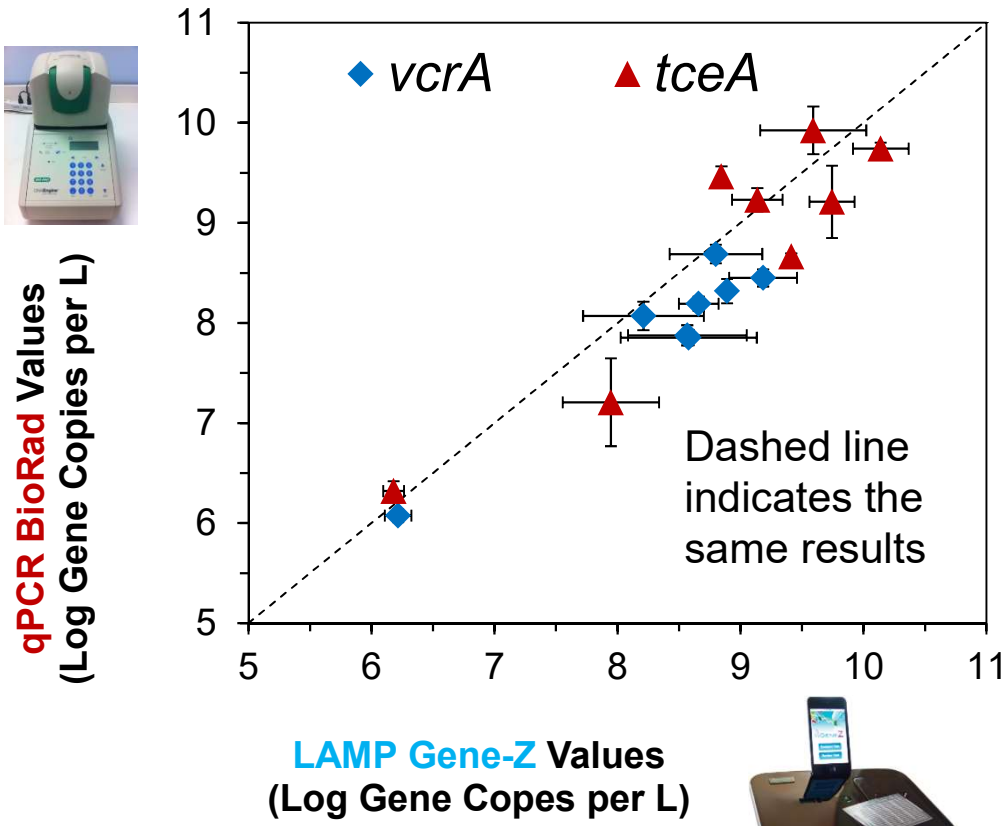
Comparison of LAMP and qPCR using DNA extracted from KB-1 (detection with BioRad)



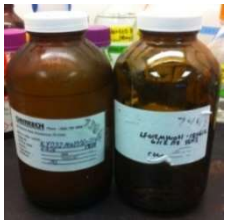
Similar results for both methods

Validation in the Gene-Z

Comparison of LAMP (Gene-Z) and qPCR (BioRad) using DNA extracted from groundwater



Tulsa, OK (SDC-9 bioaugmented)



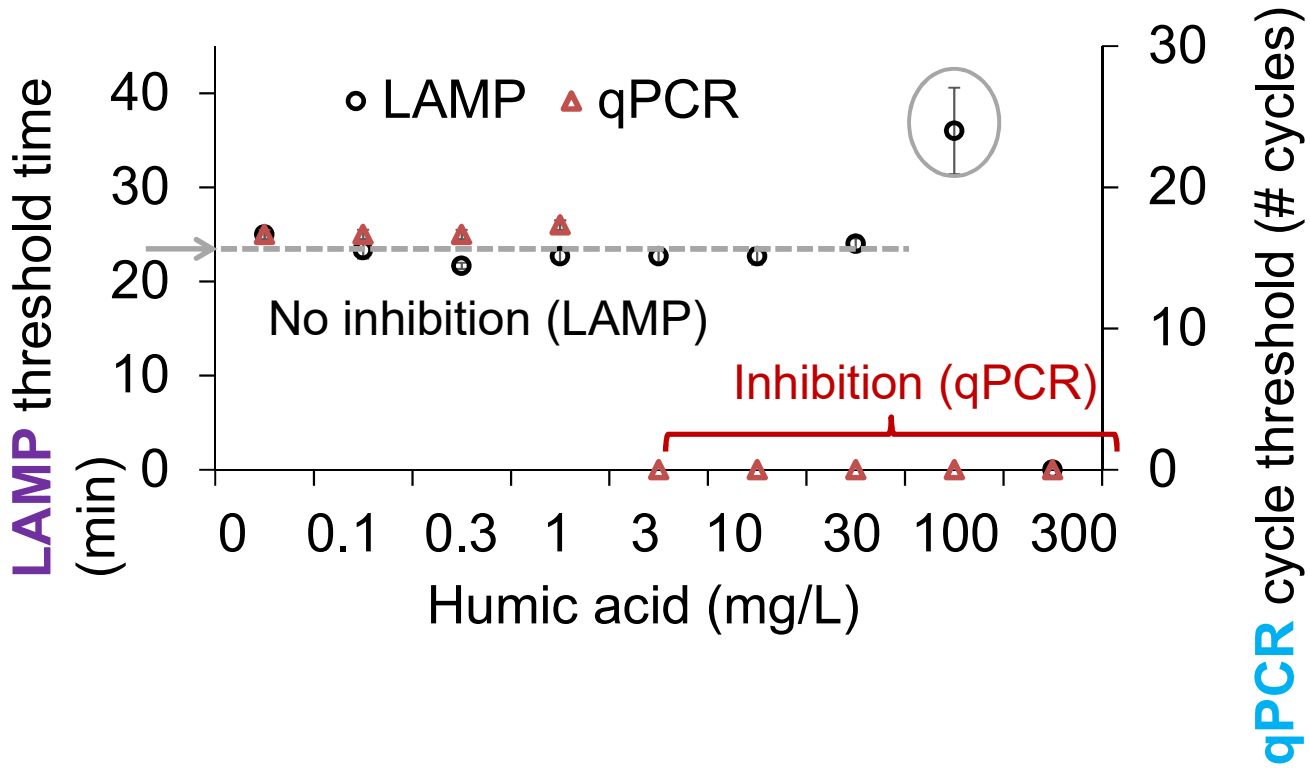
In general, the methods produced similar results

(for some samples, LAMP/Gene-Z produced higher values)

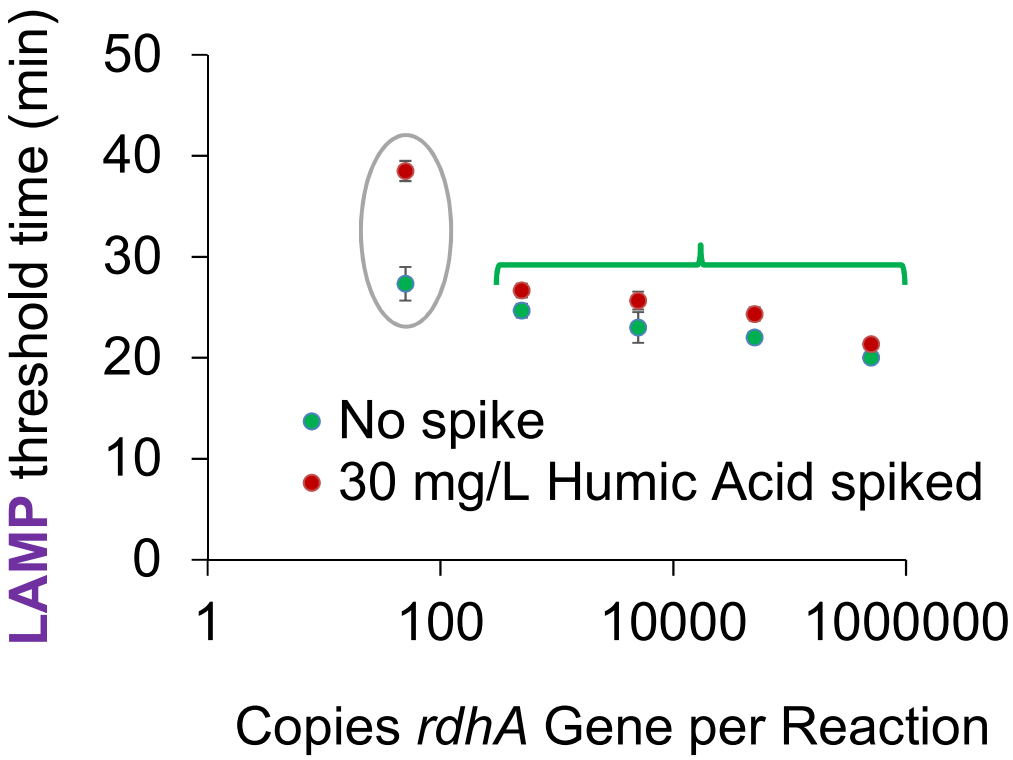
Effect of Humic Acid on Amplification

Reactions were spiked with known concentrations of *rdhA* genes (TCA-20, APTIM)

qPCR was inhibited at higher levels of humic acid

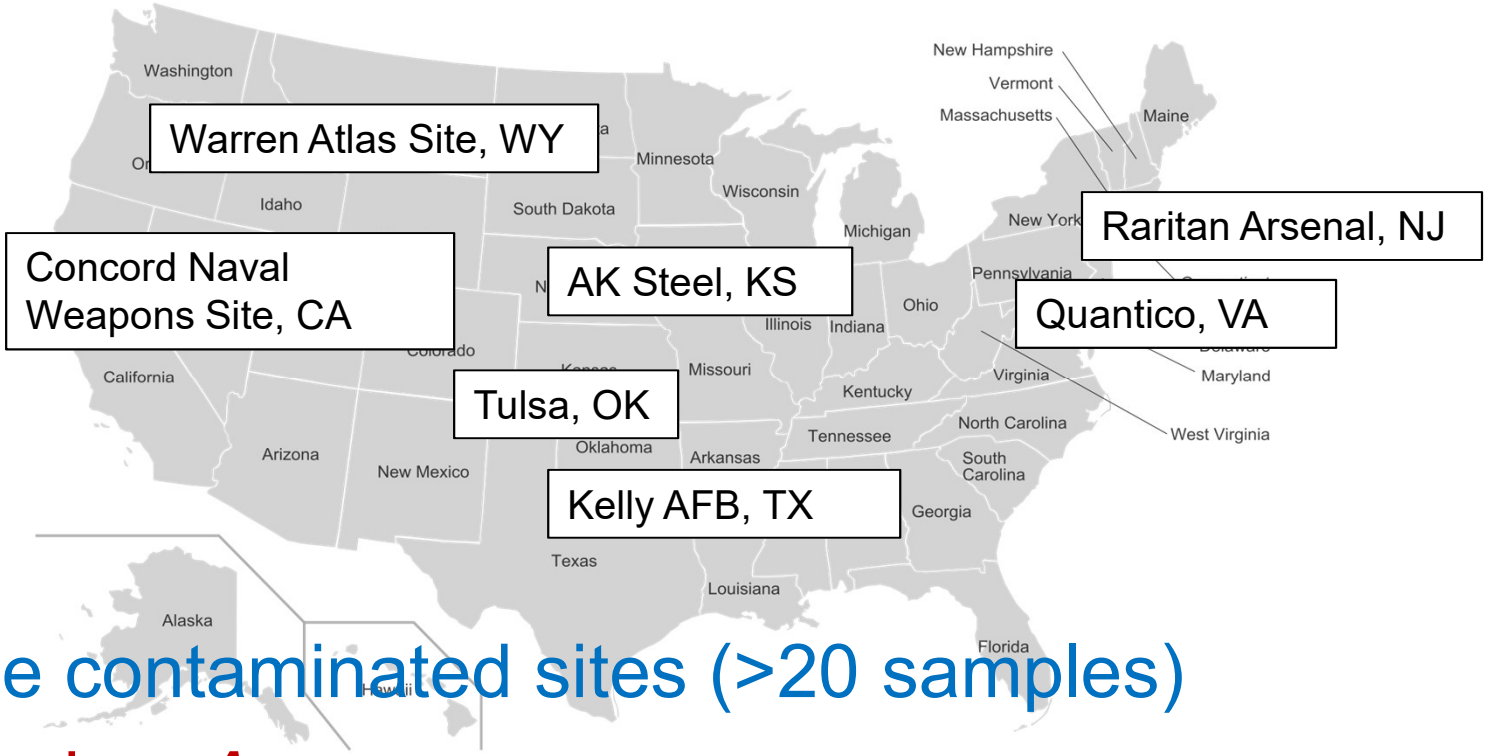


Effect of High Humic Acid Concentration on Gene Quantification with LAMP



LAMP performed the same with or without 30 mg/L of humic acid except at a low gene concentration

