Genetic Catabolic Probes for Monitored Natural Attenuation of 1,4-Dioxane

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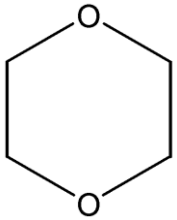
2016 DoD Environmental Monitoring & Data Quality Workshop

St. Louis, MO

April 13th, 2016

1,4-Dioxane: An Emerging Contaminant



Multi-Billion Dollar Challenge

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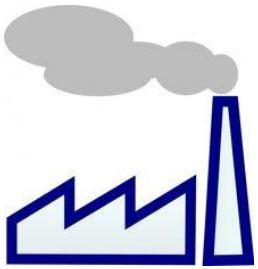
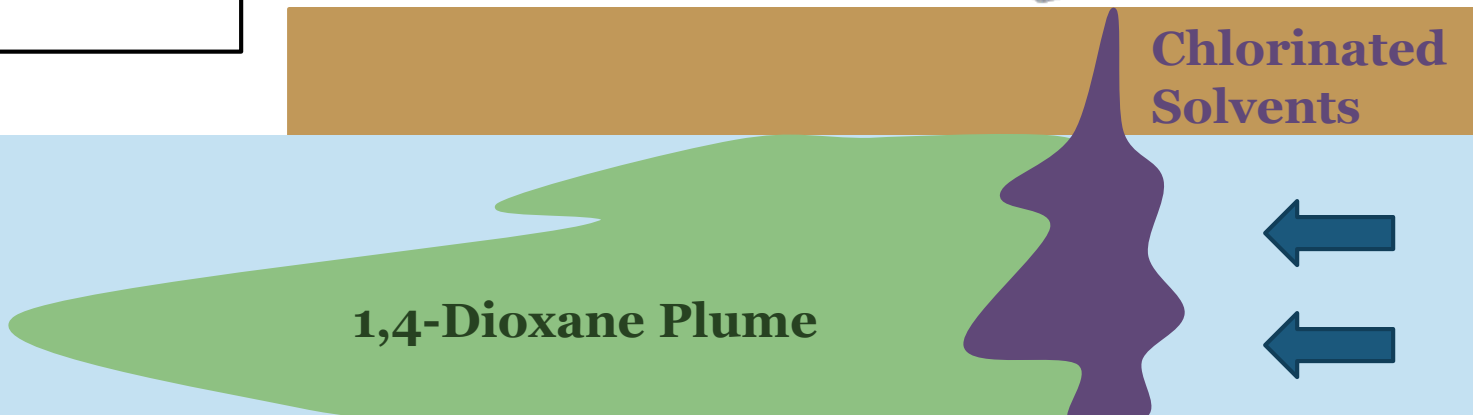




Regulatory Guidelines for Dioxane in US

State	Regulatory Concentration (µg/L)
California	1
Texas	9.13
Colorado	3.2
Connecticut	3
Maine	4
New Hampshire	3
South Carolina	70
New York	50
New Jersey	1 --> 0.4 (Oct 2015)
Massachusetts	0.3

1,4-Dioxane White Paper, Water Research Foundation, 2014

The Third Unregulated Contaminant Monitoring Rule (UCMR 3)

Contaminant	1,4-Dioxane
MRL (µg/L)	0.07
Reference Concentration (µg/L)	0.35
Total number of results	34,684
Number of results ≥MRL	4,035
% of total results ≥MRL	**12%**
Number of results >Reference Concentration	1,054
% of total results >Reference Concentration	**3%**
Total number of PWSs with results	4,773
Number of PWSs with results >Reference Concentration	333
% of PWSs with results >Reference Concentration	**7%**

Current Treatment Approaches Are Marginally Effective

Activated Carbon Adsorption

Air Stripping

Ultra-Filtration

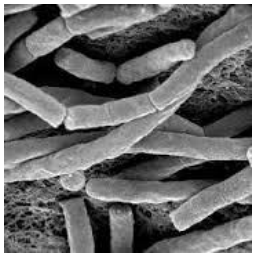
Monitored Natural Attenuation (MNA)

- Relying primarily on biodegradation by the indigenous microorganisms
- Most cost-effective approach for managing groundwater pollution
- Not yet used at dioxane-impacted sites, because
  1) Microbes that degrade dioxane have been generally perceived to be rare in the environment;
  2) No approach to quantitatively assess the presence & activity of dioxane degraders was available.

Evidence of Dioxane Biodegradation

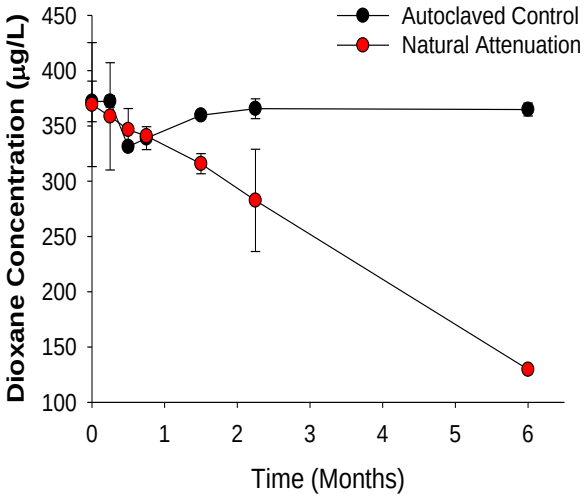
Non-biodegradable

Feasibility of Monitored Natural Attenuation?

