

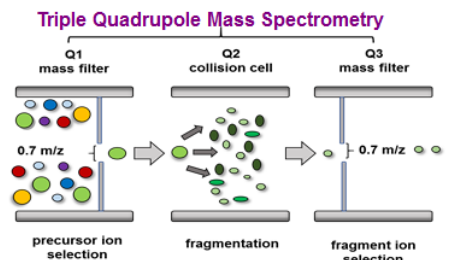
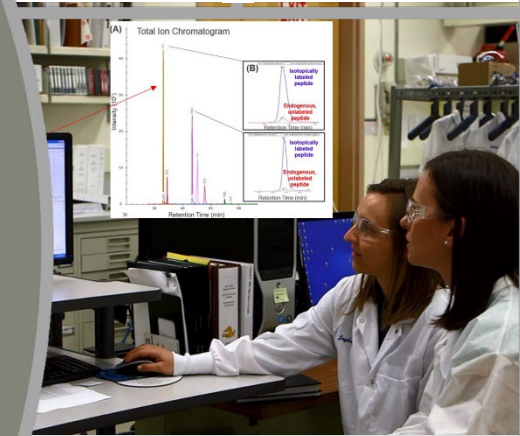
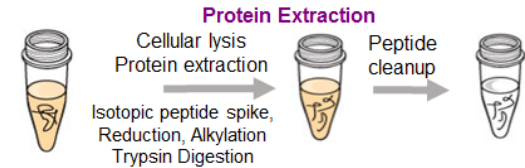
Expansion of the Molecular Biological Toolbox:

Environmental Proteomics Predicts *In Situ* Degradation Rates

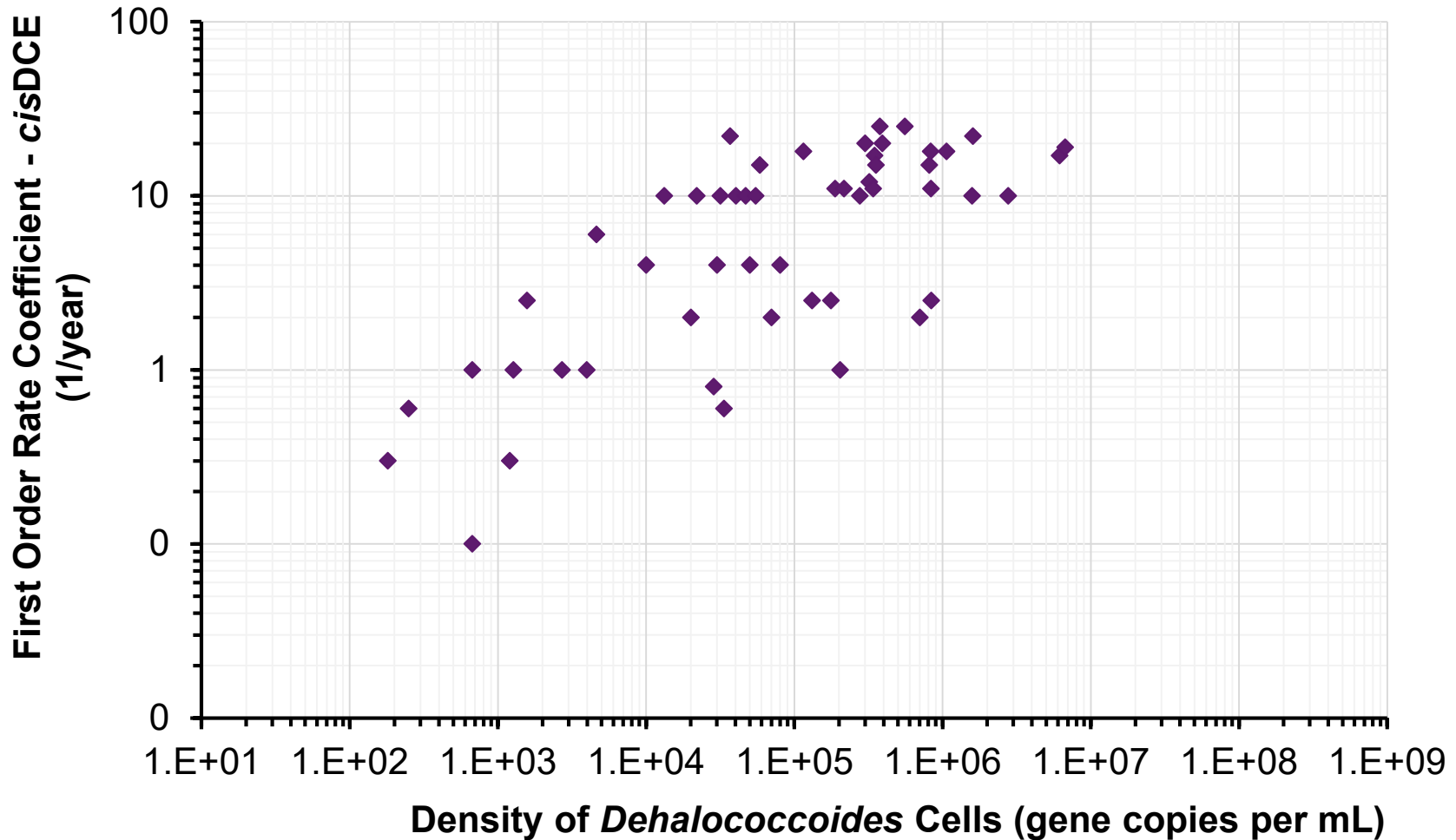
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Paul Hatzinger⁴, Frank Löffler⁵, Fadime Kara-
Murdoch⁵, John Wilson⁶, Jonathan Istok⁷