Compare qPCR and LAMP Using Contaminated Site Groundwater



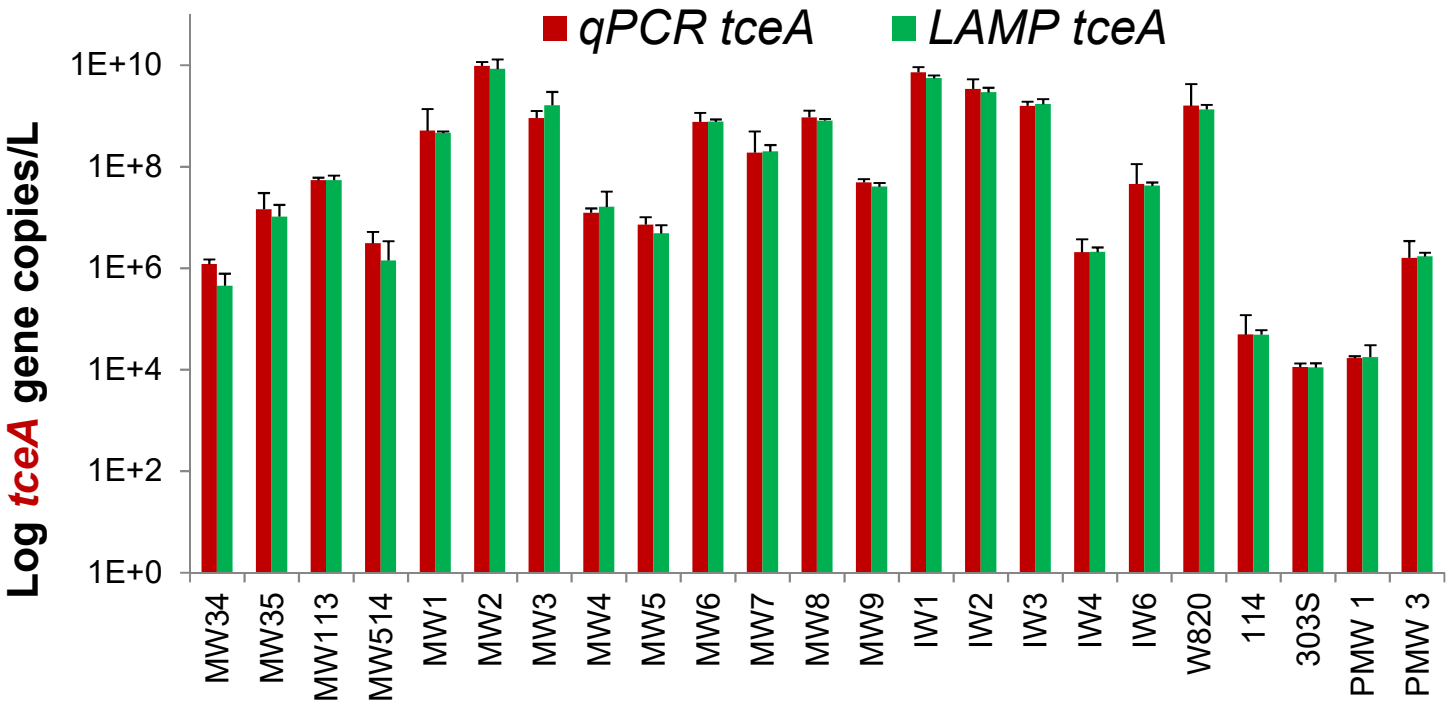
Multiple contaminated sites (>20 samples)

tceA and *vcrA*

DNA extracted from triplicate groundwater samples

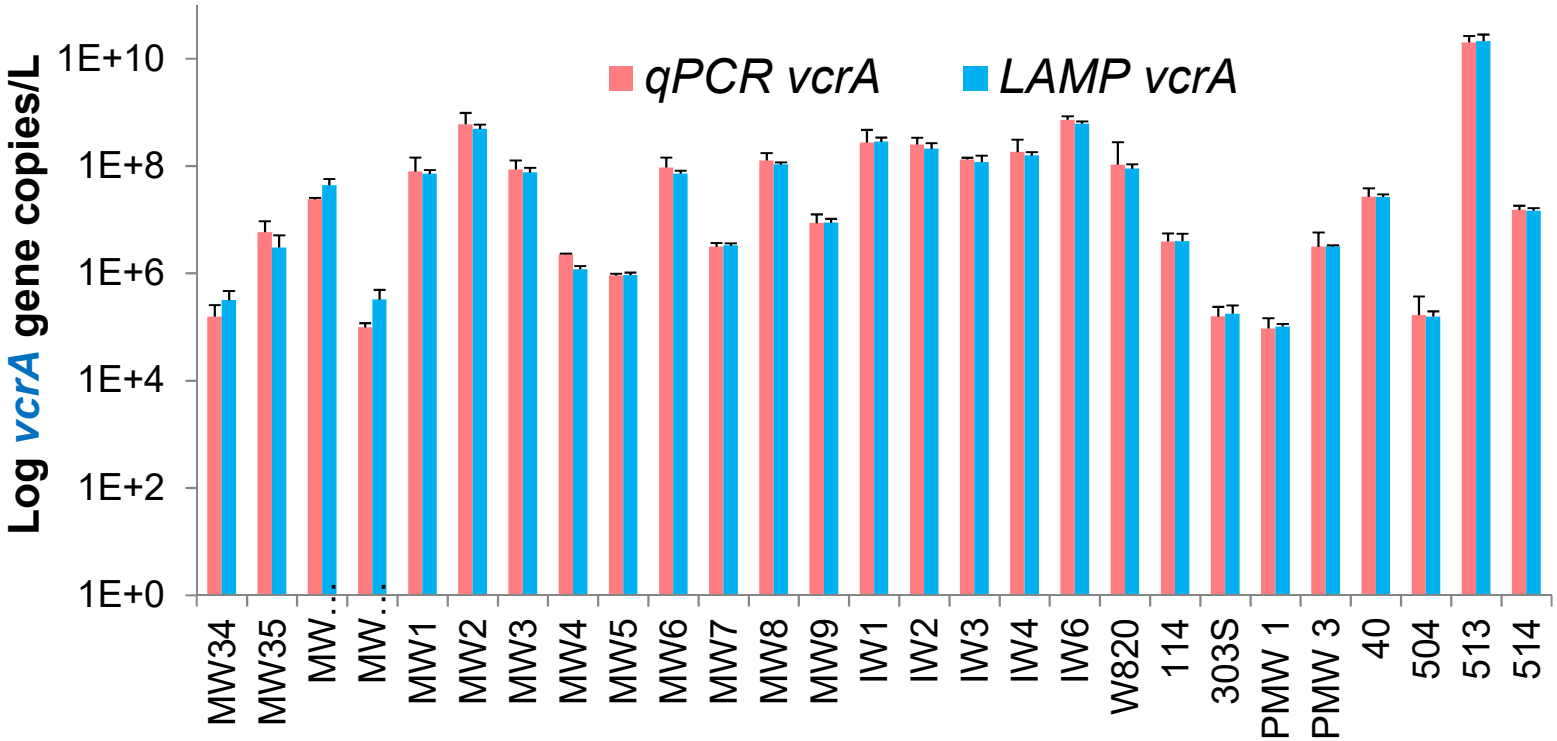
Source: By Gigillo83, original of 70.29.208.129 - w:en:Wikipedia talk: Images for upload/svg/USA-states-blank-XMLcomments-SVGnameIDs-CSSfillCLASSes.svg, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=11099373

LAMP and qPCR to Quantify *tceA* from DNA Extracted from Groundwater



qPCR and LAMP produced similar results for *tceA* ⇨ Different groundwater samples and over a range of concentrations ⇨ Good replication between triplicate samples

LAMP and qPCR to Quantify *vcrA* from DNA Extracted from Groundwater

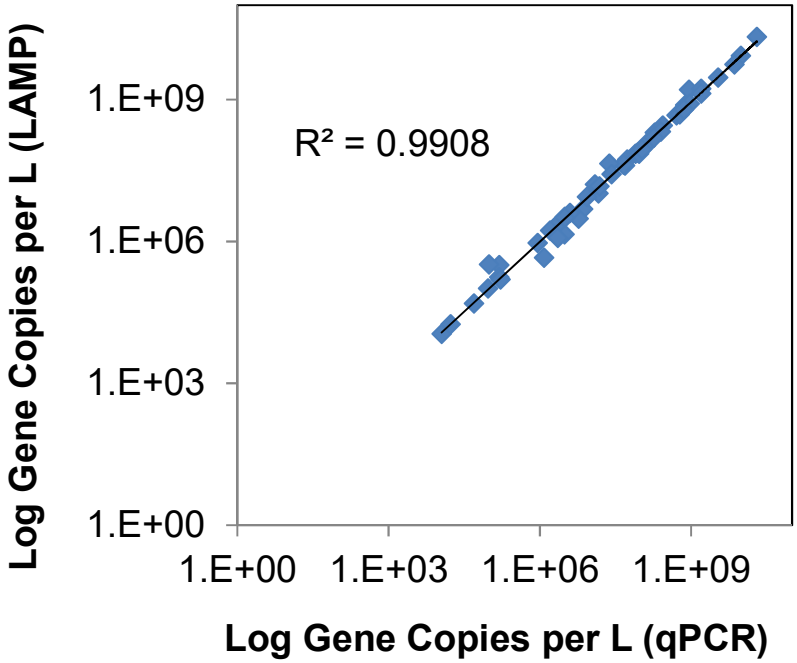


qPCR and LAMP ⇨ produced similar results for *vcrA*

Different groundwater samples and over a range of concentrations ⇨ Good replication between triplicate samples

Summary – DNA from Groundwater qPCR Compared to LAMP

LAMP vs. qPCR (*tceA* and *vcrA*)



Logarithmic Scale

qPCR and LAMP produced similar results for DNA extracted from different sites

LAMP Assay With Visual Detection

Applications for LAMP Assays?

- No DNA extraction (reduces cost and time)
- Coupled to low cost detection methods
- Coupled to potentially field deployable methods

Development of Visual Based LAMP Assay

- Requiring a water-bath and benchtop centrifuge
- Based on fluorescence of ds DNA with SYBR Green

Approach for LAMP Assays with Visual Detection

Groundwater pre-filtered (5 µm), then passed through sterivex filter

Elution buffer (1000 µL), vortex, remove buffer/cells

Centrifuge, then resuspend the pellet

Add 5 µL of sample to LAMP master mix

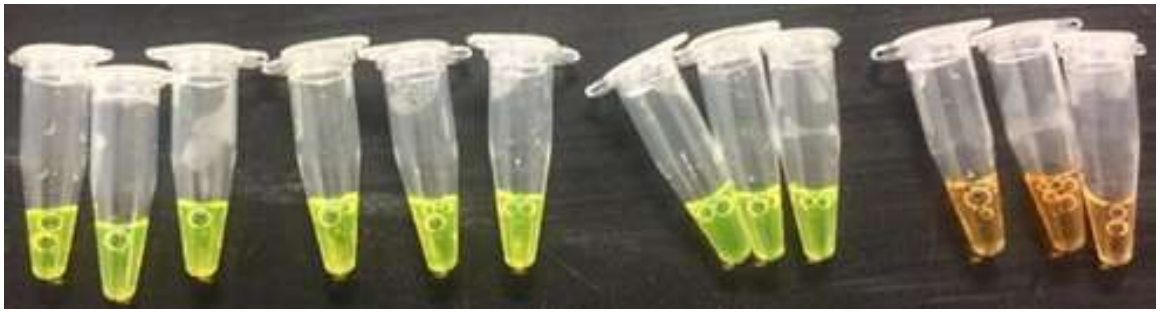
Move vials to waterbath (1 hr, 63°C)

Add SYBR Green

Sample Control

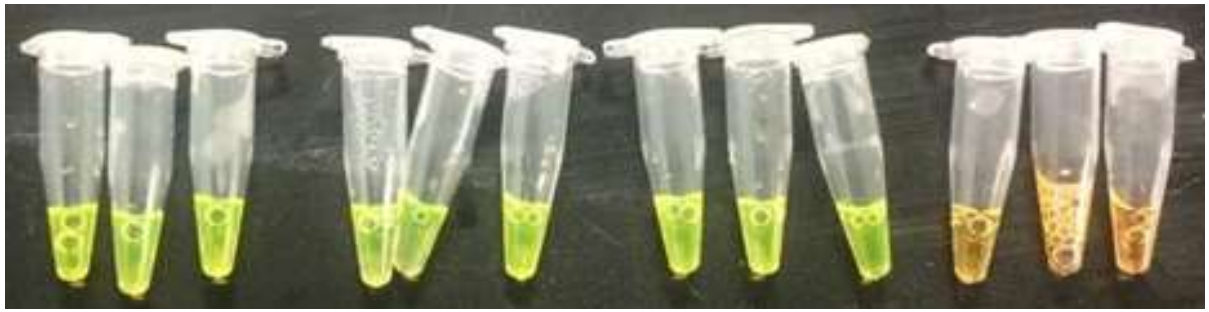
LAMP Assays with Visual Detection

Results with *vcrA* LAMP assay

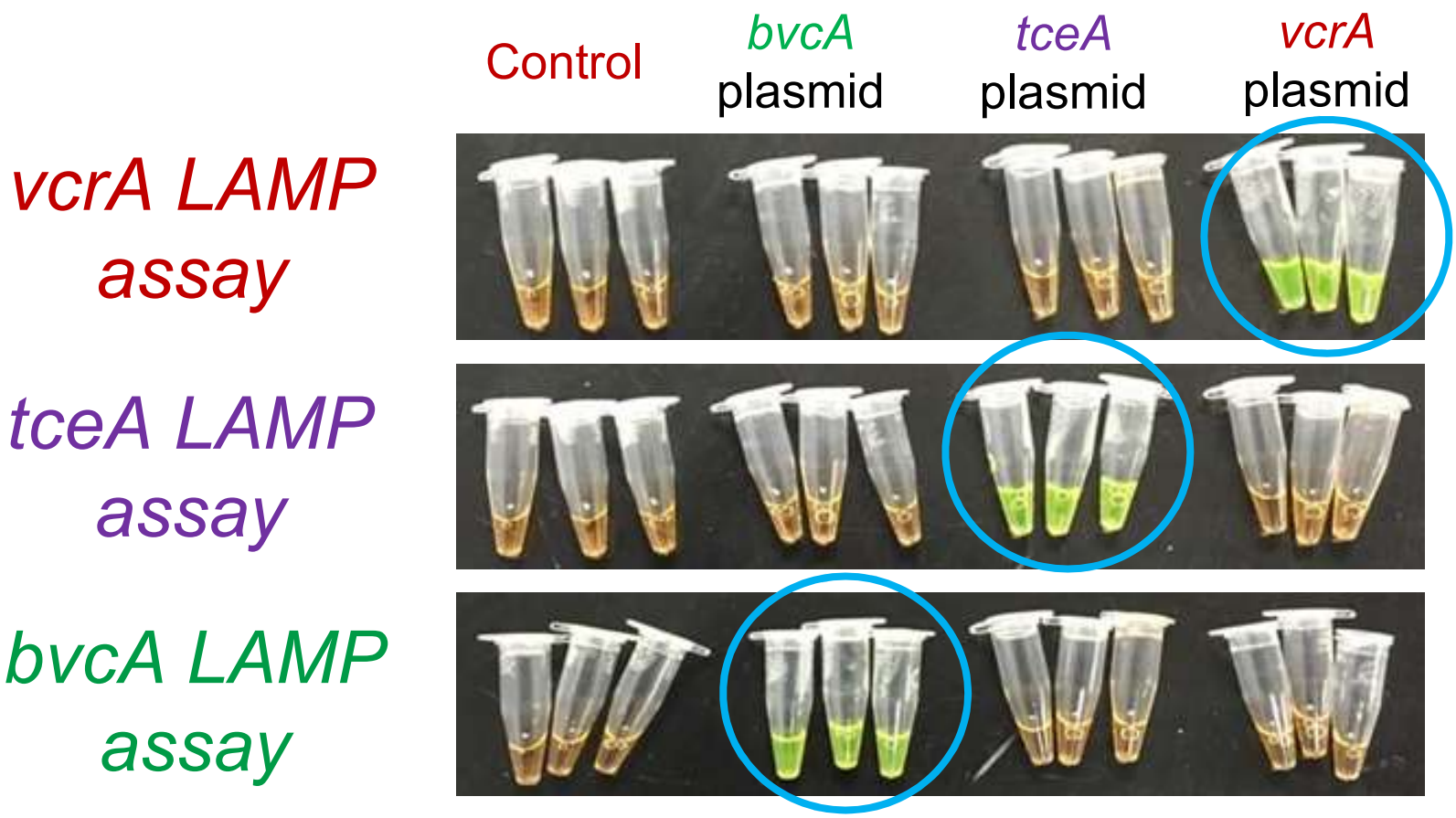


DNA Extracts | Eluted Cells | Centrifuged Cells | Control (DI water)