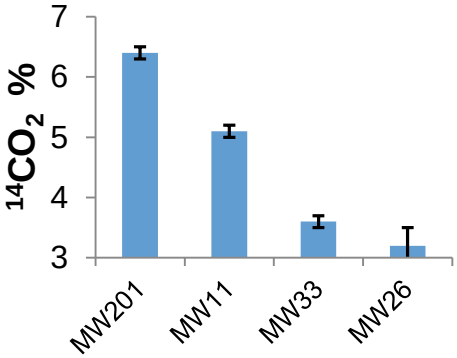


***Pseudonocardia* CB1190**

Microcosm Study



Biomineralization



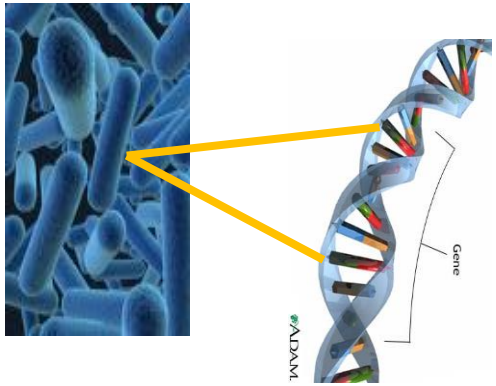
1980s → **1990s** → **2000s** → **2015**

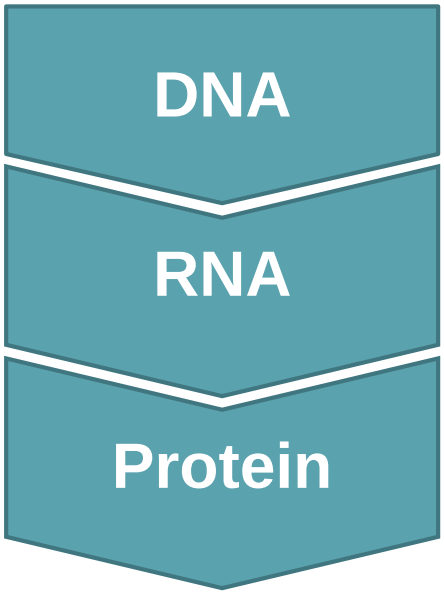
Parales *et al.*, AEM, 1994; Li *et al.*, Water Res., 2010; Li *et al.*, ES&T, 2013.

Biomarker

- A measurable molecule in an organism whose presence indicates some biological state or condition

Genetic Biomarker





- **Who is there?**
- **What can they do?**
- **What genes are being expressed?**
- **Who is active?**
- **What are they doing?**

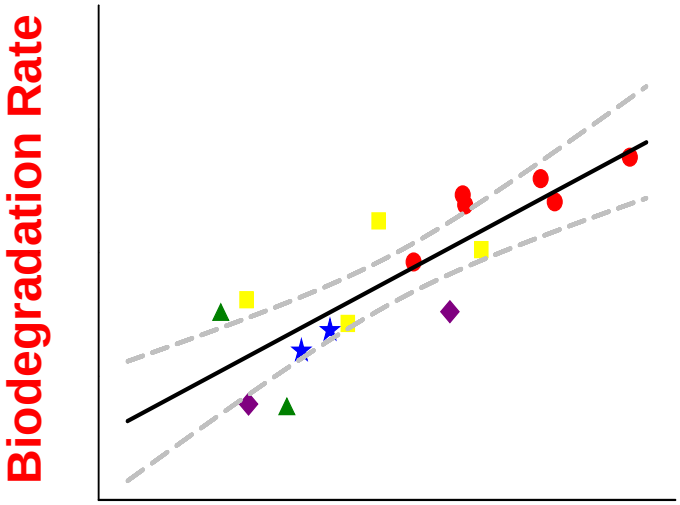
Adapted from Alvarez and Illman, Bioremediation and Natural Attenuation, 2006

Objective

- To *develop catabolic gene probe(s) to quantify the presence and expression of dioxane biodegradation capacity* to support decisions to select or reject Monitored Natural Attenuation (MNA) and Bioremediation at dioxane-impacted sites.