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April 17, 2019



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>

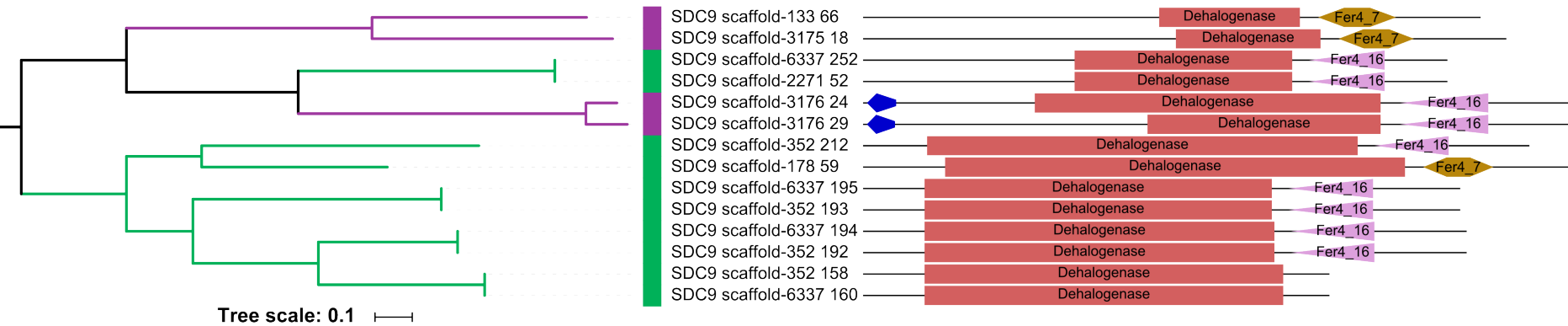
SDC-9 Metagenome Sequencing

Phylum

- *Firmicutes*
- *Chloroflexi*

Pfam Domains

- Dehalogenase
- ◆ TAT_signal
- ◆ Fer4_7
- ◀ Fer4_16



- SDC-9 metagenome sequenced;
- 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

SDC-9 Metagenome Sequencing

RDaseA Locus	RDaseB Locus	Number of TM Helices in RDaseB	Putative taxonomy	TAT Signal	Percent amino acid identity	Accession number of Best NCBI Alignment	Predicted gene
scaffold-6337_195	scaffold-6337_193	3	Dehalococcoides	Yes	99%	WP_081042195.1	--
scaffold-6337_194	scaffold-6337_193	3	Dehalococcoides	Yes	100%	WP_081042194.1	--
scaffold-352_158	ND		Dehalococcoides	Yes	100%	BAZ97963.1	--
scaffold-6337_252	scaffold-6337_251	3	Dehalococcoides	Yes	100%	WP_010935983.1	--
scaffold-352_212	scaffold-352_213	3	Dehalococcoides	Yes	99%	AEI59454.1	vcrA
scaffold-178_59	scaffold-178_58	3	Dehalococcoides	Yes	99%	WP_062900263.1	tceA
scaffold-3176_24	scaffold-3176_25	3	Dehalobacter	Yes	94%	CAD28790.2	pceA
scaffold-6337_160	ND		Dehalococcoides	Yes	100%	BAZ97963.1	--
scaffold-133_66	scaffold-133_67	3	Dehalobacter	Yes	40%	WP_015043198.1	--
scaffold-2271_52	scaffold-2271_51	3	Dehalococcoides	Yes	100%	WP_010935983.1	--
scaffold-352_192	scaffold-352_191	3	Dehalococcoides	Yes	100%	WP_081042194.1	--
scaffold-3175_18	scaffold-3175_19	3	Desulfitobacterium	Yes	100%	CDX01551.1	--
scaffold-3176_29	scaffold-3176_30	3	Dehalobacter/ Desulfitobacterium	Yes	82%	WP_025206074.1/CDX02974.1	pceA
scaffold-352_193	scaffold-352_191	3	Dehalococcoides	Yes	99%	WP_081042195.1	--

14 unique RDase genes identified

1. Measure Biomarker Abundance

2. Measure Rate Constants

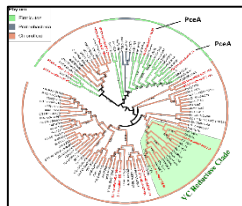
3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

Targeted Proteomics

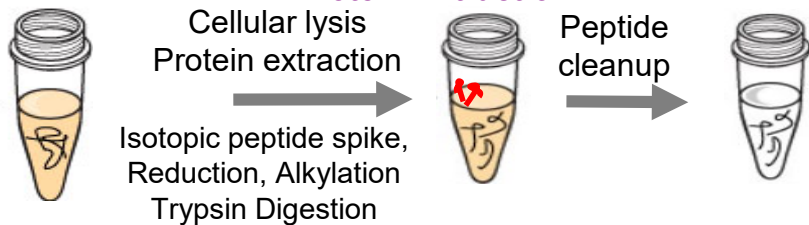
Establish MDL and IDL for Selected Peptides

RDase Identification and Down selection

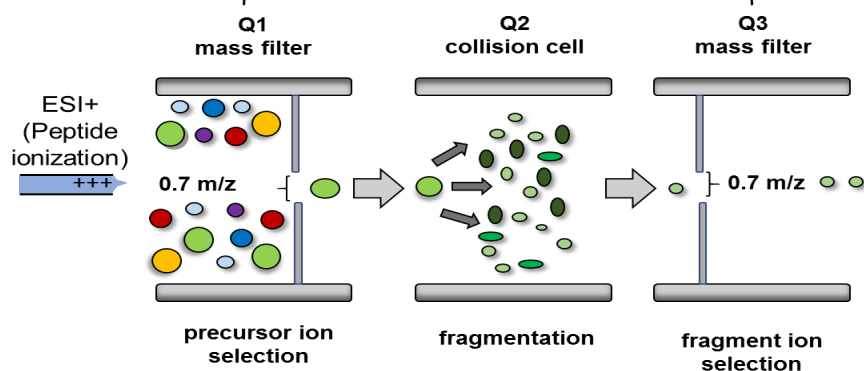


- SDC-9 metagenome sequencing
- Identified a total of 14 RDase gene sequences
- Identified at least 39 unique RDase peptides from the SDC-9 protein digests