Results with *tceA* LAMP assay



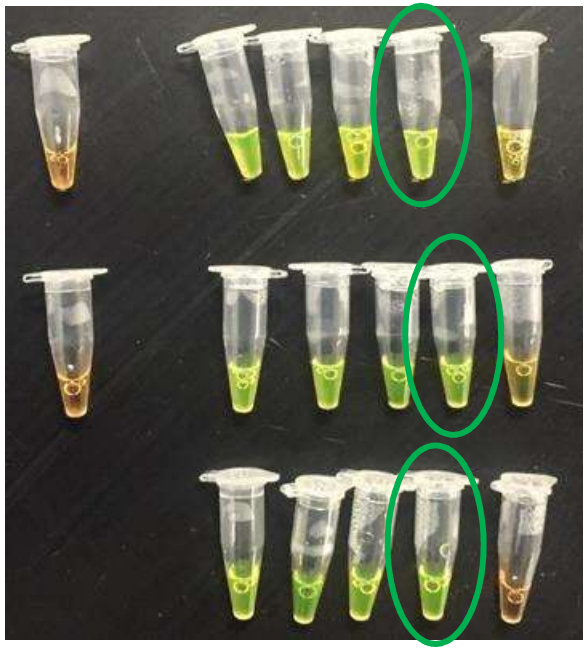
Specificity of LAMP Assays with Visual Detection



Detection Limits of LAMP Assays with Visual Detection

10^5, 10^4, 10^3, 10^2, 10
vcrA copies per reaction

Controls



Triplicates

Dilution series 1

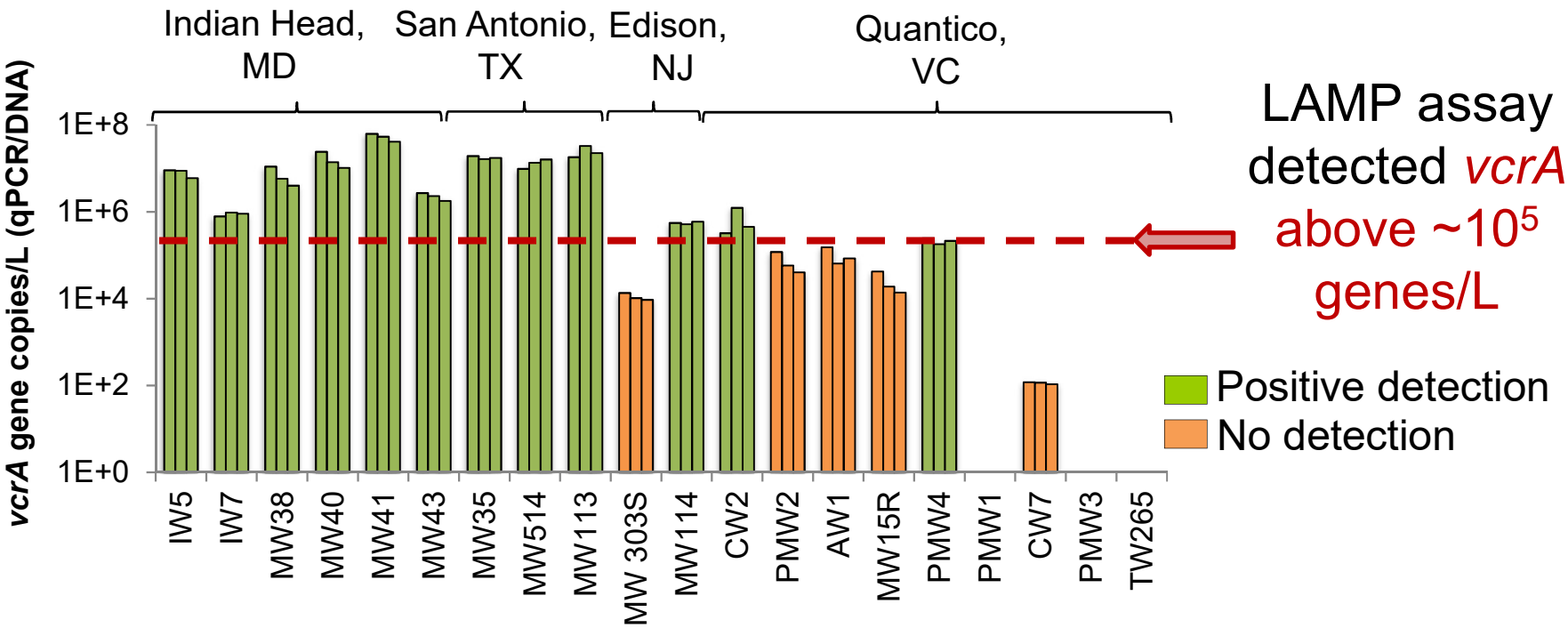
Dilution series 2

Dilution series 3

Visual detection to 10^2 *vcrA* gene copies per reaction

Similar results for *tceA* and *bvcA*

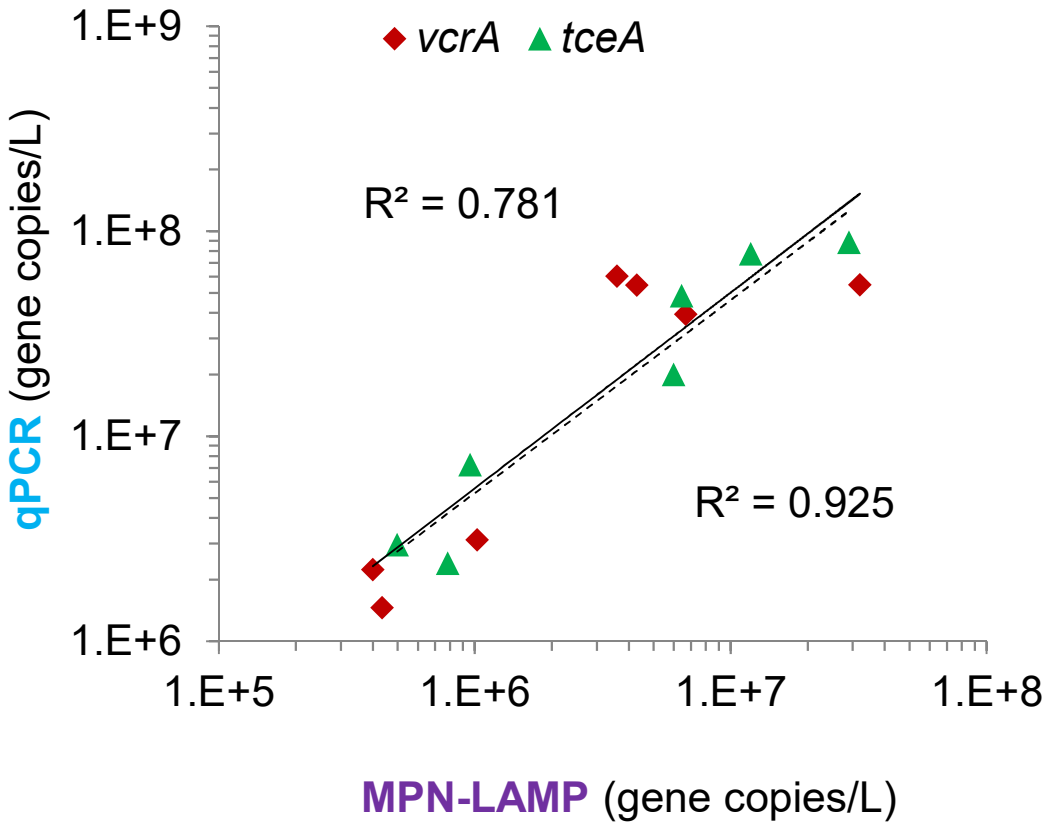
LAMP Assay with Visual Detection on Groundwater – No DNA Extraction



If all three replicates remain orange

Bioaugmentation or biostimulation may be necessary

Most Probable Number and LAMP Assays for Quantification



Comparing MPN LAMP to qPCR using groundwater

Positive correlation between qPCR and LAMP for both *vcrA* and *tceA*

Shotgun vs. 16S rRNA Amplicon Sequencing

16S rRNA Amplicon Sequencing ⇨

- Commonly used method
- Taxonomic analysis, targets a region of 16S rRNA gene
- Who is there? Is *Dehalococcoides mccartyi* present?

Shotgun Sequencing ⇨

- Less commonly used
- Whole genome sequencing, sequences random DNA strands
- Which functional genes are present? *vcrA*? *etnC*?
- Potentially limitless # target genes