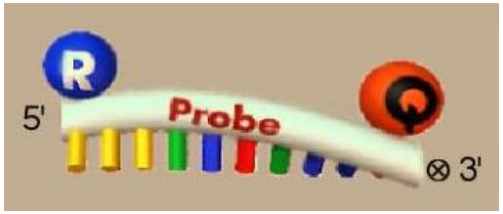
General Approach

Microcosm Studies



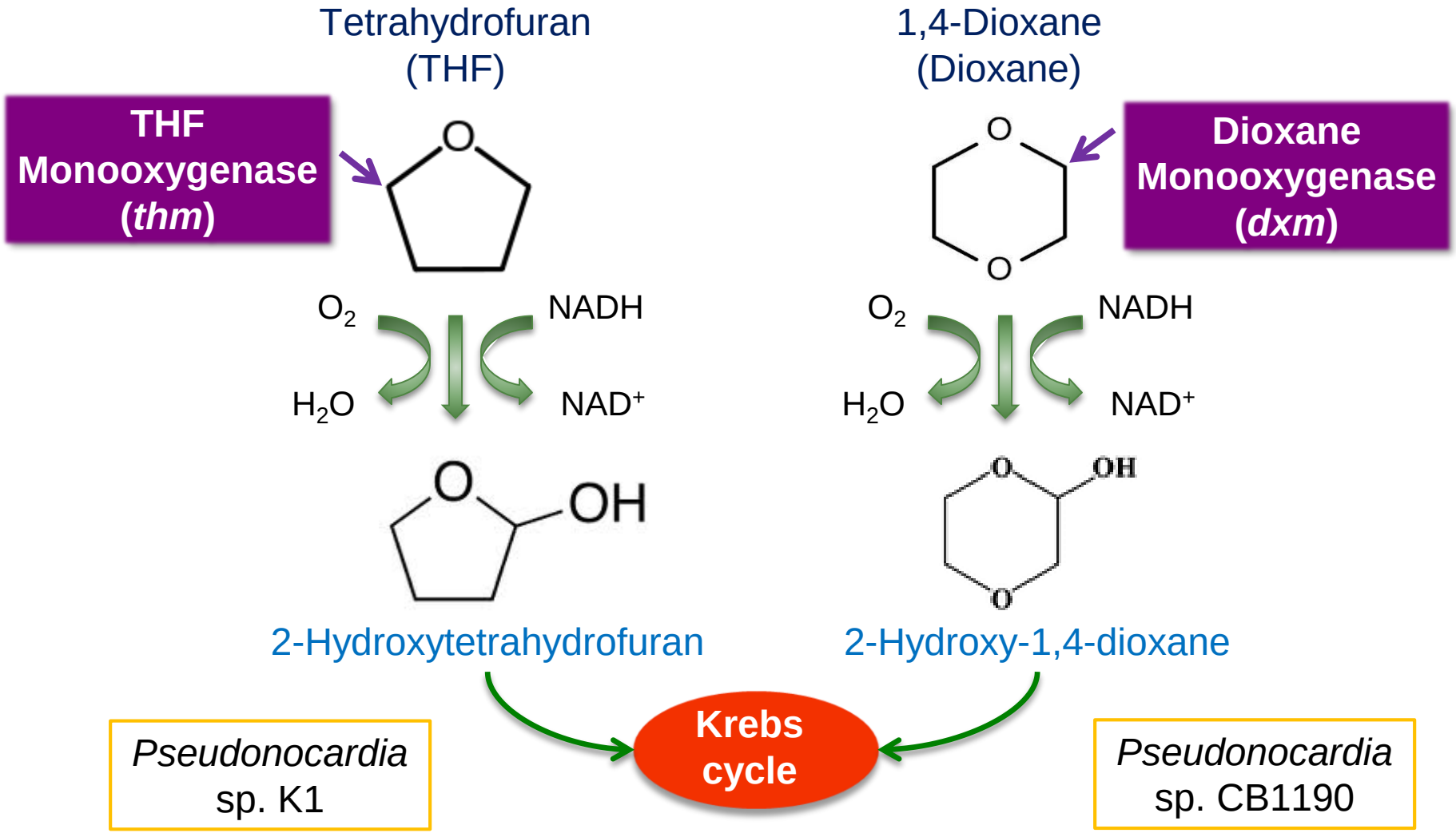
Biomarker Concentration

Biomarker Identification



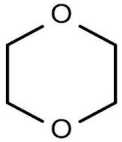
Probe Design

Dioxane/THF MOs Are the Key Enzymes Initiating the Cleavage of Cyclic Ethers



Thiemer *et al.*, Arch Microbiol, 2003; Mahendra *et al.*, ES&T, 2007

Known Degraders of Cyclic Ethers and the Key Monooxygenase Genes