Protein Extraction

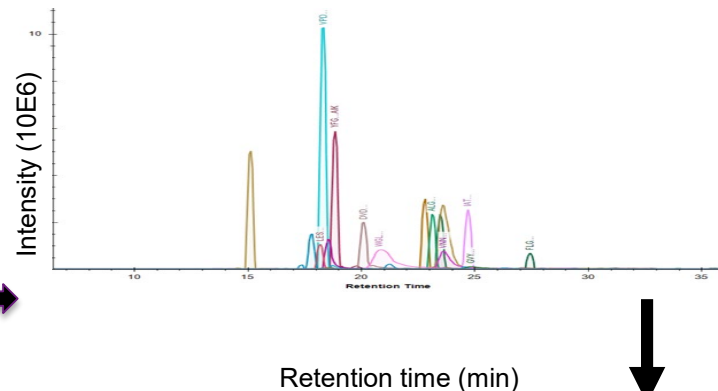


Triple Quadrupole Mass Spectrometry

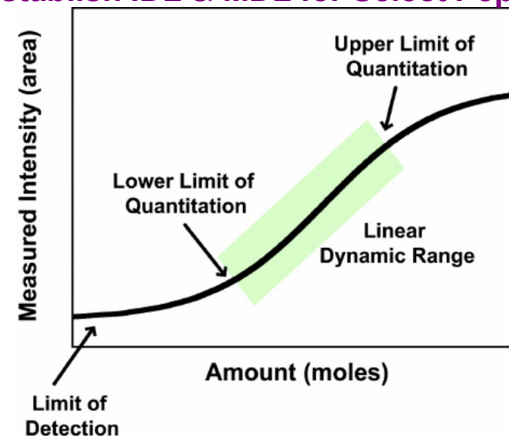


IDL = Instrument Detection Limit; MDL = Method Detection Limit

Total Ion Chromatogram



Establish IDL & MDL for Select Peptides



1. Measure Biomarker Abundance

2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

We Can “See” Them But Can We Quantify?

IDL and MDL Replicate Experiment Results

Protein	ID	Peptide ¹	MDL 1	MDL2	MDL3	Established MDL
			fmol/mL			
FdhA	FdhA2	SGSEIAFTGGLIK	3	3	3	3
	FdhA5	ALGIVYLDLSQAR	3	3	1	3
	FdhA8	NQAVSAPGEAK	3	3	3	3
PceA	PceA4	IATQIPLLQDAAR	9	9	9	9
	PceA5	LESGYVQNMVK	3	3	3	3
	PceA7	DFWNNPEPIK	1	1	1	1
	PceA8	TSPSLISSATVGK	0.3	0.3	1	1
TceA	TceA2	DVDDLLSAGK	0.3	3	3	3
	TceA3	VSSIIEPR	0.3	0.3	1	1
	TceA4	VNNEPWWVTTR	9	9	9	9
	TceA5	YFGASSVGAIK	0.3	0.3	1	1
VcrA	VcrA1	WGLYGPPHDSAPPDGSVPK	9	9	3	9
	VcrA2	YFGAGDVGALNLADPK	27	27	27	27
	VcrA3	VPDHAVPINFK	0.3	0.3	1	1
	VcrA4	GVYEGPPDAPFTSWG NR	83	27	27	83
	VcrA6	DQPWYVK	1	1	1	1

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

1. Measure Biomarker Abundance

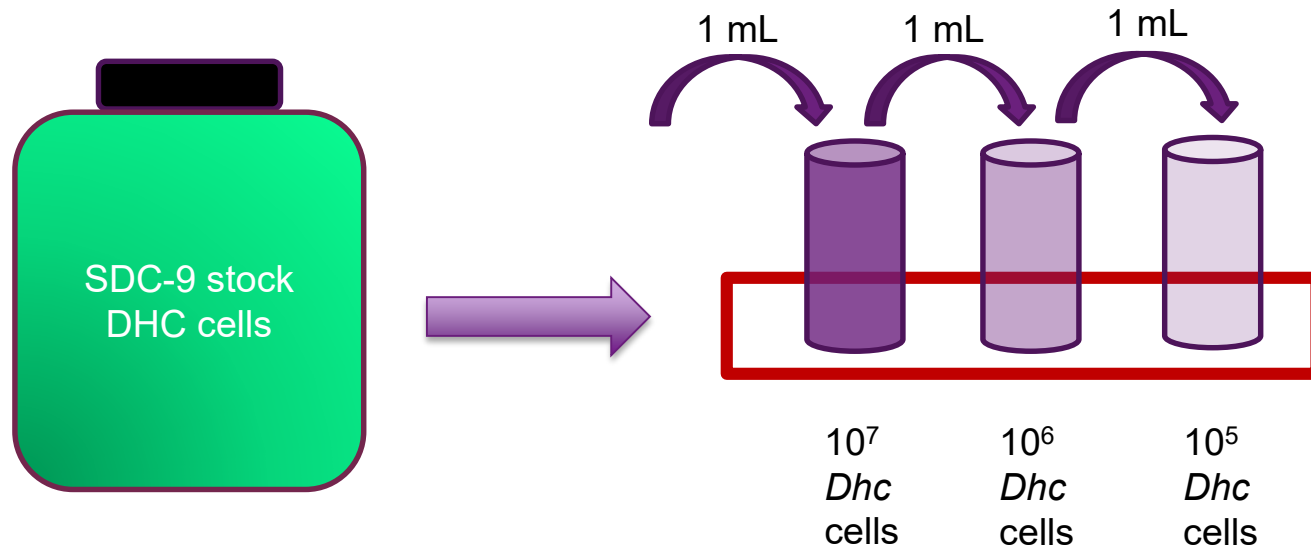
2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

How Low Can We Go?

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



qProt Assay Validation Results

Protein	Peptide ID	<i>Dhc</i> abundance in sample, <i>Dhc</i> 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				

^aQuantifier peptides used to estimate protein abundance, in cases where multiple quantifier proteins exist those abundances are averaged to obtain protein abundance, a 1:1 peptide to protein ratio is assumed; ^b15mL of each cell density were extracted, corresponding to 2x10⁶ to 5x10⁸ *Dhc* cells extracted; ^cDetected proteins were expressed in both fmol/mL and protein/cell concentration units; ^dWerner et al. 2009. Environmental microbiology, 11(10), 2687-2697; ^eRowe, A. et al. (2012). Environmental science & technology, 46(17), 9388-9397.