Shotgun Sequencing for Gene Analysis in Groundwater Samples

- Groundwater samples from sites bioaugmented with SDC-9

Illumina HiSeq 4000 → ~4-5 million sequences per sample

1. Taxonomic analysis using MG-RAST

https://www.mg-rast.org/

http://fungene.cme.msu.edu/

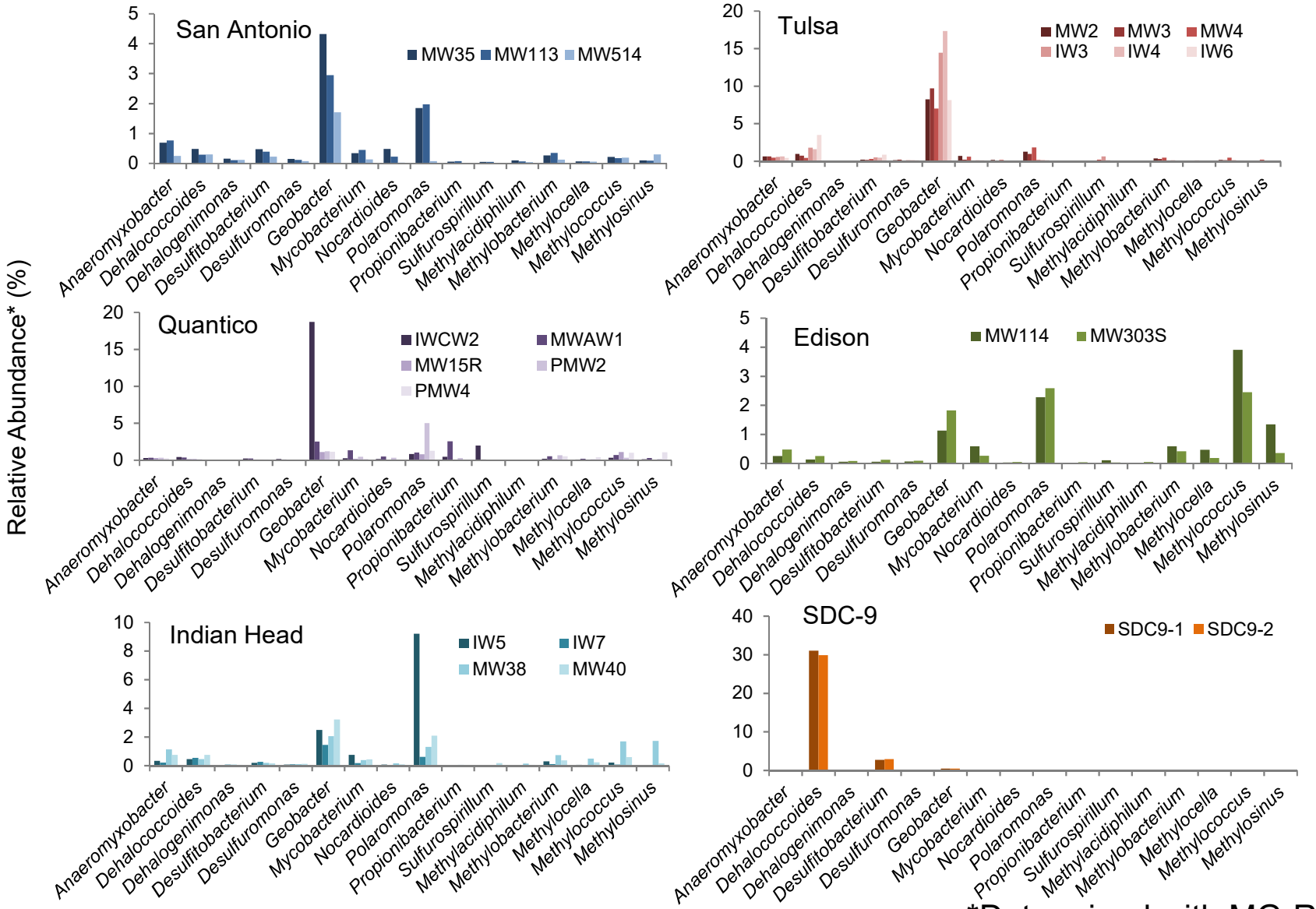
https://www.ncbi.nlm.nih.gov/

2. Functional analysis using FunGene and NCBI

Alignment of functional sequences using DIAMOND

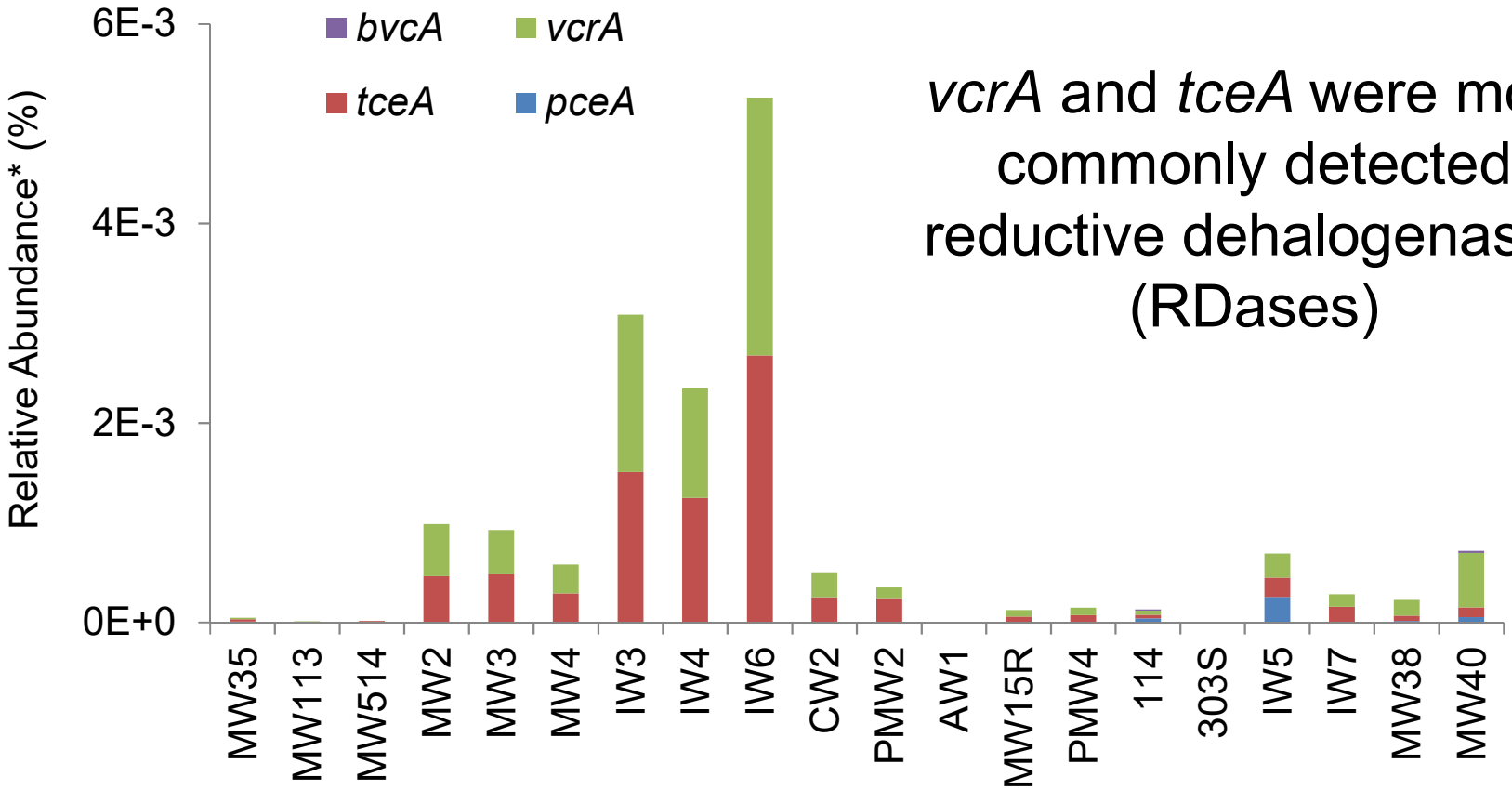
MG-RAST: Meta Genome Rapid Annotation using Subsystem Technology; **FunGene**: Functional Gene Pipeline, RDP, MSU; **NCBI**: National Center for Biotechnology Information; **DIAMOND**: Double Index Alignment of Next-Generation Sequencing Data

Genera Associated w/ Chlorinated Solvent Degradation



*Determined with MG-RAST

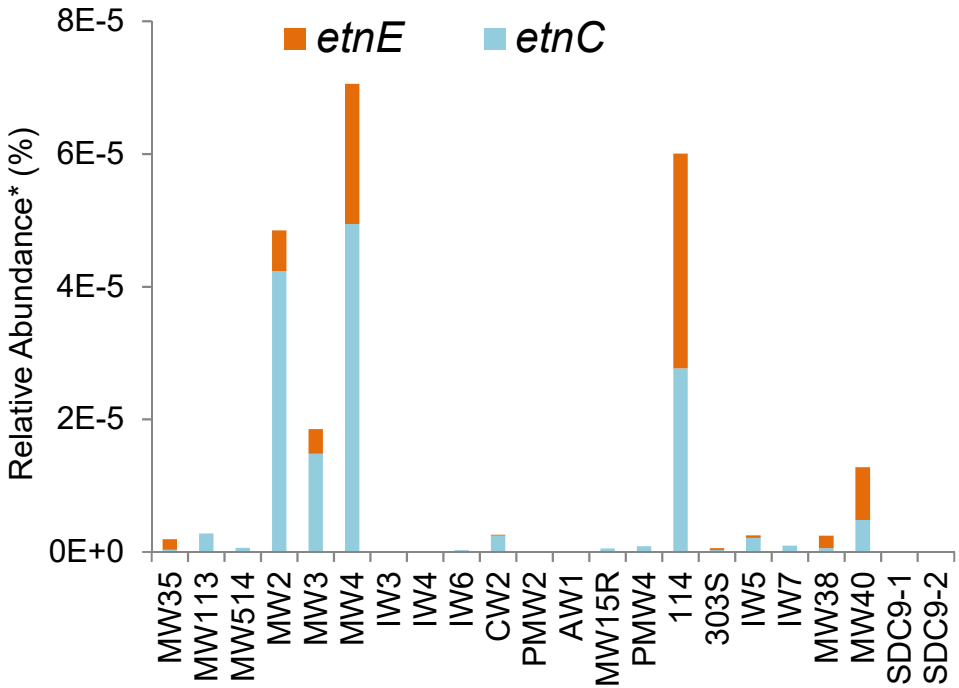
Anaerobic Functional Genes Associated with Chlorinated Solvent Degradation

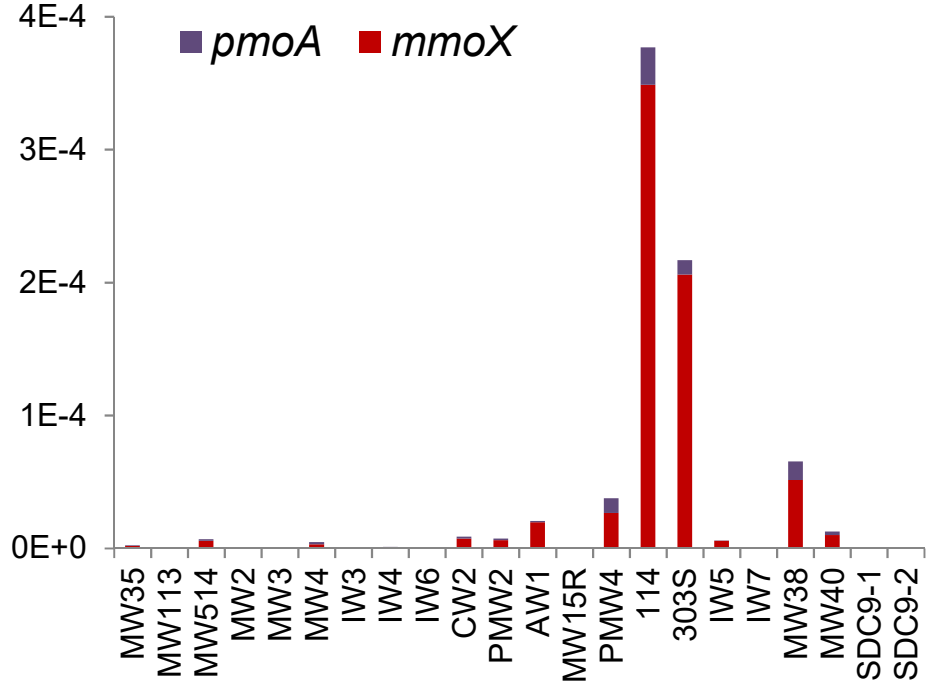


vcrA and *tceA* were most commonly detected reductive dehalogenases (RDases)

*Determined with DIAMOND

Aerobic Functional Genes Associated with Chlorinated Solvent Degradation

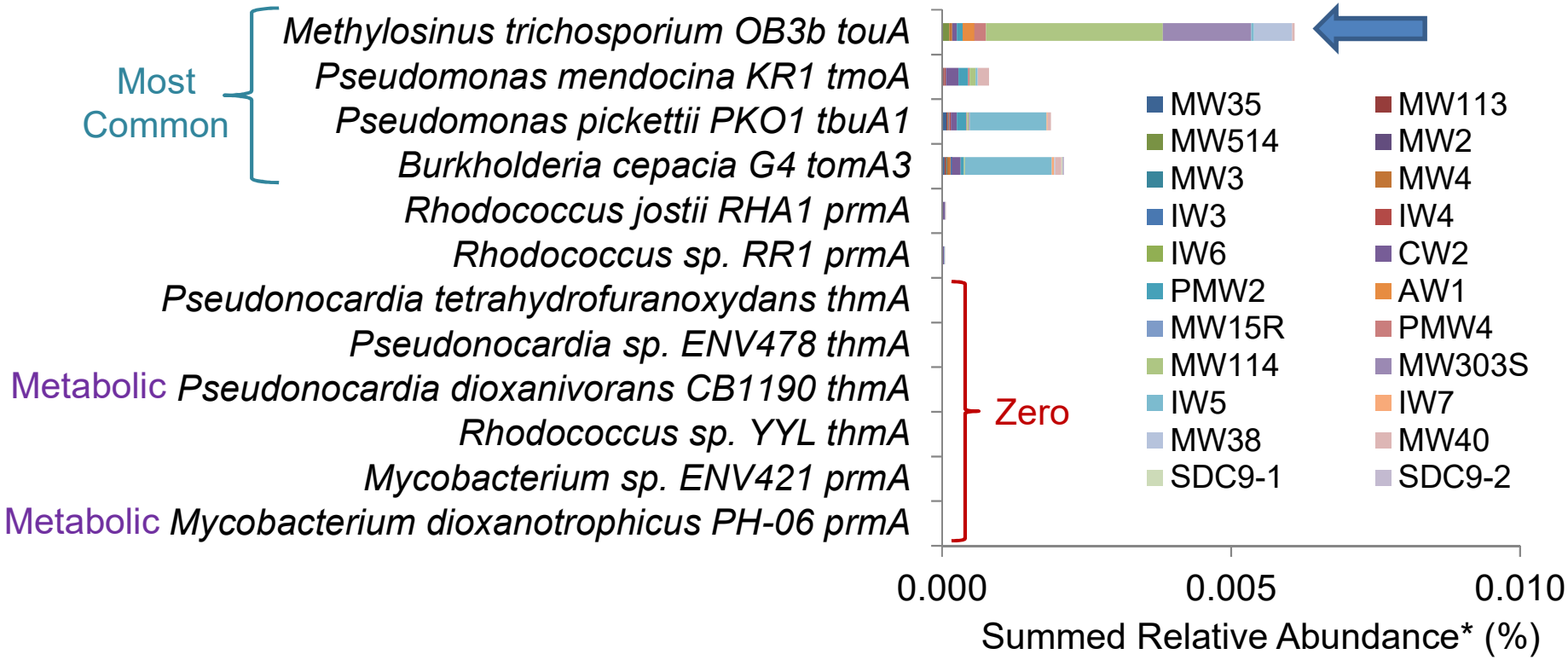




- These aerobic genes were detected in at least one sample from each site
- Aerobic and anaerobic genes were found together
- Highest values for *etnC* and *etnE* were 2 orders of magnitude < *vcrA* or *tceA*

**Determined with DIAMOND*

1,4-Dioxane Degrading Functional Genes



Methylosinus trichosporium OB3b mmoX was the most abundant

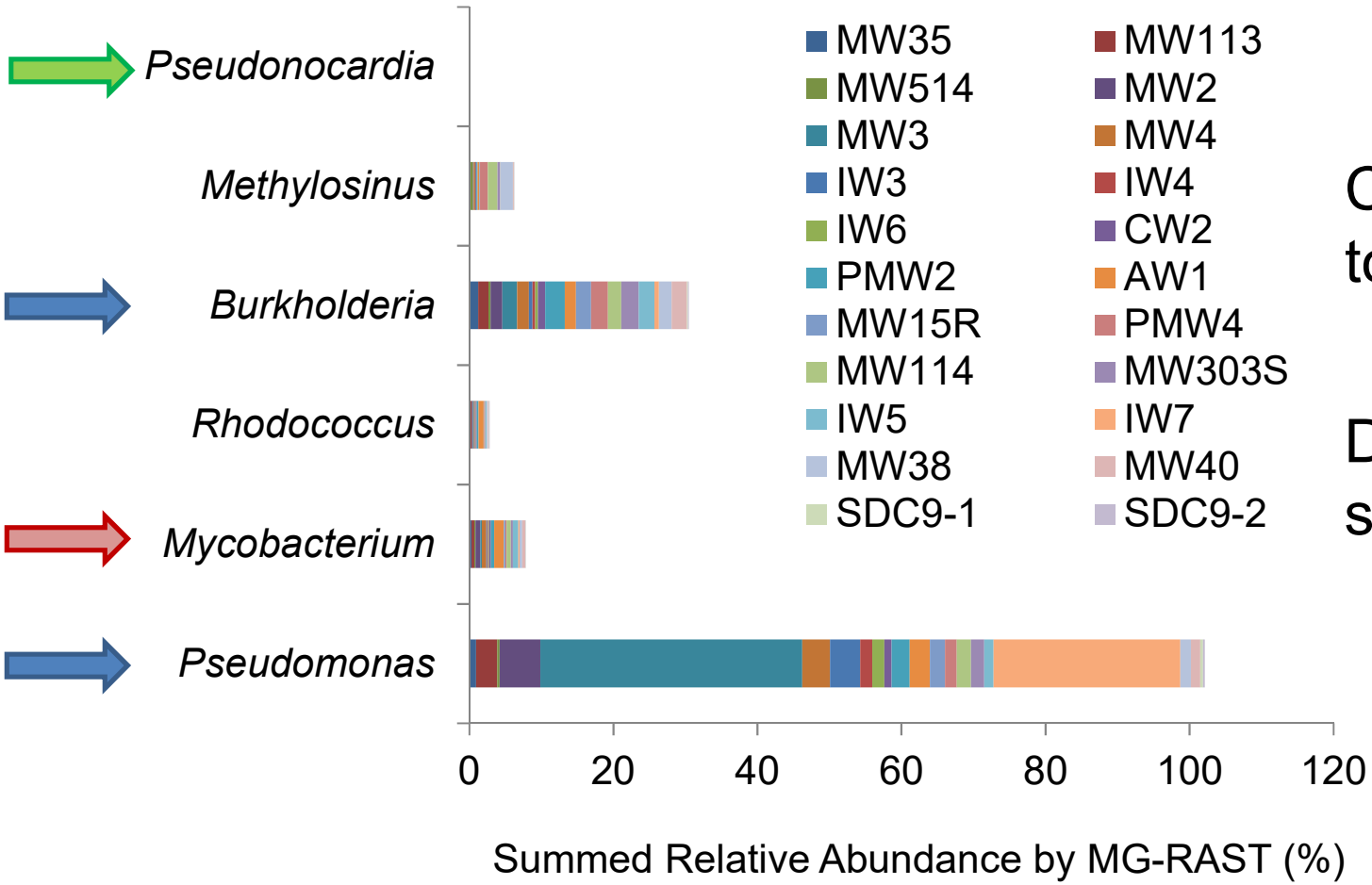
Genes from *Methylosinus, Pseudomonas and Burkholderia* ⇨ most common

Genes from *Pseudonocardia* or *Mycobacterium* ⇨ not detected

He et al, 2017, EST Letters, 4, 494

**Determined with DIAMOND*

1,4-Dioxane Degrading Genera in Groundwater



Classification to genus level

Degrading species/strains?