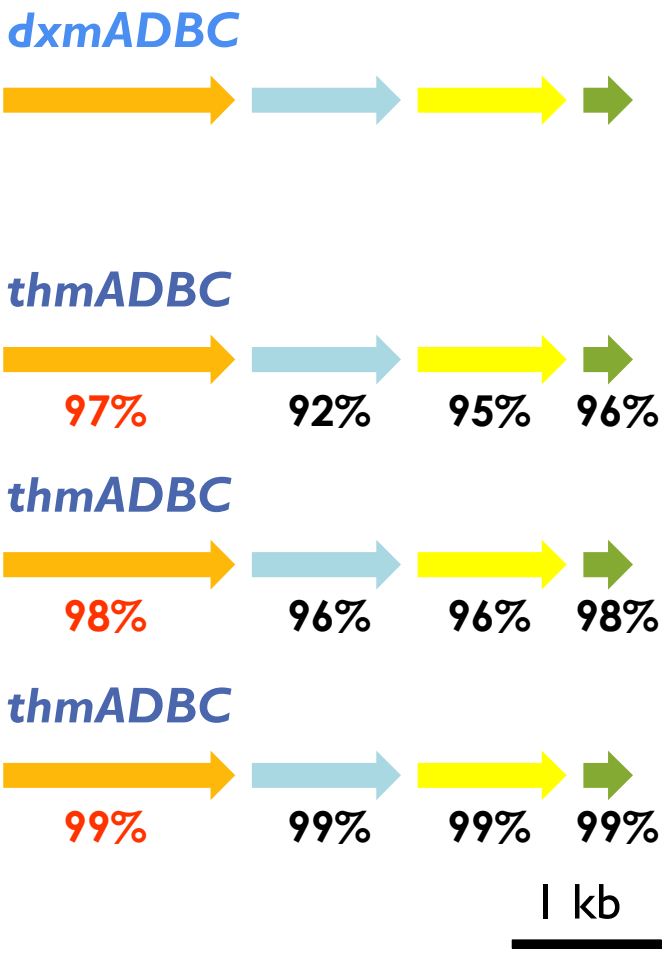


Dioxane Degrader

- *Pseudonocardia* sp. CB1190

THF Degrader

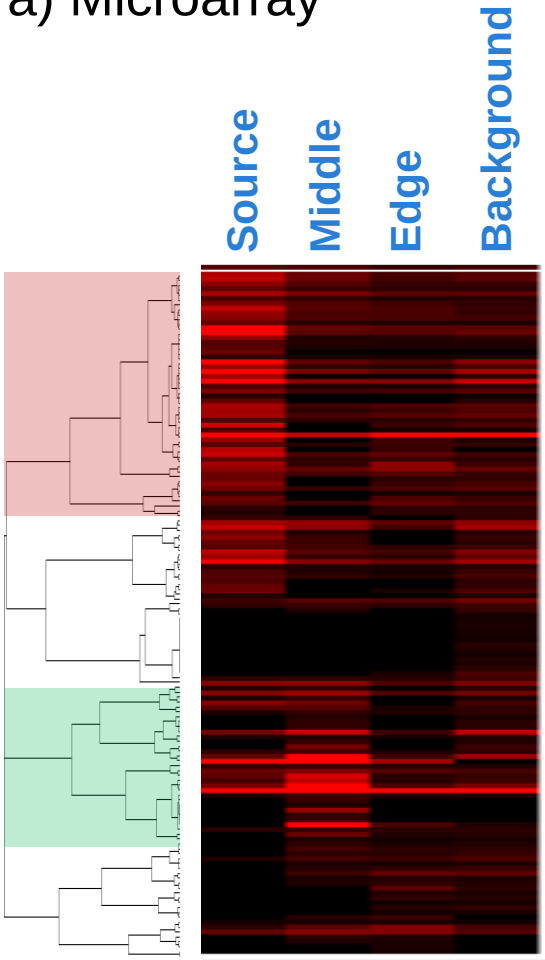
- *Pseudonocardia* sp. K1
- *Pseudonocardia* sp. ENV478
- *Rhodococcus* sp. YYL

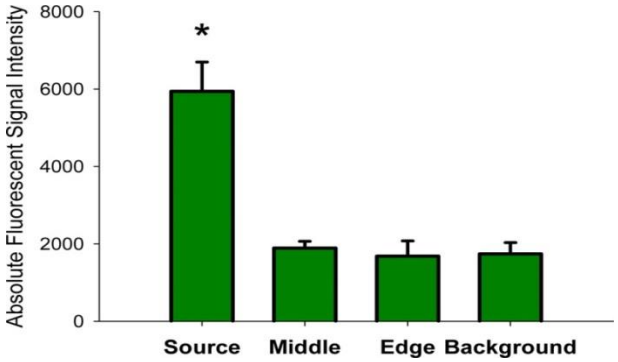


Li *et al.*, in preparation

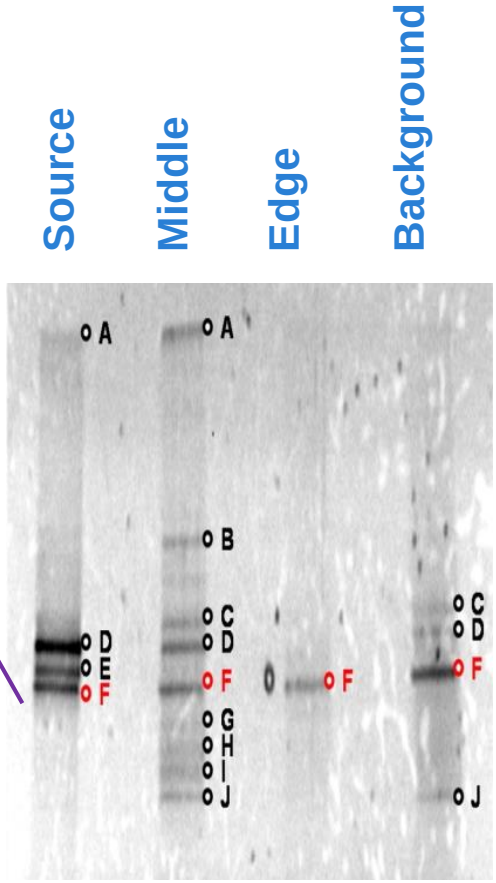
ThmA-like Genes Are Enriched near the Source Zone