1. Measure Biomarker Abundance

2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

qProt Assay Validation Results

Protein	Peptide ID	<i>Dhc</i> abundance in sample, <i>Dhc</i> 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				
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	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				
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	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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1. Measure Biomarker Abundance

2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

qProt Assay Validation Results

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		Peptide concentration in culture, fmol/mL			Protein concentration in culture, fmol/mL (protein/cell) ^c			
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	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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1. Measure Biomarker Abundance

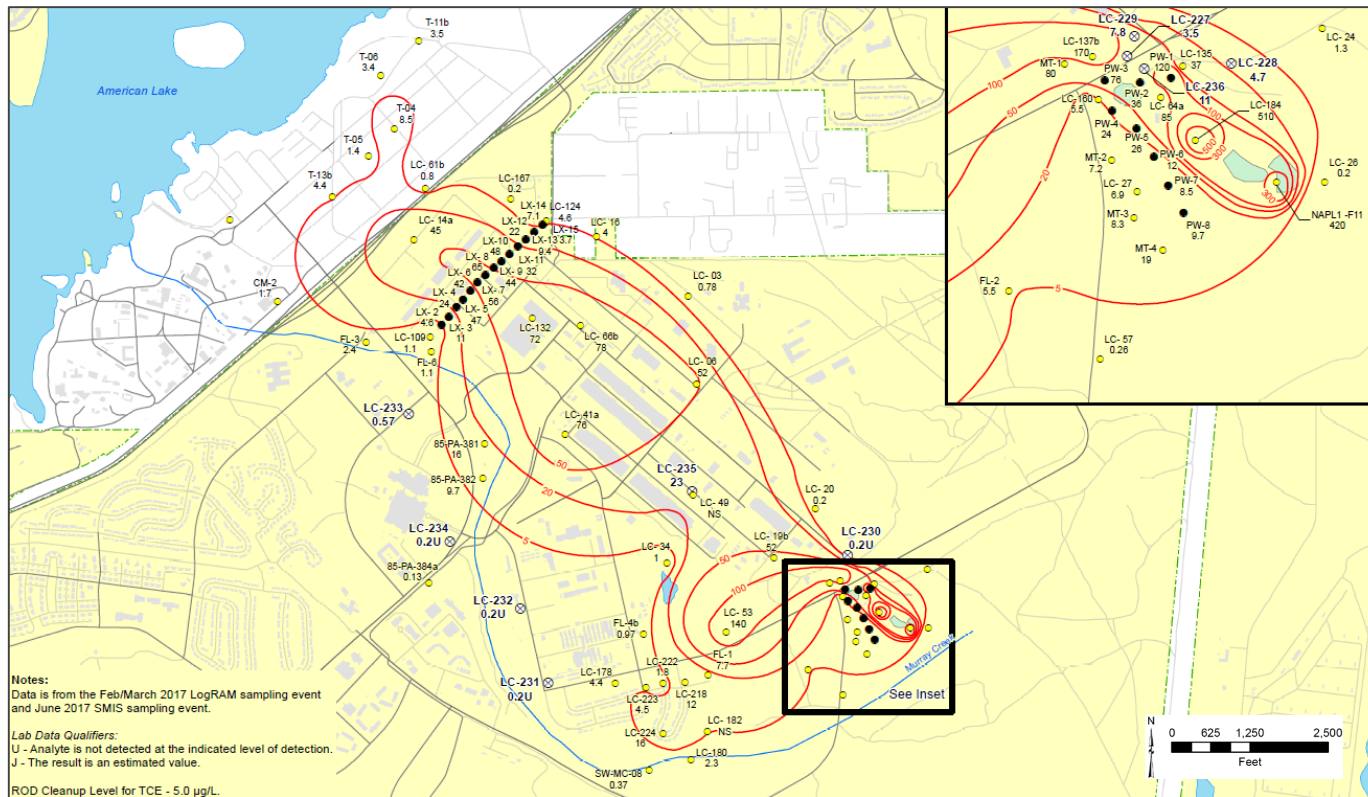
2. Measure Rate Constants






3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

Joint Base Lewis-McChord Landfill 2

Sediment and groundwater were collected on May 30, 2017



-  New Monitoring Well
-  Monitoring Well
-  P&T System Extraction Well
-  TCE Concentration
-  Landfill 2 NAPL Areas



1. Measure Biomarker Abundance

2. Measure Rate Constants

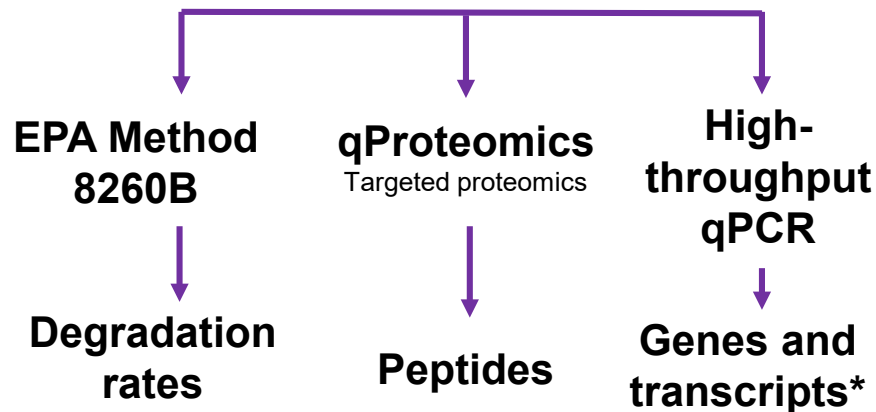
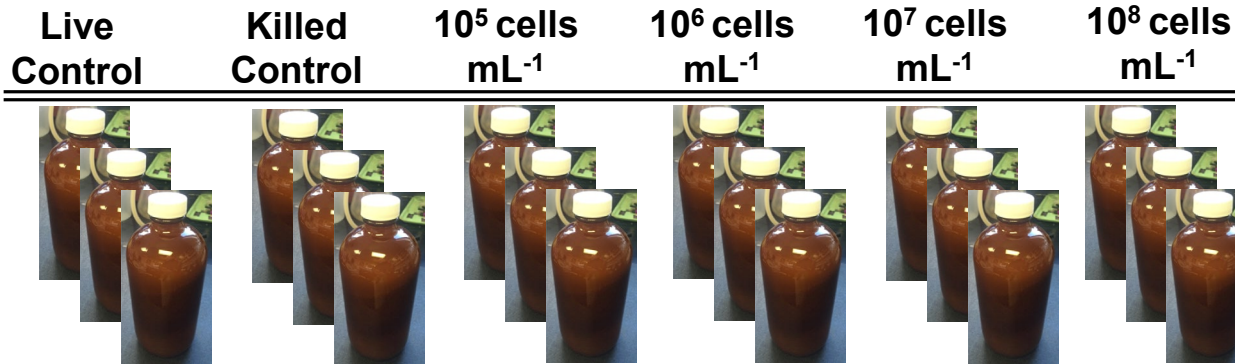
3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