Shotgun Sequencing Key Points

- Reads aligning with both aerobic and anaerobic biomarkers were found across all sites
- In some wells, RDases and 1,4-dioxane degrading genes were both present
  - Effective environment for co-contaminant degradation?
- Shotgun sequencing offers a greater depth of data compared to current methods … however …
  - How do these values compare with qPCR?
  - What is the cost and availability?

Overall Conclusions

- LAMP assays provided similar results to qPCR
- LAMP assays were less sensitive to inhibition by humic acid
- The visual-based LAMP assay (without DNA extraction) is a low-cost and potentially field deployable approach
- Shotgun sequencing provides relative abundance data for a limitless number of functional genes

Benefits to DoD

- Natural attenuation, biostimulation or bioaugmentation often more cost effective
- Determine trends across sites and over time
- LAMP assays offer a potentially field deployable approach for monitoring bioremediation at DoD sites
- Shotgun sequencing for DoD sites that have multiple contaminants

Project Team

- Syed Hashsham, Robert Stedtfeld, Yogendra Kanitkar, Hongyu Dang, Michigan State University
- Dr. Paul Hatzinger, APTIM

Acknowledgments

- Phil Dennis (SiREM) supplied the KB-1 culture
- Frank Löffler (UTK) provided the tceA plasmid
- Simon Vainberg, Sheryl Streger, Robert E. Mayer, Michael Martinez and David Lippincott (APTIM)
- Yanlyang Pan,Tiffany Stedtfeld, James Cole and Benli Chai (MSU)

For additional information, please visit

https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-2309

Speaker Contact Information

cupplesa@msu.edu; 517-432-3370

SERDP DOD • EPA • DOE | ESTCP

Q&A Session 1

SERDP DOD • EPA • DOE | ESTCP

SERDP & ESTCP Webinar Series

Validation of Advanced Molecular Biological Tools to Monitor Chlorinated Solvent Bioremediation and Estimate cVOC Degradation Rates

Mandy Michalsen, Ph.D.
Engineer Research Development Center

Kate Kucharzyk, Ph.D.
Battelle Memorial Institute

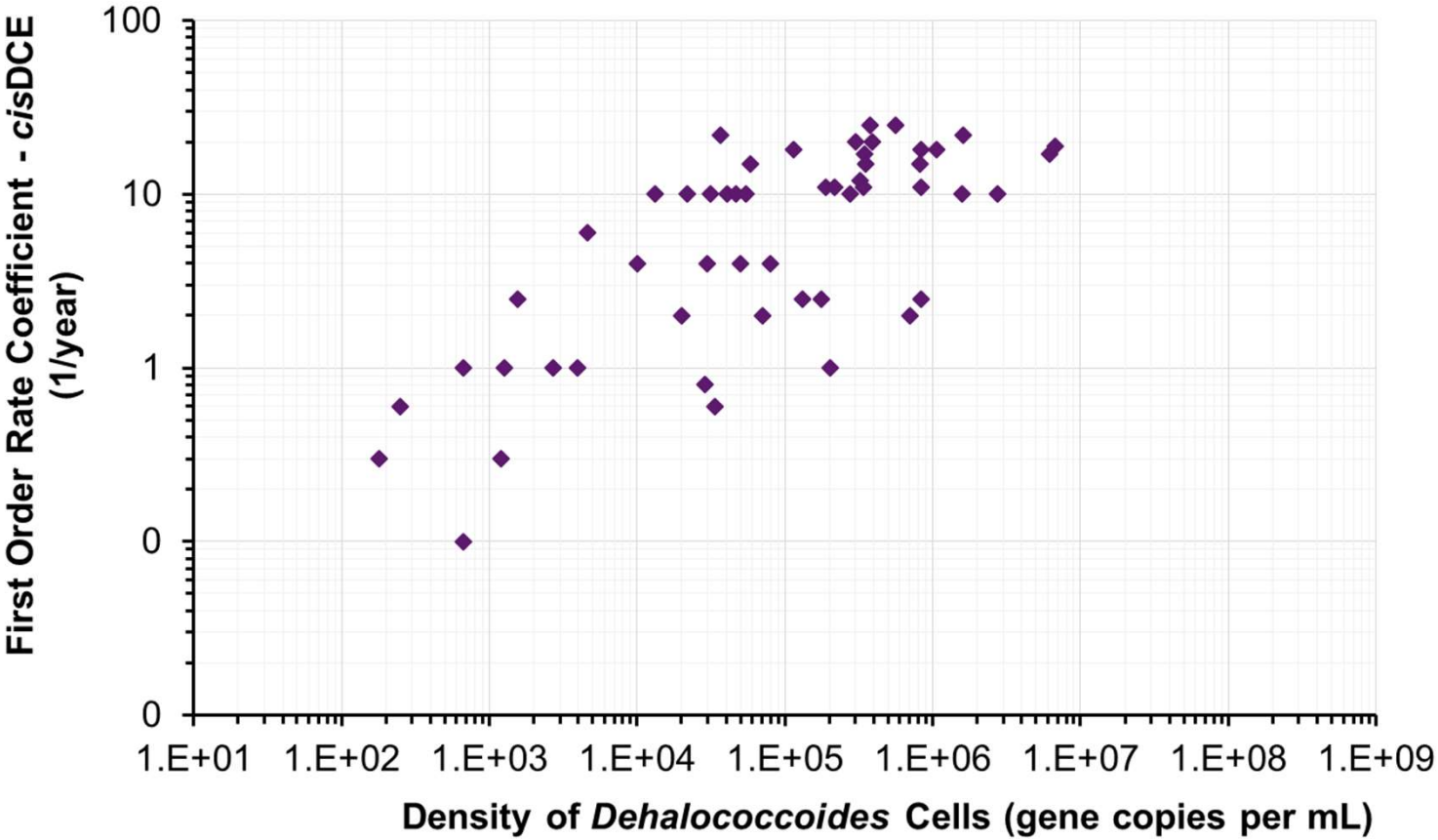
SERDP DOD • EPA • DOE | ESTCP

Technical Objectives

- **Quantify** reductive dechlorination (RD) **rates** of *cis*-DCE and VC and RDase biomarker abundances (target RDase genes and proteins) in aquifer microcosms
- **Quantify** RD rates and RDase biomarker abundances **correlation** in the aquifer materials
- **Develop** a RD **rate-predictive model**, whereby RDase biomarker abundance inputs can be used to predict RD rates – then test the predictive model using experimental results
- Empower end users to utilize quantitative proteomics for MNA and in situ bioremediation at cVOC-contaminated sites

DISTRIBUTION A. Approved for public release: distribution unlimited

k_{cisDCE} vs. *Dehalococcoides* Cell Abundance: Can We Do Better?



◆ Data from BioPIC available online: https://www.serdp-estcp.org/content/download/37095/354098/file/ER-201129-FR.pdf

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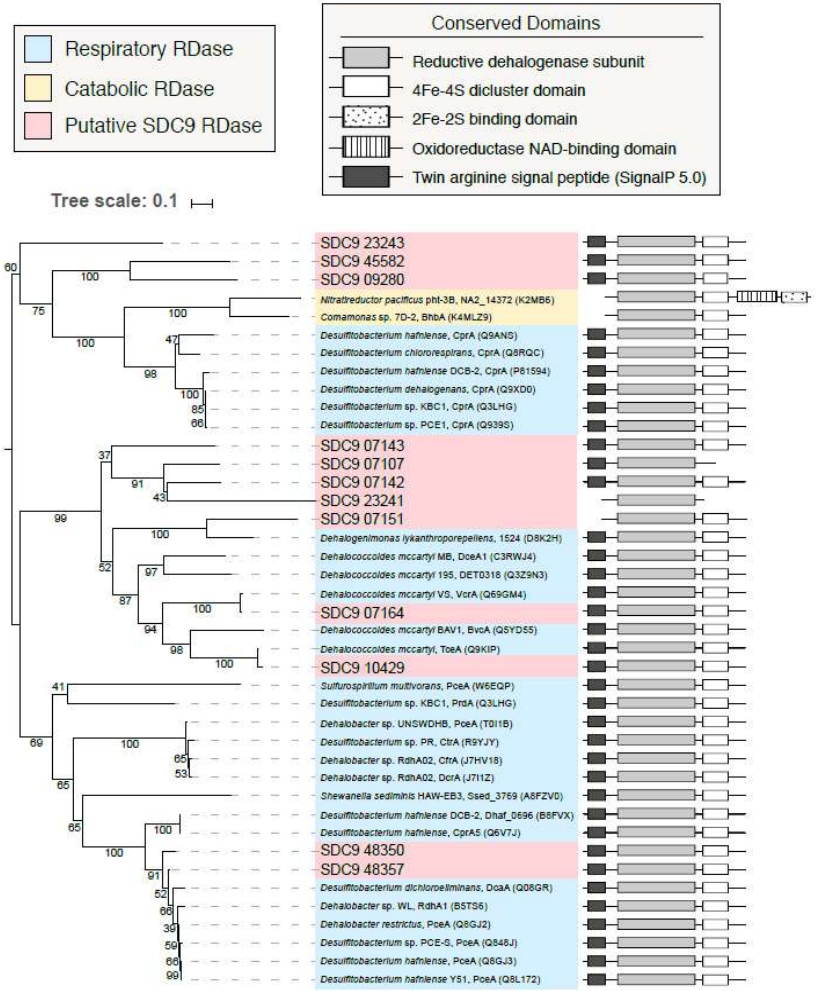
SDC-9 Metagenome Sequencing



- RDase genes identified and RDase peptides annotated
- Correct* RDase peptides used to identify peptide targets in SDC-9 proteomics
- Approach avoids false negative peptide detection results

* *Metagenomic guided proteomics reduces probability of misalignment of peptides or using incorrectly annotated sequences from public databases*

14 unique RDase genes identified

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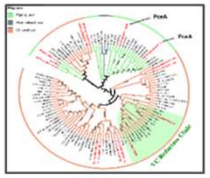
Targeted Proteomics

Establish MDL and IDL for Selected Peptides

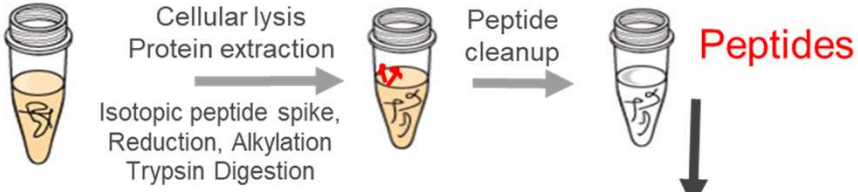
RDase Identification and Down selection



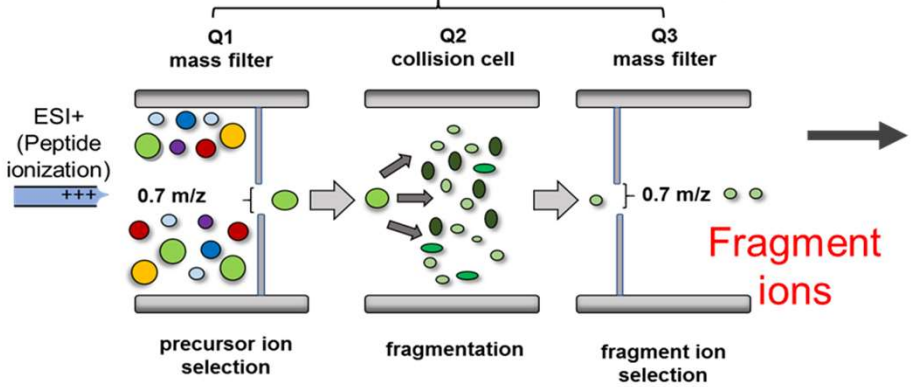
- SDC-9 metagenome sequencing
- Identified a total of 14 RDase gene sequences
- Identified at least 39 unique RDase peptides from the SDC-9 protein digests

Proteins

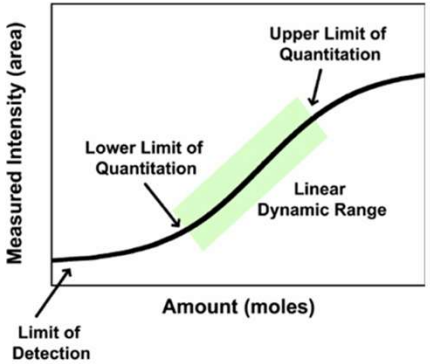
Protein Extraction



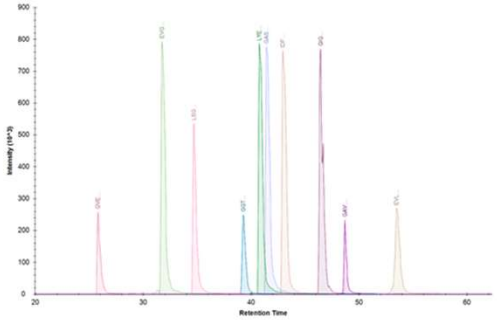
Triple Quadrupole Mass Spectrometry



Establish IDL and MDL for Selected Peptide Targets



Bioinformatic Analysis



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Detection vs. Quantification

IDL and MDL Replicate Experiment Results

Protein	ID	Peptide[1]	MDL 1	MDL2	MDL3	Established MDL
			fmol/mL			
FdhA	FdhA2	SGSEIAFTGGLI**K**	3	3	3	**3**
	FdhA5	ALGIVYLDSQA**R**	3	3	1	**3**
	FdhA8	NQAVSAPGEA**K**	3	3	3	**3**
PceA	PceA4	IATQIPLLQDAA**R**	9	9	9	**9**
	PceA5	LESGYVQNMV**K**	3	3	3	**3**
	PceA7	DFWNNPEPI**K**	1	1	1	**1**
	PceA8	TSPSLISSATVG**K**	0.3	0.3	1	**1**
TceA	TceA2	DVDDLLSAG**K**	0.3	3	3	**3**
	TceA3	VSSIIEP**R**	0.3	0.3	1	**1**
	TceA4	VNNEPWWVTT**R**	9	9	9	**9**
	TceA5	YFGASSVGAI**K**	0.3	0.3	1	**1**
VcrA	VcrA1	WGLYGPPHDSAPPDGSVP**K**	9	9	3	**9**
	VcrA2	YFGAGDVGALNLADP**K**	27	27	27	**27**
	VcrA3	VPDHAVPINF**K**	0.3	0.3	1	**1**
	VcrA4	GVYEGPPDAPFTSWGN**R**	83	27	27	**83**
	VcrA6	DQPWYV**K**	1	1	1	**1**