a) Microarray





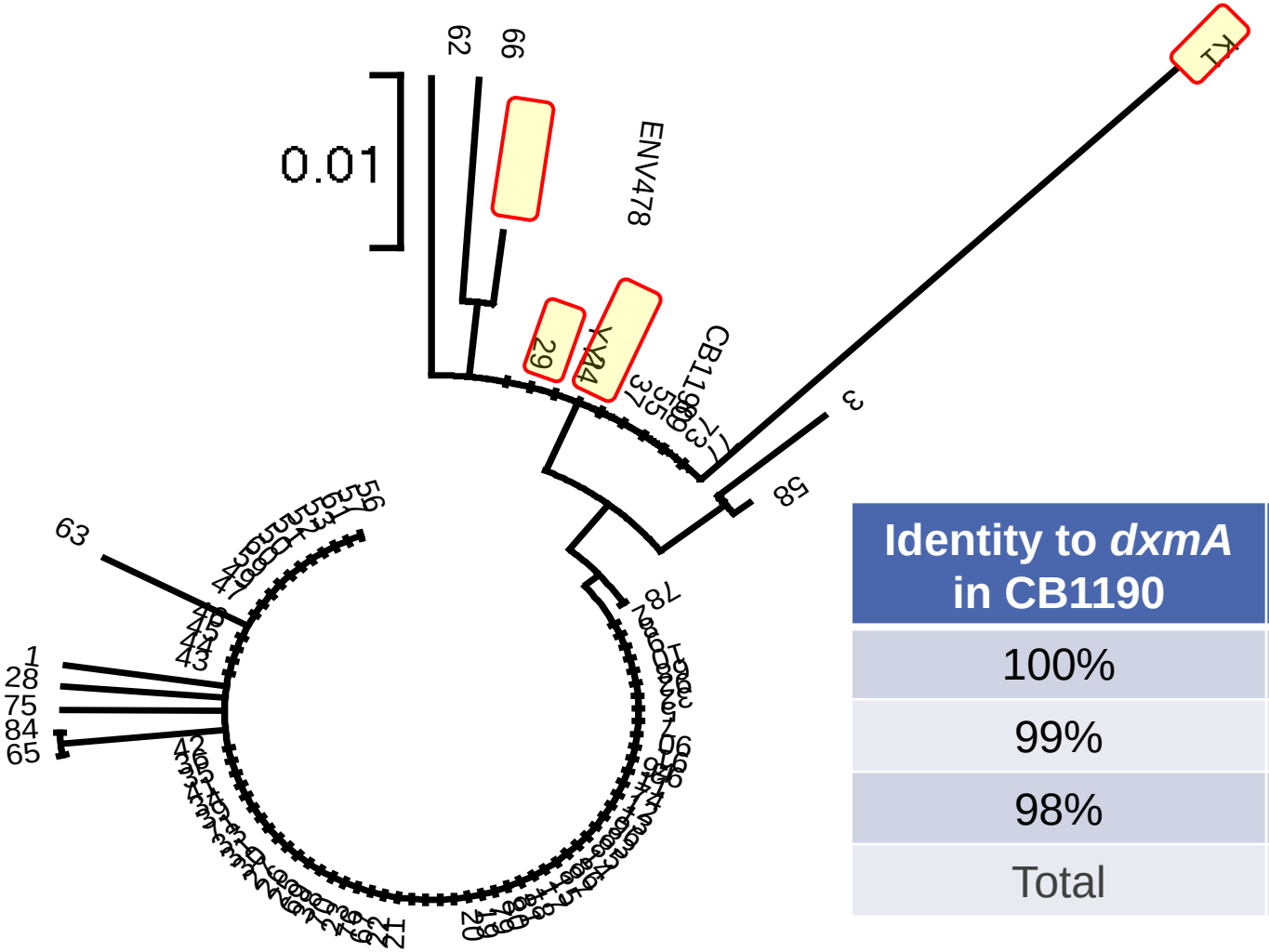
b) DGGE



thmA-like genes

Li *et al.*, ES&T, 2013

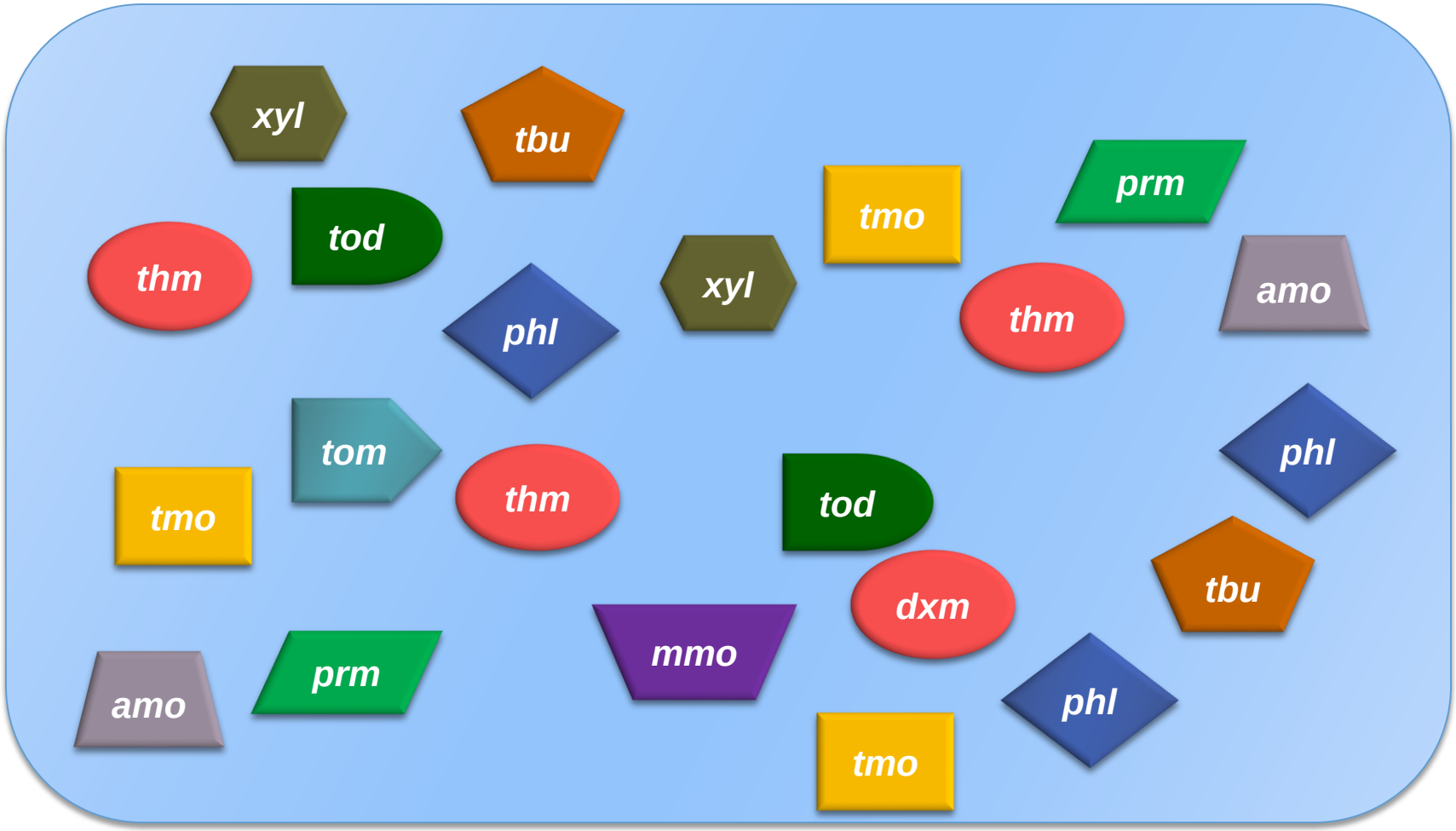
ThmA/dxmA Genes in Environment Share High Similarity to Previously Reported Ones