JBLM Groundwater Microcosm Study

SDC-9 spiked microcosms

e- donor and SDC-9 cells



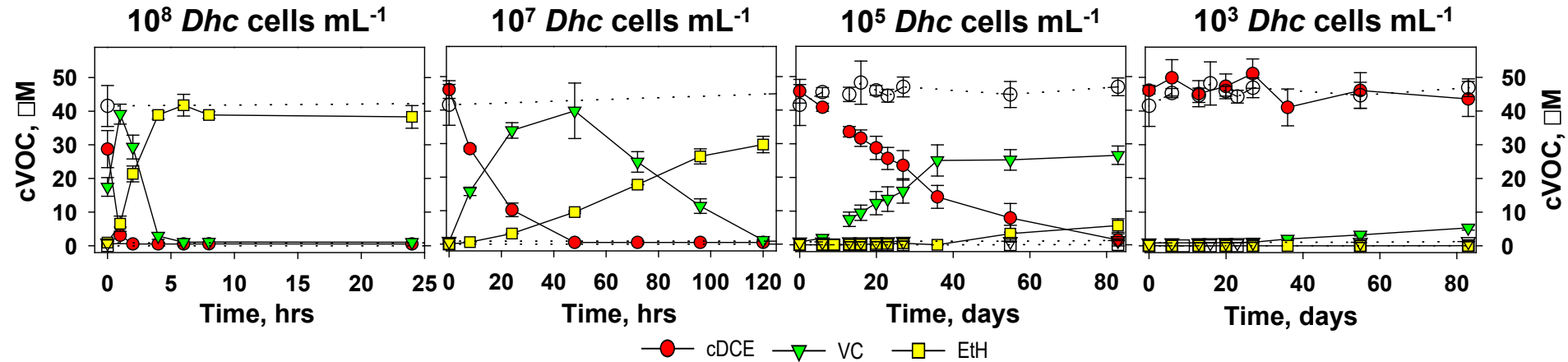
1. Measure Biomarker Abundance

2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

JBLM Microcosm Study Example Results



<i>Dhc</i> 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

1. Measure Biomarker Abundance

2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

Obtained good-quality rate coefficients

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 ± 1.20x10 ⁻⁸	0.00058 ± 6.6x10 ⁻⁸	88%	0.0%
JBLM1_Set3C	0.026 ± 2.20x10 ⁻⁷	0.0037 ± 6.6x10 ⁻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; ^aBest fit rate constants ± the 95% confidence interval on the rate constant; ^bNo 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

example subset of rate coefficient data analysis provided here; complete results are included in lab demonstration report.

1. Measure Biomarker Abundance

2. Measure Rate Constants

3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

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

Biomarker and Rate Correlations Confirmed

Biomarker abundance correlations with rate coefficients (biomarker abundance, microcosm **all time points)**