[1] Bolded letters denote heavy ^{13}C and ^{15}N labeled amino acid;
the maximum of three MDL test replicates was established as the MDL

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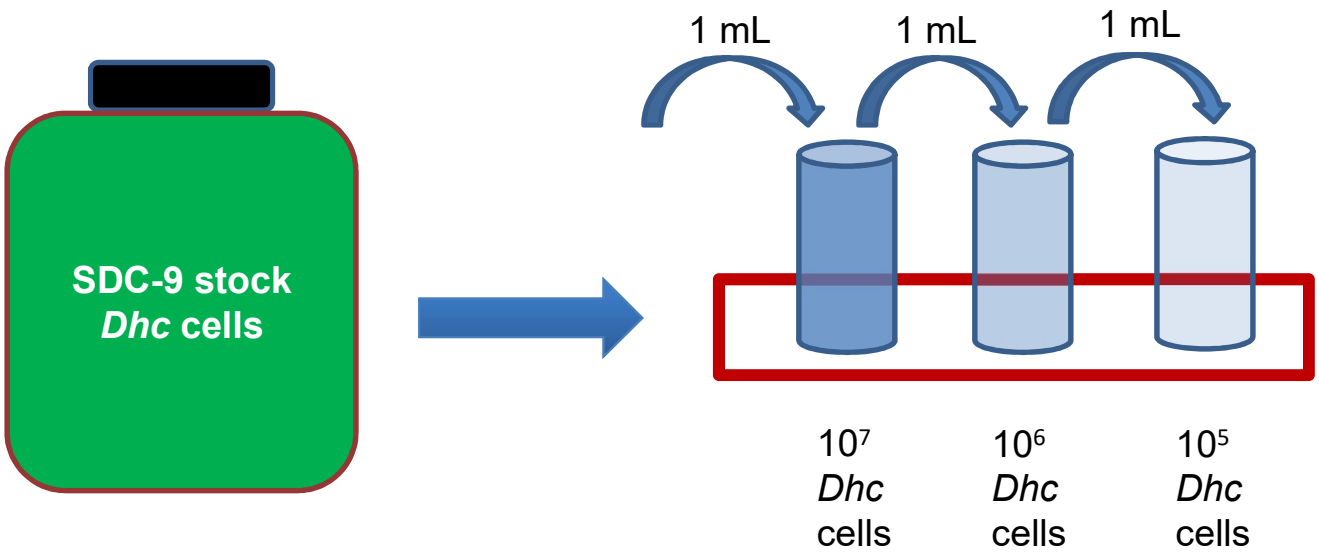
How Low Can We Go?

Prepare Dehalococcoides titers using SDC-9 and validate quantitative qProt assay!



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qProt Assay Validation - Results

Protein	Peptide ID	*Dhc* abundance in sample, *Dhc* cells/mL[b]						Previously reported[d-e] approx. protein concentrations in culture, fmol/mL (protein/cell)[c]
		$1.3x10^5$	$2.2x10^6$	$3.1x10^7$	$1.3x10^5$	$2.2x10^6$	$3.1x10^7$	
		Peptide concentration in culture, fmol/mL			Protein concentration in culture, fmol/mL (protein/cell)[c]			
FdhA	**FdhA2[a]**	$<3.0x10^0$	$<3.0x10^0$	$8.5x10^0$	$<3.0 x10^0$	$<3.0 x10^0$	$8.5x10^0$ ($3.8x10^3$)	[d]KB1, D2 culture(TCE): $9.0x10^1 – 1.0x10^2$ ($2.3x10^3 - 3.5x10^3$)
	FdhA5	$<3.0x10^0$	$<3.0x10^0$	$1.1x10^1$				
PceA	**PceA4[a]**	$<9.0x10^0$	$<9.0x10^0$	$2.1x10^1$	$6.3x10^2$	$1.9x10^2$	$4.4x10^1$	
	PceA5[a]	$6.3x10^2$	$1.9x10^2$	$6.8x10^1$				
	PceA7	$<1.0x10^0$	$2.2x10^1$	$1.9x10^1$				
	PceA8	$<1.0x10^0$	$1.7x10^1$	$1.2x10^1$				
TceA	**TceA2[a]**	$<3.0x10^0$	$1.9x10^1$	$1.3x10^1$	$<1.0x10^0$	$2.5x10^1$ ($1.1x10^4$)	$1.7x10^1$ ($7.7x10^3$)	([e]$1.1x10^3$)
	TceA3[a]	$<1.0x10^0$	$3.1x10^1$	$2.1x10^1$				
	TceA4	$<9.0x10^0$	$<9.0x10^0$	$2.3x10^1$				
	TceA5	$<1.0x10^0$	$2.4x10^1$	$1.7x10^1$				
VcrA	VcrA1	$<9.0x10^0$	$1.7x10^2$	$1.8x10^1$	$<1.0x10^0$	$5.7x10^1$ ($2.6x10^4$)	$9.3x10^0$ ($4.2x10^3$)	[d]Difficult to quantify due to low peptide sensitivity
	VcrA2	$<2.7x10^1$	$7.6x10^1$	$4.9x10^1$				
	VcrA3[a]	$<1.0x10^0$	$5.7x10^1$	$9.3x10^1$				
	VcrA4	$<8.3x10^1$	$<8.3x10^1$	$<8.3x10^1$				
	VcrA6	$<1.0x10^0$	$5.8x10^1$	$1.1x10^1$				

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qProt Assay Validation - Results

Protein	Peptide ID	*Dhc* abundance in sample, *Dhc* cells/mL[b]						Previously reported[d-e] approx. protein concentrations in culture, fmol/mL (protein/cell)[c]
		$1.3x10^5$	$2.2x10^6$	$3.1x10^7$	$1.3x10^5$	$2.2x10^6$	$3.1x10^7$	
		Peptide concentration in culture, fmol/mL			Protein concentration in culture, fmol/mL (protein/cell)[c]			
FdhA	**FdhA2[a]**	$<3.0x10^0$	$<3.0x10^0$	$8.5x10^0$	$<3.0 x10^0$	$<3.0 x10^0$	$8.5x10^0$ ($3.8x10^3$)	[d]KB1, D2 culture(TCE): $9.0x10^1 – 1.0x10^2$ ($2.3x10^3 - 3.5x10^3$)
	FdhA5	$<3.0x10^0$	$<3.0x10^0$	$1.1x10^1$				
PceA	**PceA4[a]**	$<9.0x10^0$	$<9.0x10^0$	$2.1x10^1$	$6.3x10^2$	$1.9x10^2$	$4.4x10^1$	
	PceA5[a]	$6.3x10^2$	$1.9x10^2$	$6.8x10^1$				
	PceA7	$<1.0x10^0$	$2.2x10^1$	$1.9x10^1$				
	PceA8	$<1.0x10^0$	$1.7x10^1$	$1.2x10^1$				
TceA	**TceA2[a]**	$<3.0x10^0$	$1.9x10^1$	$1.3x10^1$	$<1.0x10^0$	$2.5x10^1$ ($1.1x10^4$)	$1.7x10^1$ ($7.7x10^3$)	([e]$1.1x10^3$)
	TceA3[a]	$<1.0x10^0$	$3.1x10^1$	$2.1x10^1$				
	TceA4	$<9.0x10^0$	$<9.0x10^0$	$2.3x10^1$				
	TceA5	$<1.0x10^0$	$2.4x10^1$	$1.7x10^1$				
VcrA	VcrA1	$<9.0x10^0$	$1.7x10^2$	$1.8x10^1$	$<1.0x10^0$	$5.7x10^1$ ($2.6x10^4$)	$9.3x10^0$ ($4.2x10^3$)	[d]Difficult to quantify due to low peptide sensitivity
	VcrA2	$<2.7x10^1$	$7.6x10^1$	$4.9x10^1$				
	VcrA3[a]	$<1.0x10^0$	$5.7x10^1$	$9.3x10^1$				
	VcrA4	$<8.3x10^1$	$<8.3x10^1$	$<8.3x10^1$				
	VcrA6	$<1.0x10^0$	$5.8x10^1$	$1.1x10^1$				

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qProt Assay Validation - Results

Protein	Peptide ID	*Dhc* abundance in sample, *Dhc* cells/mL[b]						Previously reported[d-e] approx. protein concentrations in culture, fmol/mL (protein/cell)[c]
		1.3×10^5	2.2×10^6	3.1×10^7	1.3×10^5	2.2×10^6	3.1×10^7	
		Peptide concentration in culture, fmol/mL			Protein concentration in culture, fmol/mL (protein/cell)[c]			
FdhA	**FdhA2[a]**	$<3.0\times10^0$	$<3.0\times10^0$	8.5×10^0	$<3.0\times10^0$	$<3.0\times10^0$	8.5×10^0 (3.8×10^3)	[d]KB1, D2 culture(TCE): $9.0\times10^1 – 1.0\times10^2$ ($2.3\times10^3 – 3.5\times10^3$)
	FdhA5	$<3.0\times10^0$	$<3.0\times10^0$	1.1×10^1				
PceA	**PceA4[a]**	$<9.0\times10^0$	$<9.0\times10^0$	2.1×10^1	6.3×10^2	1.9×10^2	4.4×10^1	
	PceA5[a]	6.3×10^2	1.9×10^2	6.8×10^1				
	PceA7	$<1.0\times10^0$	2.2×10^1	1.9×10^1				
	PceA8	$<1.0\times10^0$	1.7×10^1	1.2×10^1				
TceA	**TceA2[a]**	$<3.0\times10^0$	1.9×10^1	1.3×10^1	$<1.0\times10^0$	2.5×10^1 (1.1×10^4)	1.7×10^1 (7.7×10^3)	([e]1.1×10^3)
	TceA3[a]	$<1.0\times10^0$	3.1×10^1	2.1×10^1				
	TceA4	$<9.0\times10^0$	$<9.0\times10^0$	2.3×10^1				
	TceA5	$<1.0\times10^0$	2.4×10^1	1.7×10^1				
VcrA	VcrA1	$<9.0\times10^0$	1.7×10^2	1.8×10^1	$<1.0\times10^0$	5.7×10^1 (2.6×10^4)	9.3×10^0 (4.2×10^3)	[d]Difficult to quantify due to low peptide sensitivity
	VcrA2	$<2.7\times10^1$	7.6×10^1	4.9×10^1				
	VcrA3[a]	$<1.0\times10^0$	5.7×10^1	9.3×10^1				
	VcrA4	$<8.3\times10^1$	$<8.3\times10^1$	$<8.3\times10^1$				
	VcrA6	$<1.0\times10^0$	5.8×10^1	1.1×10^1				

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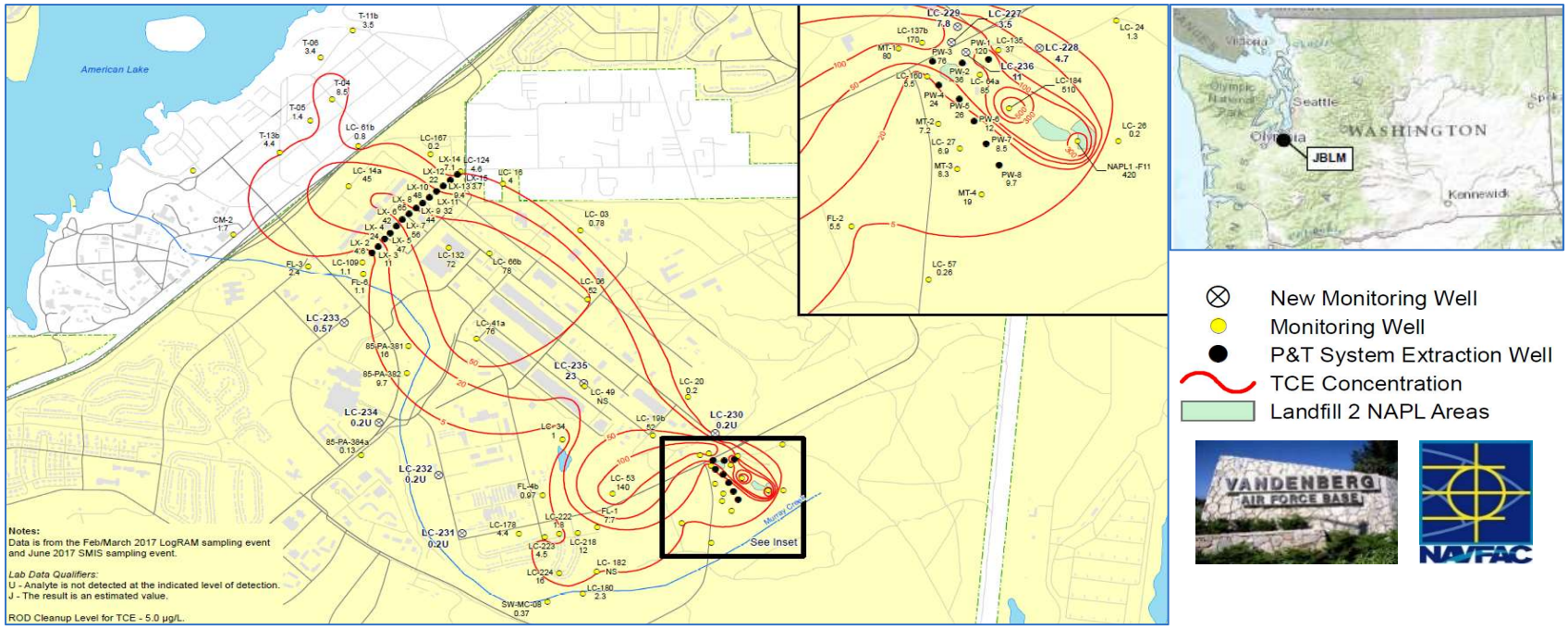
Joint Base Lewis-McChord Landfill 2

Sediment and groundwater were collected on May 30, 2017



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JBLM Groundwater Microcosm Study