Identity to *dxmA* in CB1190	Number of clones
100%	7
99%	69
98%	10
Total	86

Li *et al.*, ES&T Letters, 2014

Other Genes in Environment Could Hinder Detection of *thmA/dxmA*

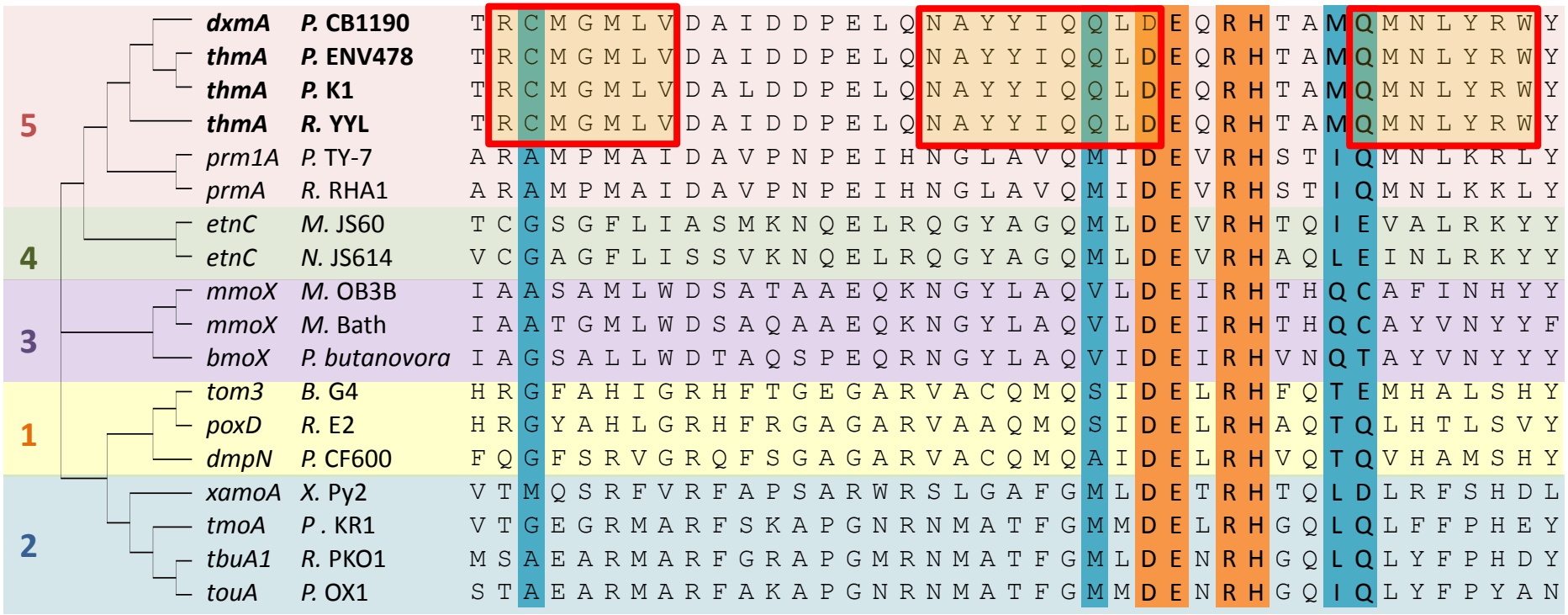


Design Criteria of Oligonucleotide Probes

i. Avoid the **di-iron centers (active site)** conserved by all SDIMOs;