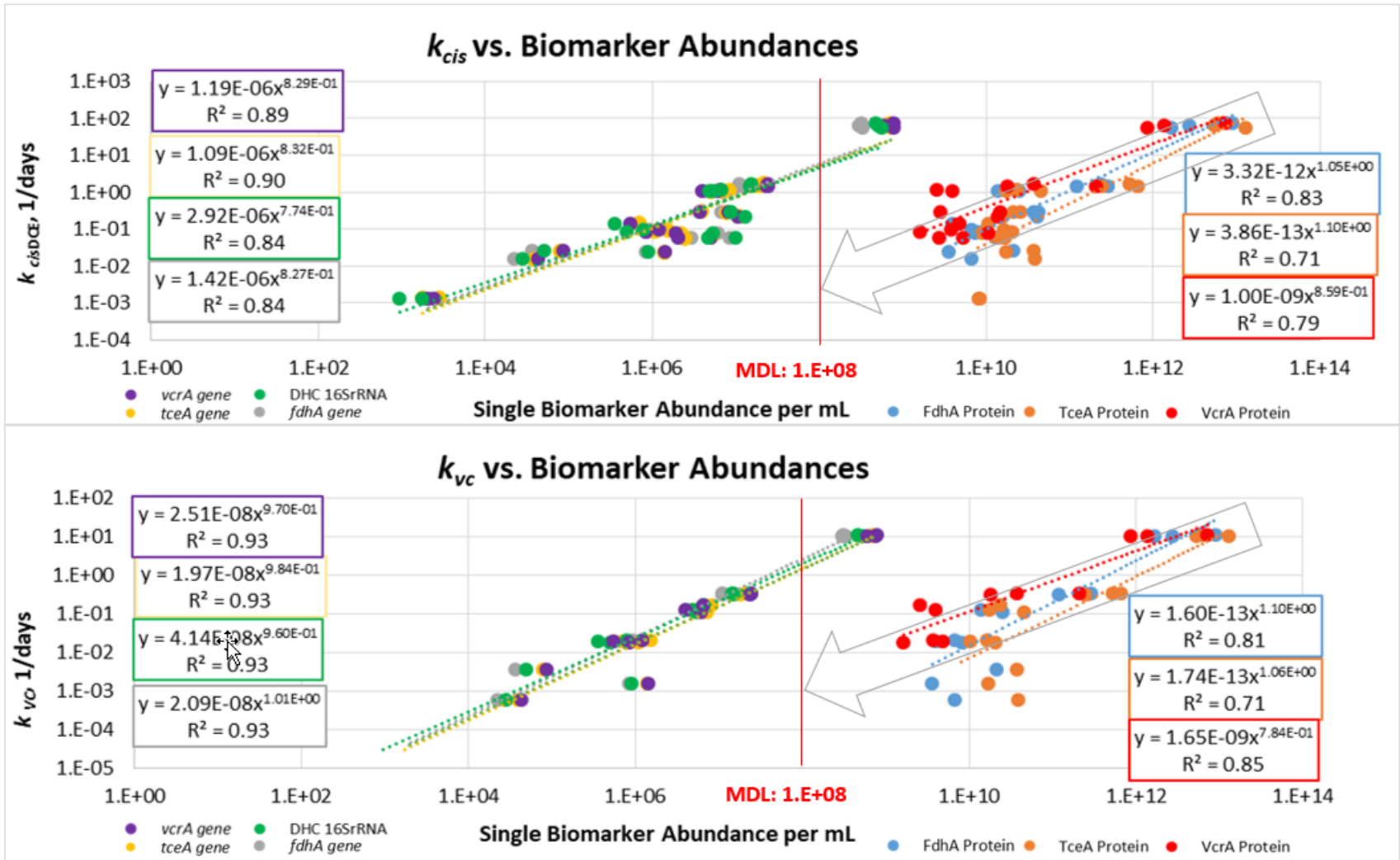
	Log Biomarker Abundance								
	FdhA	PceA	TceA	VcrA	DHC_16S gene	tceA	vcrA	fdhA	pceA
Log k_{cis}	0.737	0.571	0.575	0.374	0.844	0.859	0.856	0.801	0.804
p value	2E-07	6.29E-06	1.2E-06	0.00548	2E-07	2E-07	2E-07	2E-07	2E-07
n	57	55	62	54	64	64	64	64	62
Log k_{VC}	0.774	0.797	0.652	0.678	0.932	0.934	0.93	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

Regression Results



Applying the standard qProt assay yields a MDL of $\sim 10^8$ protein/mL, which translates to predicted k_{cis} and k_{vc} in the MNA-relevant range of $\geq 10^{-3}$ day $^{-1}$ (0.3 yr $^{-1}$)

1. Measure Biomarker Abundance

2. Measure Rate Constants

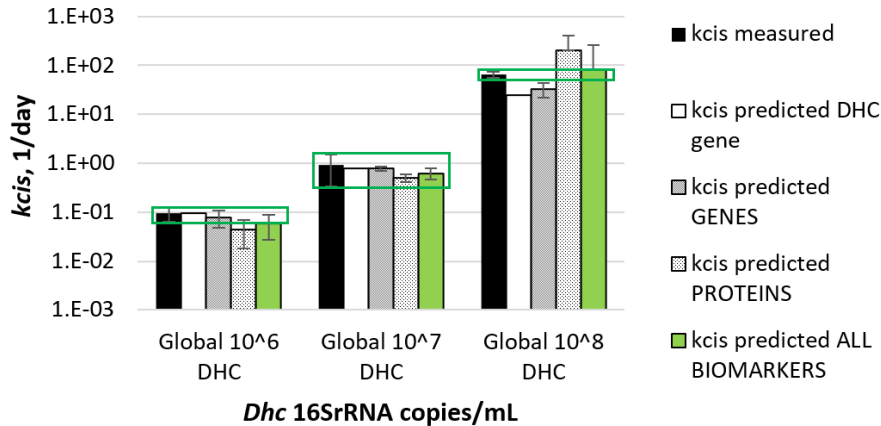
3. Evaluate Correlation between Rate Constants and Biomarker Abundance

4. Regression Analysis

RDase Biomarkers Can Predict Rate Constants

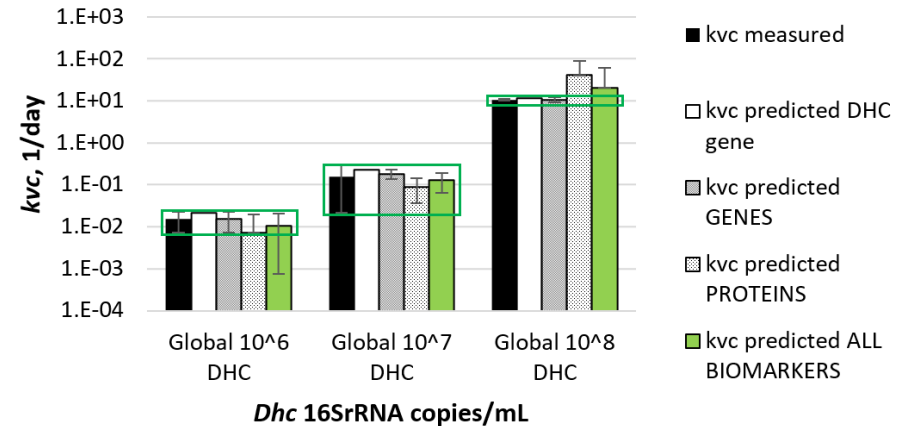
Global Averages of Set DHC Cell Abundances --