SDC-9 spiked microcosms | **e- donor and SDC-9 cells**

Live Control	Killed Control	10^5 cells mL^{-1}	10^6 cells mL^{-1}	10^7 cells mL^{-1}	10^8 cells mL^{-1}

EPA Method 8260B → **Degradation rates**

qProteomics
Targeted proteomics → **Peptides**

High-throughput qPCR → **Genes and transcripts***

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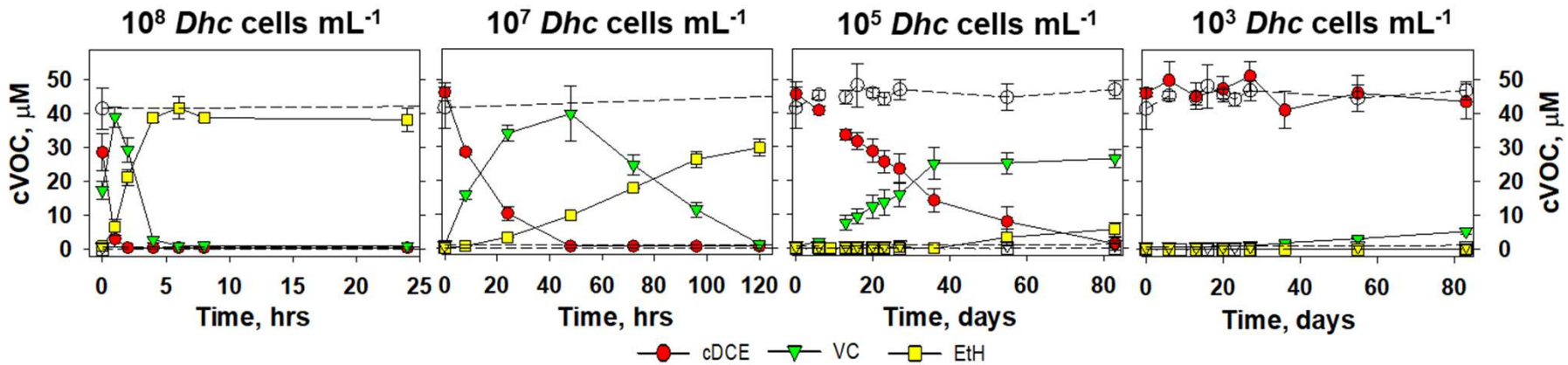
JBLM Microcosm Study Example Results



Dhc cells mL^{-1}	k_{cis}, day^{-1}	k_{VC}, day^{-1}
10^8	68.0 ± 27.0	9.6 ± 2.6
10^7	1.6 ± 0.48	0.28 ± 0.044
10^5	0.023 ± 0.0068	0.0046 ± 0.0014
10^3	0.0030 ± 0.0007	0.0010 ± 0.0063

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Good-Quality Rate Coefficients Obtained

Microcosm Test Replicate	[a]k_{cis}, 1/day	[a]k_{VC}, 1/day	R2 model	Average Ratio of 95% Confidence Interval to Rate Constant for k_{cis}, and k_{VC}
JBLM1				
JBLM1_Set1A	62.6 ± 73.1	10.2 ± 3.9	85%	77%
JBLM1_Set1B	57.0 ± 53.7	10.2 ± 3.6	86%	65%
[a]JBLM1_Set1C	73.6 ± 146.0	11.3 ± 5.9	77%	125%
JBLM1_Set2A	1.48 ± 0.50	0.31 ± 0.073	88%	29%
JBLM1_Set2B	1.49 ± 0.48	0.34 ± 0.078	89%	28%
[b]JBLM1_Set2C	1.68 ± 0.53	0.34 ± 0.070	91%	26%
JBLM1_Set3A	0.024 ± 0.0026	0.0015 ± 0.0018	90%	65%
JBLM1_Set3B	0.016 ± 1.20×10^{-8}	0.00058 ± 6.6×10^{-8}	88%	0.0%
JBLM1_Set3C	0.026 ± 2.20×10^{-7}	0.0037 ± 6.6×10^{-7}	89%	0.0%
JBLM1_Set4A	--	--	--	--
JBLM1_Set4B	0.0013 ± 0.00013	--[b]	91%	--
JBLM1_Set4C	0.0014 ± 0.0014	0.000001 ± 0.026	91%	--
JBLM2				
JBLM2_Set1A	1.05 ± 0.94	0.11 ± 0.018	94%	53%
JBLM2_Set1B	1.05 ± 1.14	0.13 ± 0.028	92%	65%
JBLM2_Set1C	1.13 ± 1.28	0.17± 0.041	83%	68%
JBLM2_Set1A_Dup	0.28 ± 0.038	0.013 ± 0.031	99%	128%

'—" indicates no rate was computed; [a]Best fit rate constants ± the 95% confidence interval on the rate constant; [b]No ethane production was measured; grey-highlighted values did not meet acceptance criteria and were excluded from further analysis

In total, 26 and 15 microcosm tests respectively yielded acceptable quality k_{cis} and k_{VC} rate coefficients to carry forward in correlation analysis

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Biomarker and Rate Correlations Confirmed

Biomarker abundance correlations with rate coefficients (biomarker abundance, microcosm all time points)									
	Log Biomarker Abundance								
	FdhA	PceA	TceA	VcrA	DHC_16S gene	*tceA*	*vcrA*	*fdhA*	*pceA*
Log k_{cis}	0.737	0.571	0.575	0.374	0.844	0.859	0.856	0.801	0.804
p value	2E-07	6.29E-06	1.2E-06	0.00548	2E-07	2E-07	2E-07	2E-07	2E-07
n	57	55	62	54	64	64	64	64	62
Log k_{VC}	0.774	0.797	0.652	0.678	0.932	0.934	0.93	0.905	0.91
p value	2E-07	2E-07	2.54E05	0.000026	2E-07	2E-07	2E-07	2E-07	2E-07
n	35	33	34	30	36	36	36	36	36
Biomarker abundance correlations with rate coefficients (biomarker abundance, microcosm mid-points only)									
	Log Biomarker Abundance								
	FdhA	PceA	TceA	VcrA	DHC_16S gene	*tceA*	*vcrA*	*fdhA*	*pceA*
Log k_{cis}	0.852	0.793	0.755	0.725	0.863	0.905	0.918	0.881	0.854
p value	2E-07	2E-07	2E-07	0.000706	2E-07	2E-07	2E-07	2E-07	2E-07
n	21	21	23	17	23	23	23	23	23
Log k_{VC}	0.925	0.836	0.765	0.916	0.934	0.953	0.962	0.966	0.943
p value	2E-07	2E-07	0.000391	2E-07	2E-07	2E-07	2E-07	2E-07	2E-07
n	15	15	15	11	15	15	15	15	15

Improved correlations across the board when using mid-point biomarker abundances only; carried mid-point abundances forward for regression analysis

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Biomarker and Rate Correlations Confirmed

Biomarker abundance correlations with rate coefficients (biomarker abundance, microcosm all time points)									
	Log Biomarker Abundance								
	FdhA	PceA	TceA	VcrA	DHC_16S gene	*tceA*	*vcrA*	*fdhA*	*pceA*
Log k_{cis}	0.737	0.571	0.575	0.374	0.844	0.859	0.856	0.801	0.804
p value	2E-07	6.29E-06	1.2E-06	0.00548	2E-07	2E-07	2E-07	2E-07	2E-07
n	57	55	62	54	64	64	64	64	62
Log k_{VC}	0.774	0.797	0.652	0.678	0.932	0.934	0.93	0.905	0.91
p value	2E-07	2E-07	2.54E05	0.000026	2E-07	2E-07	2E-07	2E-07	2E-07
n	35	33	34	30	36	36	36	36	36
Biomarker abundance correlations with rate coefficients (biomarker abundance, microcosm mid-points only)									
	Log Biomarker Abundance								
	FdhA	PceA	TceA	VcrA	DHC_16S gene	*tceA*	*vcrA*	*fdhA*	*pceA*
Log k_{cis}	0.852	0.793	0.755	0.725	0.863	0.905	0.918	0.881	0.854
p value	2E-07	2E-07	2E-07	0.000706	2E-07	2E-07	2E-07	2E-07	2E-07
n	21	21	23	17	23	23	23	23	23
Log k_{VC}	0.925	0.836	0.765	0.916	0.934	0.953	0.962	0.966	0.943
p value	2E-07	2E-07	0.000391	2E-07	2E-07	2E-07	2E-07	2E-07	2E-07
n	15	15	15	11	15	15	15	15	15

Improved correlations across the board when using mid-point biomarker abundances only; carried mid-point abundances forward for regression analysis

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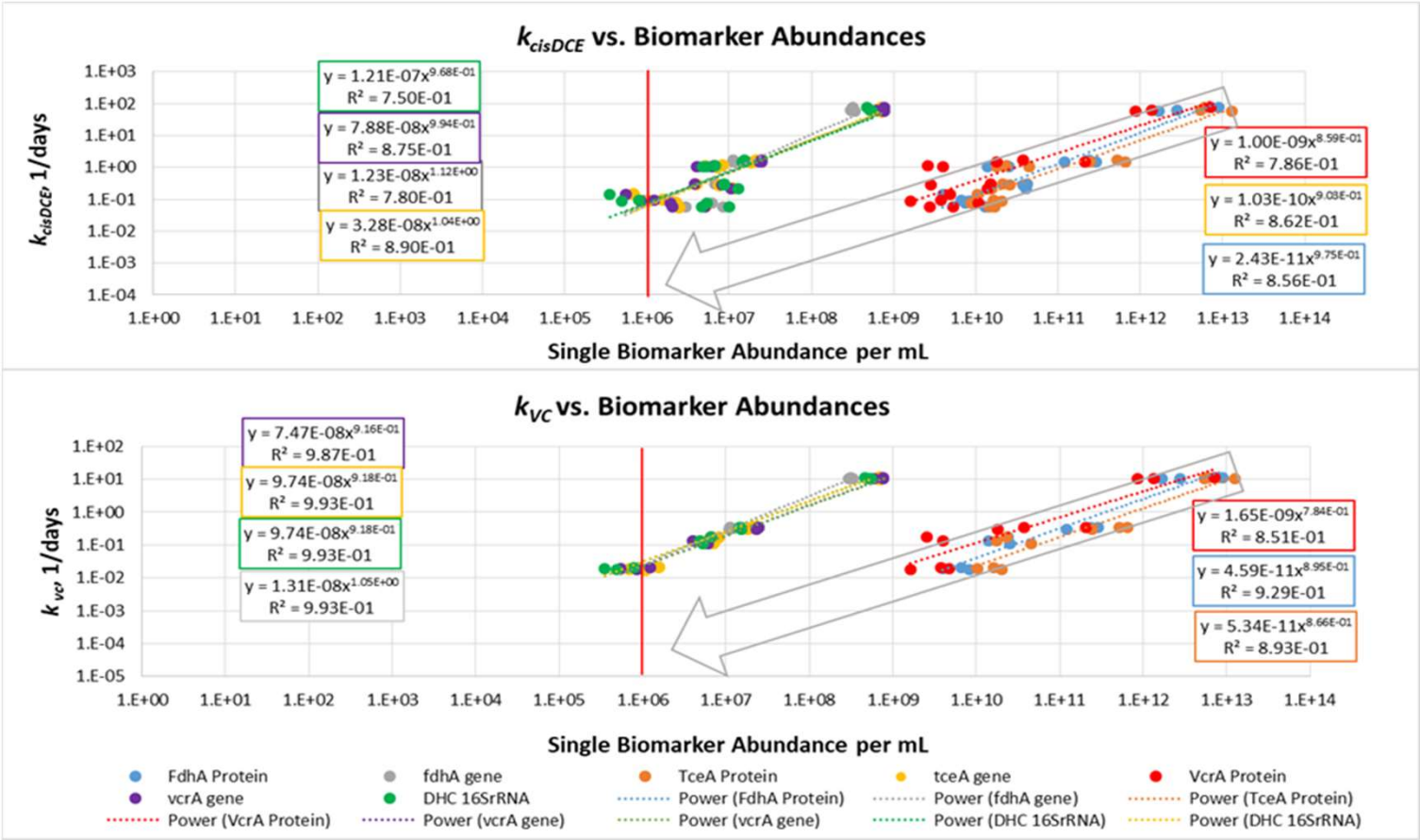
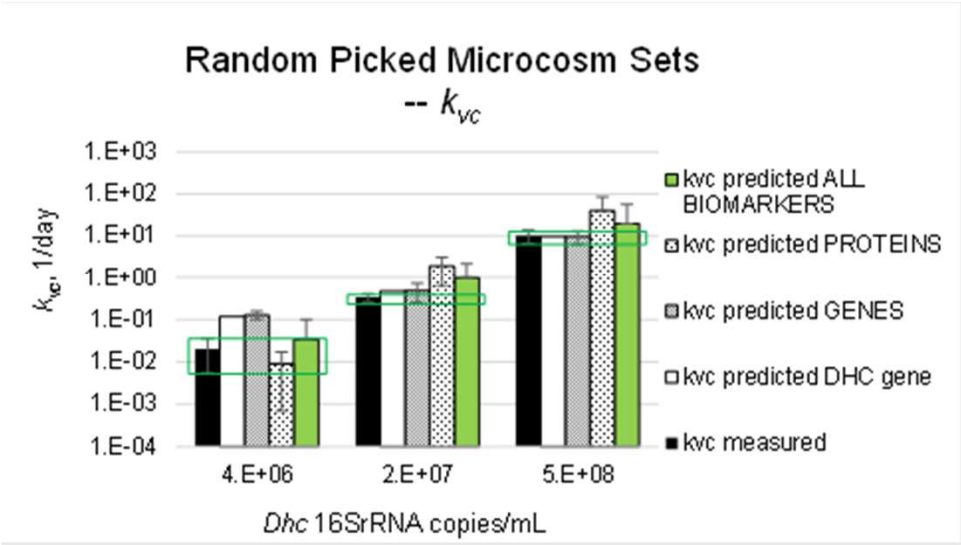
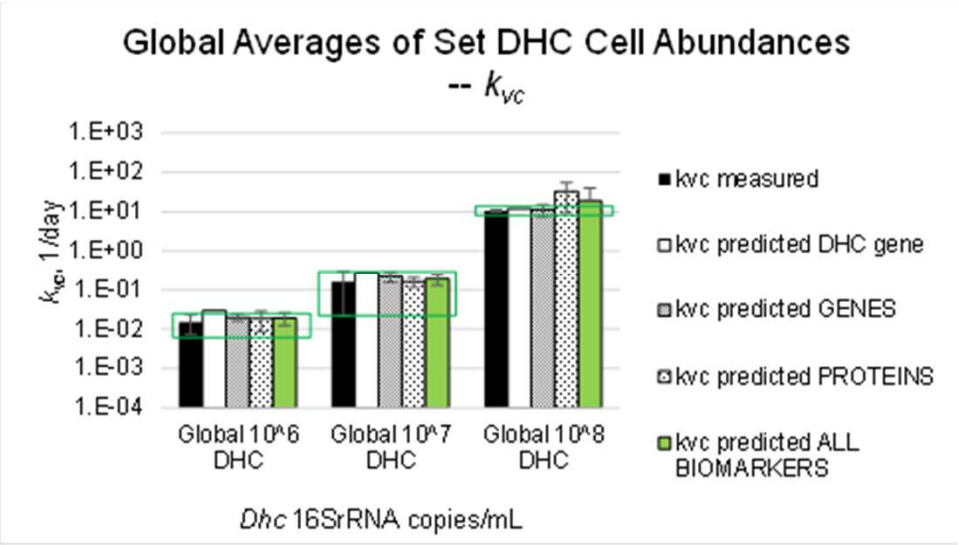
Regression Results