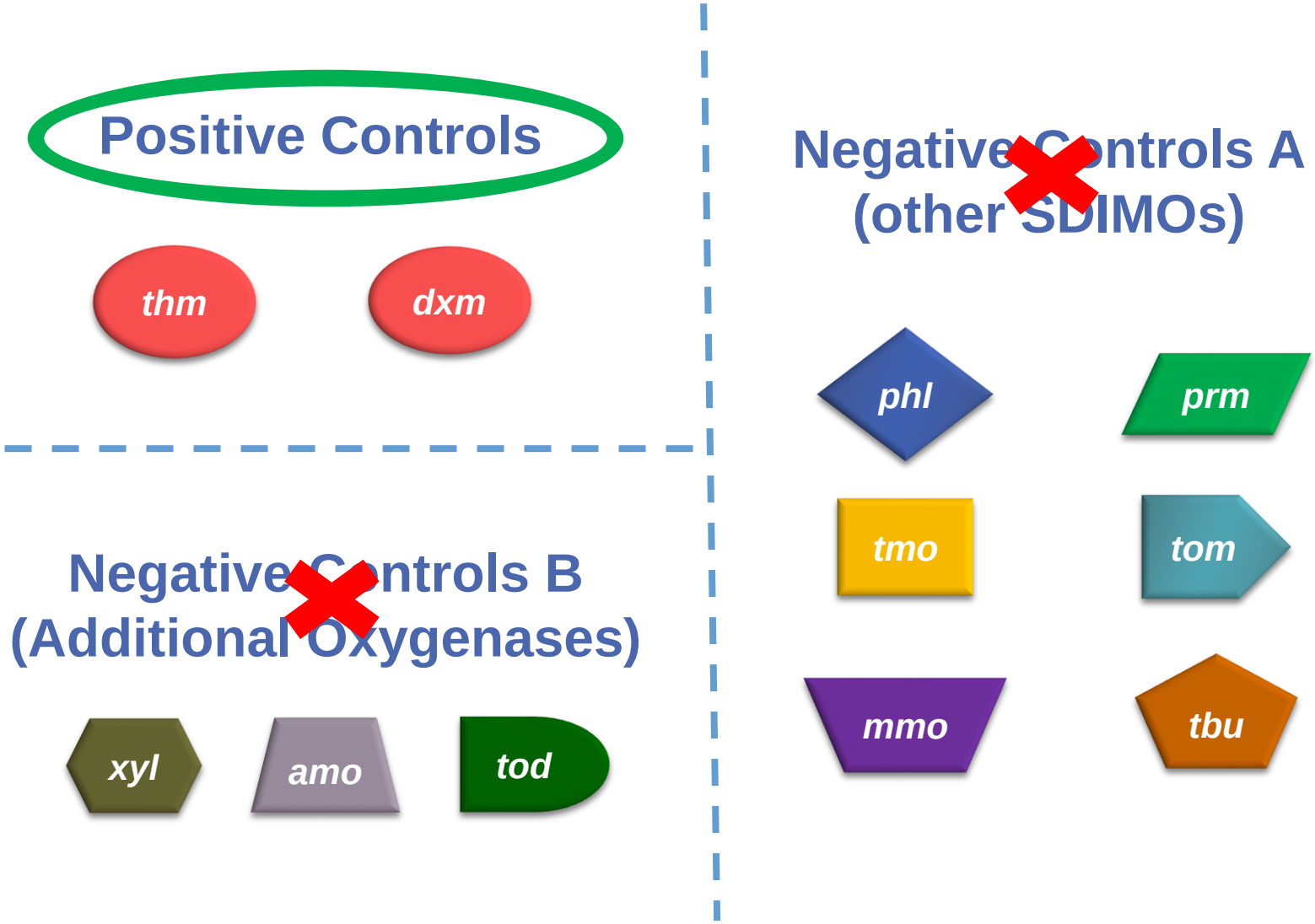
ii. Target the surrounding **hydrophobic residues (specificity)** shared by only THF/dioxane monooxygenases.

Soluble Di-Iron Monooxygenases (SDIMOs)