k_{cis}



Global Averages of Set DHC Cell Abundances --

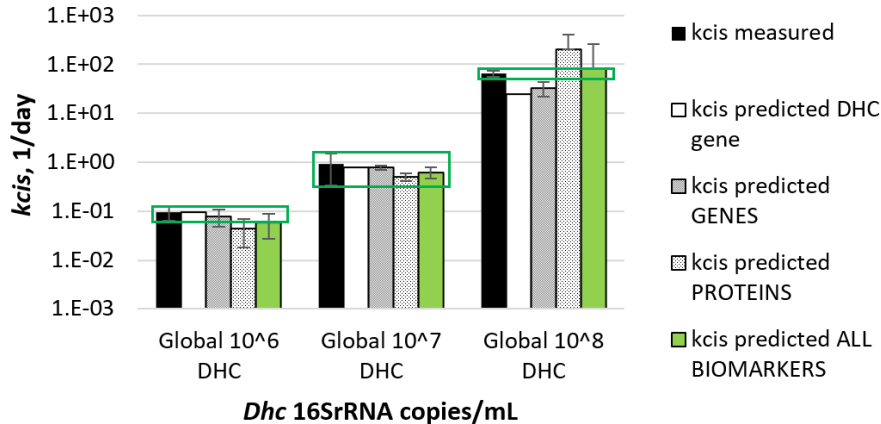
k_{vc}



RDase Biomarkers Can Predict Rate Constants

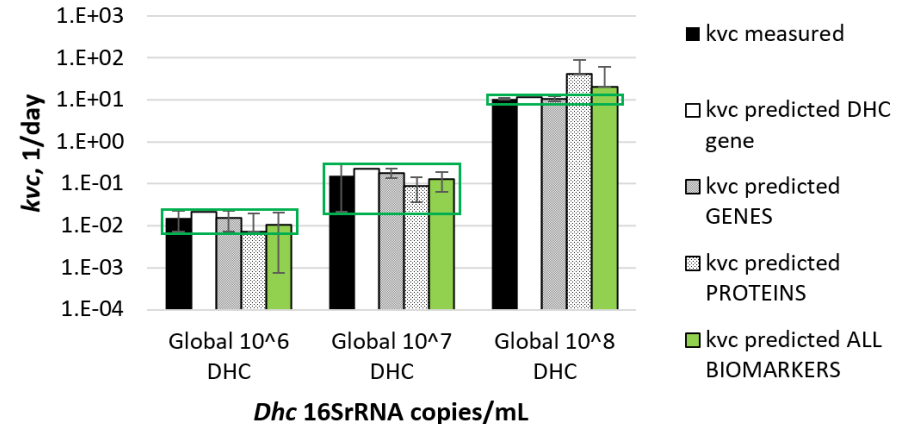
Global Averages of Set DHC Cell Abundances --

k_{cis}



Global Averages of Set DHC Cell Abundances --

k_{vc}



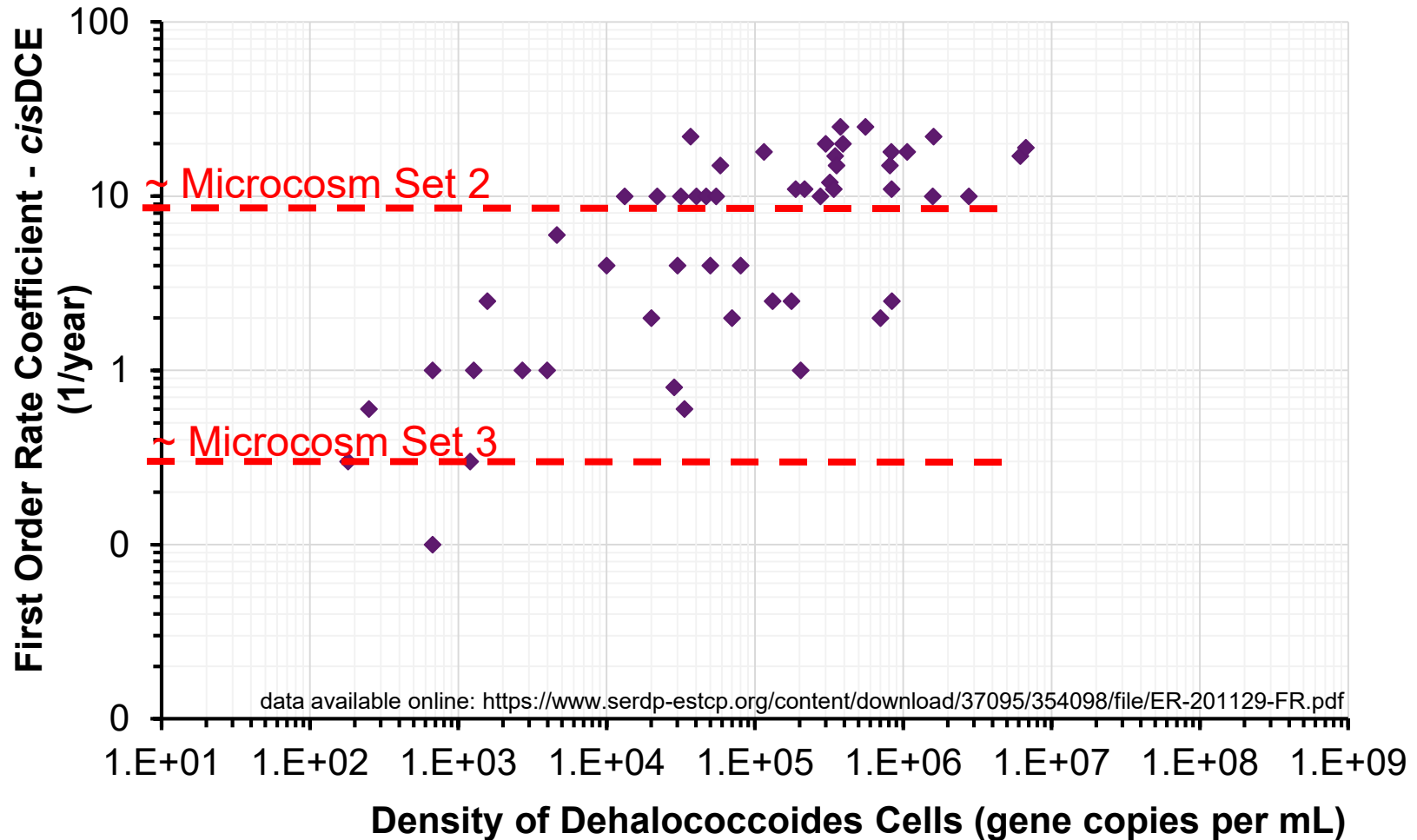
"Unfortunately, not all *Dehalococcoides* organisms act on chloroethenes and their presence does not guarantee efficient ethene formation." **Loffler and Edwards (2006)** *Current Opinion in Biotechnology* 17:274-284