Applying the standard qProt assay yields a MDL of ~ 106 protein/mL, which translates to predicted k_{cis} and k_{vc} in the MNA-relevant range of 0.0001 day-1 (~ 0.04 yr^{-1})

RDase Biomarkers Can Predict Rate Constants





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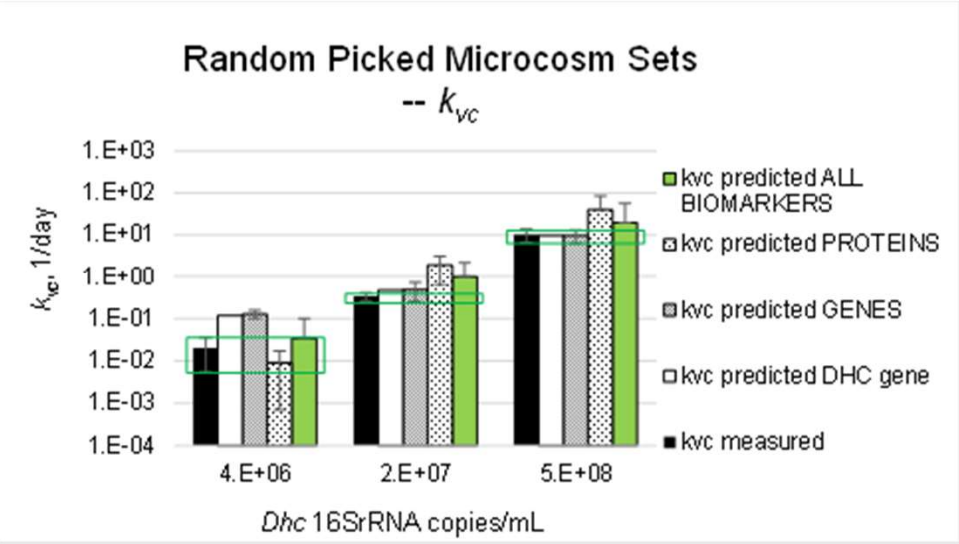
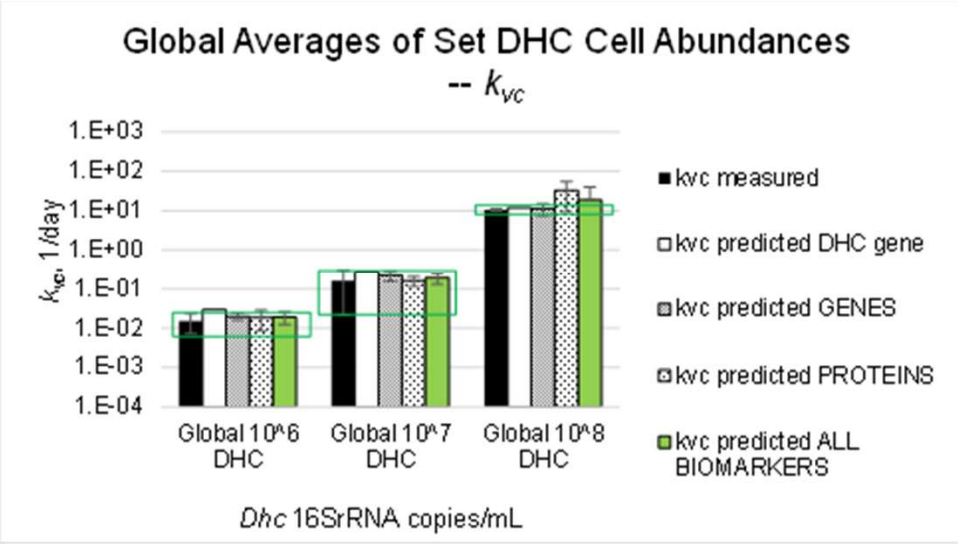
RDase Biomarkers Can Predict Rate Constants





"Unfortunately, not all *Dehalococcoides* organisms act on chloroethenes and their presence does not guarantee efficient ethene formation." **Loffler and Edwards (2006)** *Current Opinion in Biotechnology* 17:274-284

"...ethene production was not enhanced in samples exhibiting VC RDase genes abundances 10- to 10 000-fold higher than the Dhc 16S rRNA gene abundance.." **Clark et al. (2018)** *Environmental Science & Technology* 52:13410-13420

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Sensitive Enough for MNA Assessment?

We only extracted 15 mL of sample from the JBLM microcosms. What could we "see" if we extracted 1000 mL from a field sample?

Dhc cells/mL	Microcosm Set	Volume Extracted, mL	VcrA, fmol/extract	TceA, fmol/extract	Rate constant, per year
10^7	2(B)	15	1.0E+04	1.2E+04	580
10^5	3(C)	15	7.6E+02	9.9E+02	8.4
	Condition in a Field Sample with an Equivalent Amount of VcrA and TceA Extracted				
		1000	1.0E+04	1.2E+04	8.8
		1000	7.6E+02	9.9E+02	0.13
	Method Detection Limit				
			1.0E+00	3.0E+00	

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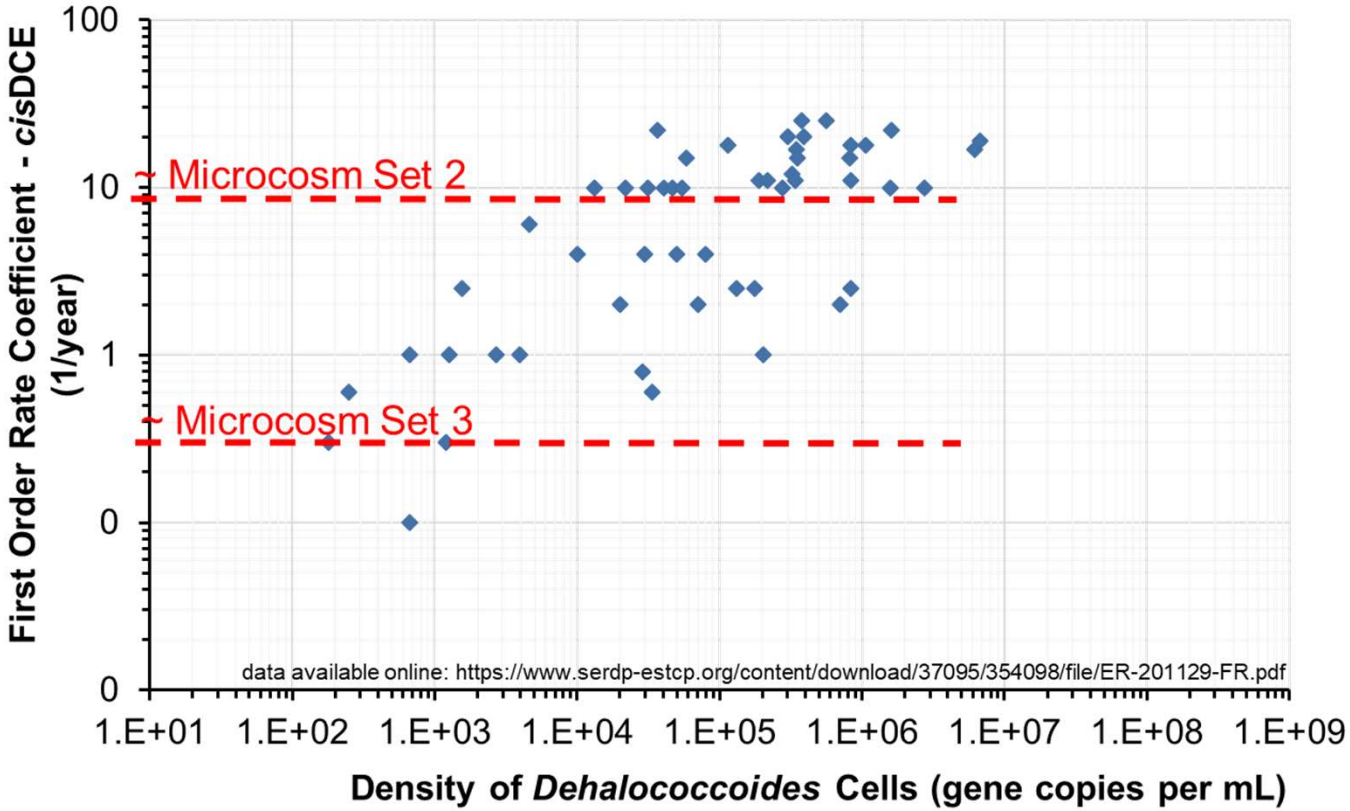

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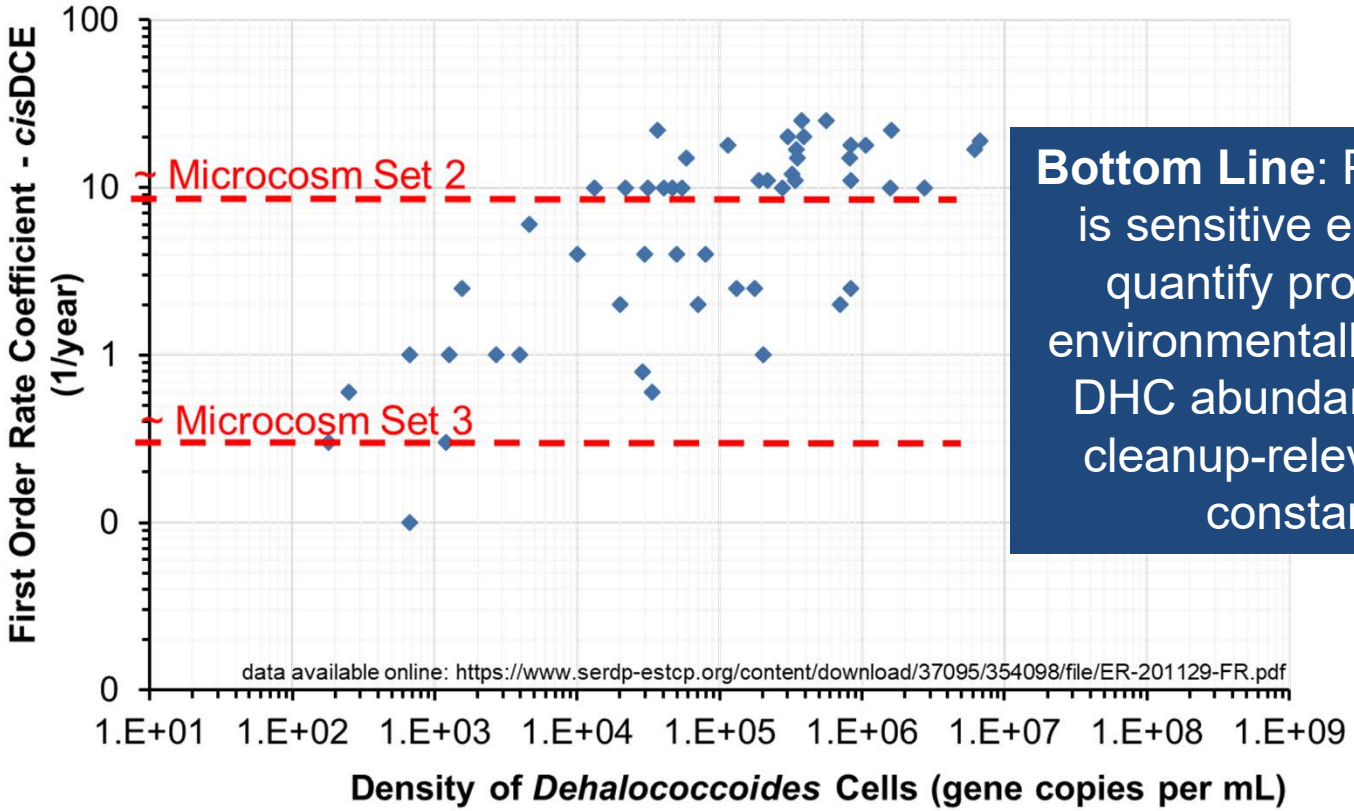

Bottom Line: Proteomics is sensitive enough to quantify proteins at environmentally-relevant DHC abundances *and* cleanup-relevant rate constants

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Key Demonstration Findings

- Confirmed quantification of genes and protein biomarkers at environmentally-relevant Dhc abundances
- Confirmed positive and significant correlation between gene and protein biomarkers; quantified relationship via regression analysis
- Rate predictive power of proteins and genes were confirmed over a range of cell abundance and corresponding activity levels

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Project Team

- Mandy Michalsen[1], Michelle Aylward[2]
  - [1]U.S. Engineer Research Development Center, Environmental Laboratory; [2]USACE Seattle District
- Kate H. Kucharzyk, Jayda E. Meisel
  - Battelle Memorial Institute
- Paul Hatzinger
  - APTIM Federal Services
- Frank Loeffler, Fadime Kara-Murdoch
  - University of Tennessee
- John Wilson, Technical Advisor
  - Scissortail Environmental Solutions
- Jack Istok, Technical Advisor
  - Oregon State University

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Air Force, Navy, Army Participants

NAVFAC

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SOLDIERS & AIRMEN DEFENDING AMERICA

Project Team

OSU Oregon State UNIVERSITY

THE UNIVERSITY OF TENNESSEE KNOXVILLE

Battelle

Scissortail Environmental Solutions

APTIM

U.S. ARMY ENGINEER RESEARCH AND DEVELOPMENT CENTER ERDC

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https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Monitoring/ER-201726

Speaker Contact Information
mandy.m.michalsen@usace.army.mil; 206-764-3324
kucharzyk@battelle.org

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Q&A Session 2

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