"...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

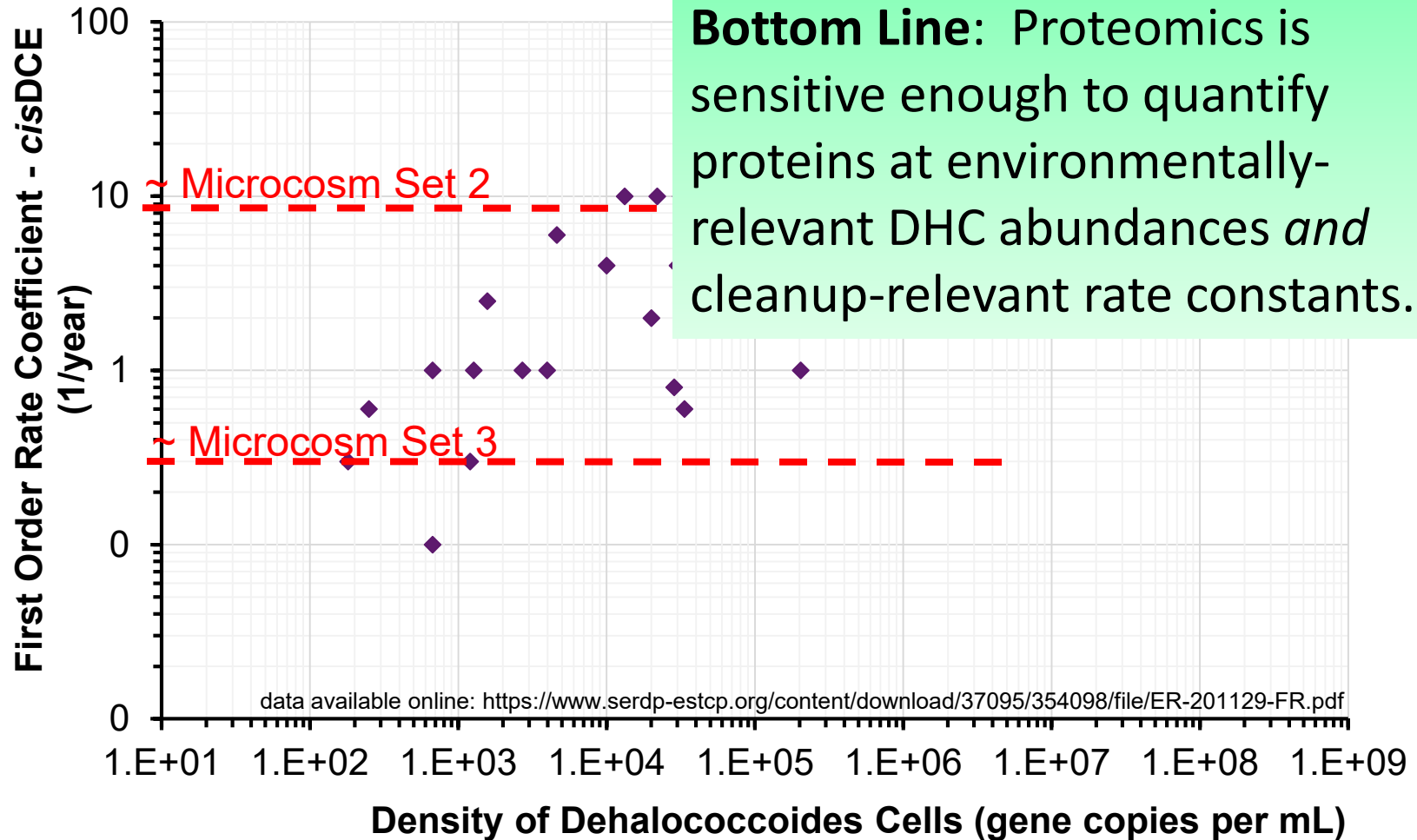
We only extracted 15 mL of sample from the JBLM microcosms.
 What could we “see” if we extracted 1000 mL from a field sample?

<i>Dhc</i> 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	

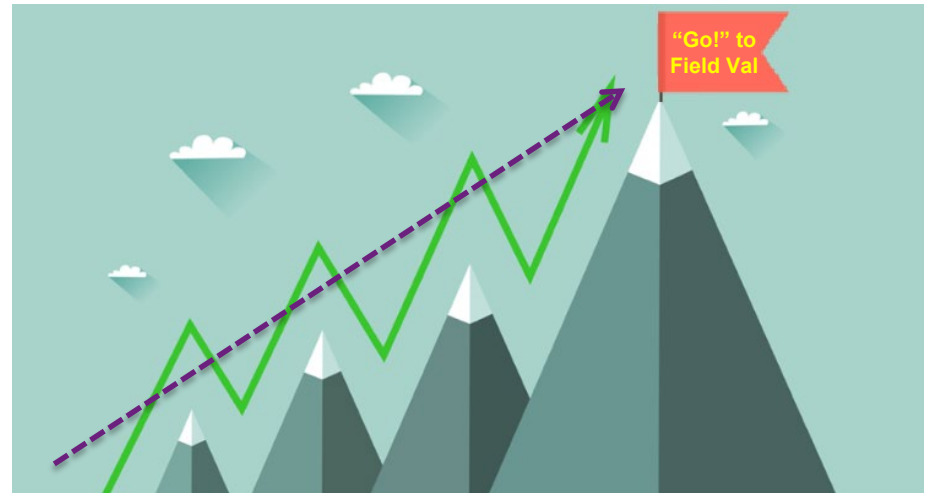
We only extracted 15 mL of sample from the JBLM microcosms.
 What could we “see” if we extracted 1000 mL from a field sample?



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



Key Points



- 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
- Recommend “**GO**” decision to proceed with field validation



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