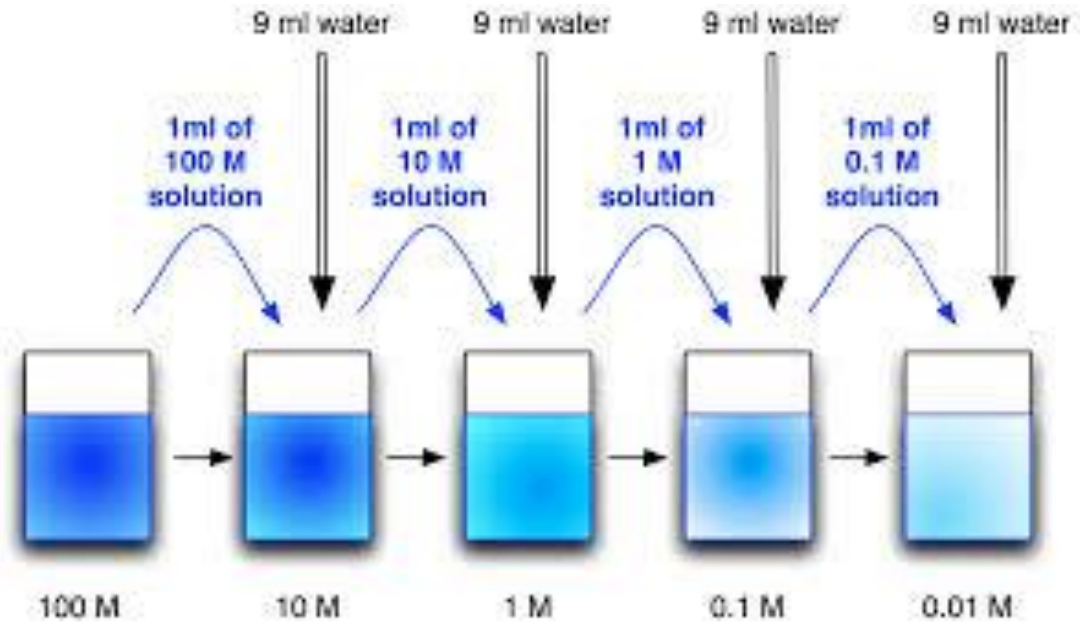
Li *et al.*, ES&T Letters, 2014

The *thmA/dxmA* Probes Are Selective



The *thmA/dxmA* Probes Are Sensitive

10 ng/µL gDNA of CB1190

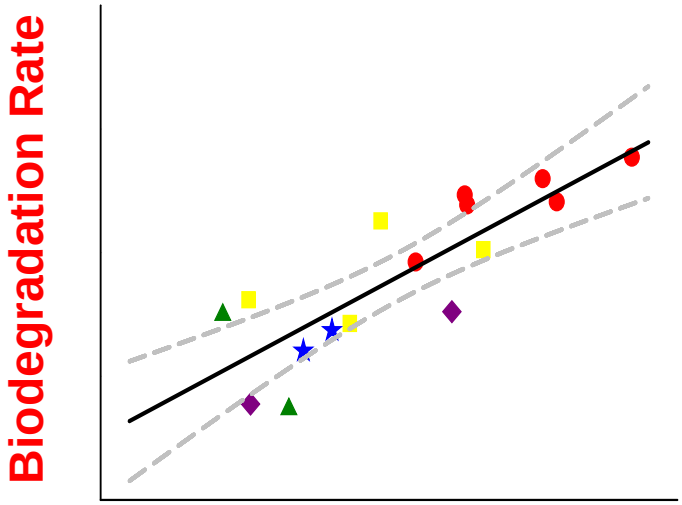


The qPCR instrument method detection limit (MDL) of the *thmA/dxmA* genes was **~100** copies/reaction mixture, and the overall MDL was **7,000 ~ 8,000** copies/gram of soil.

Li *et al.*, ES&T Letters, 2014

General Approach

Microcosm Studies



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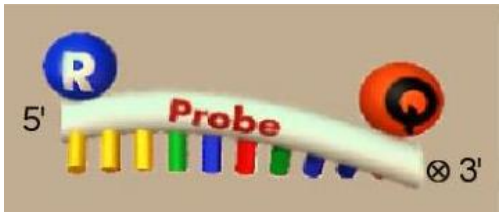
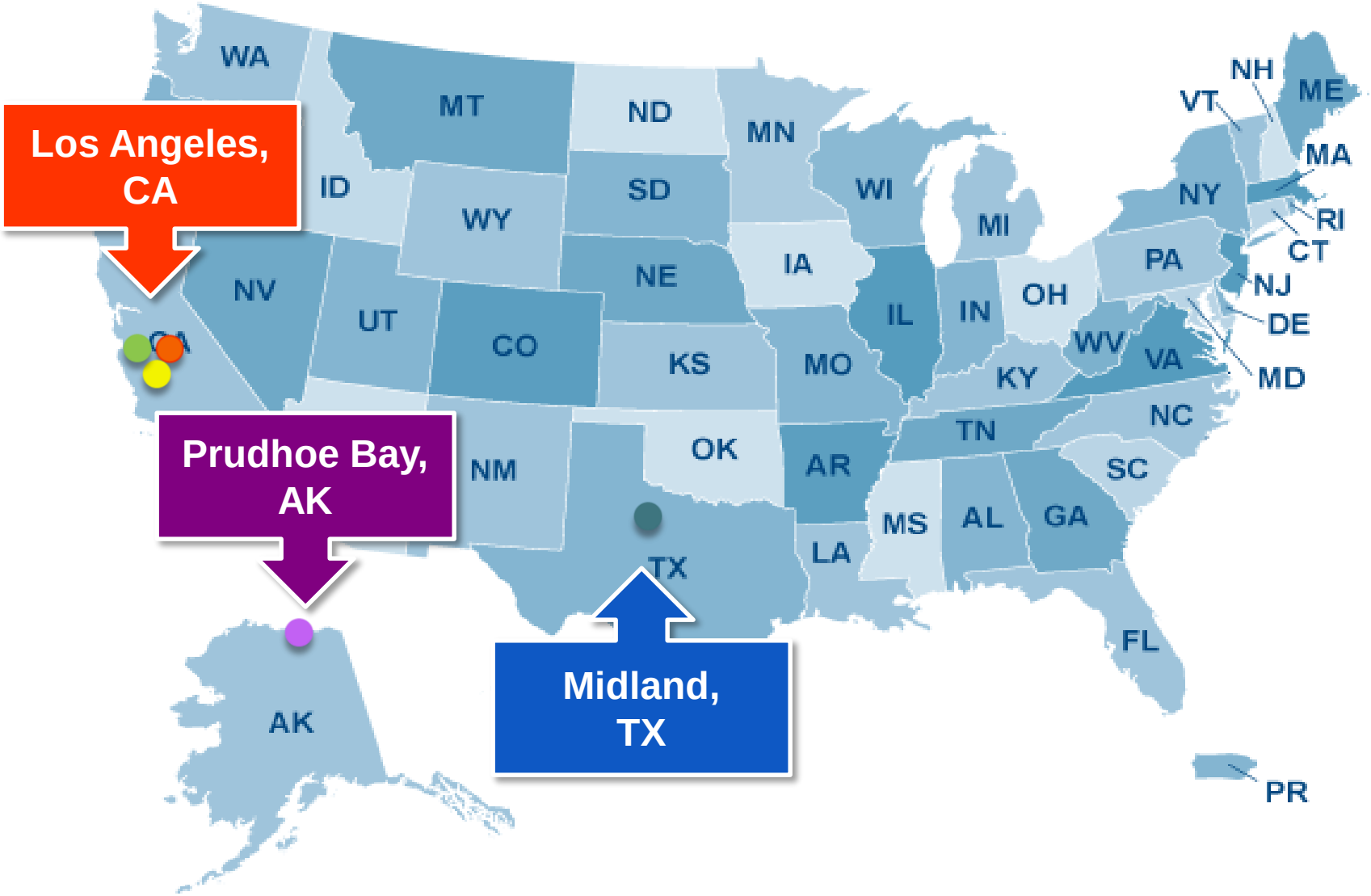
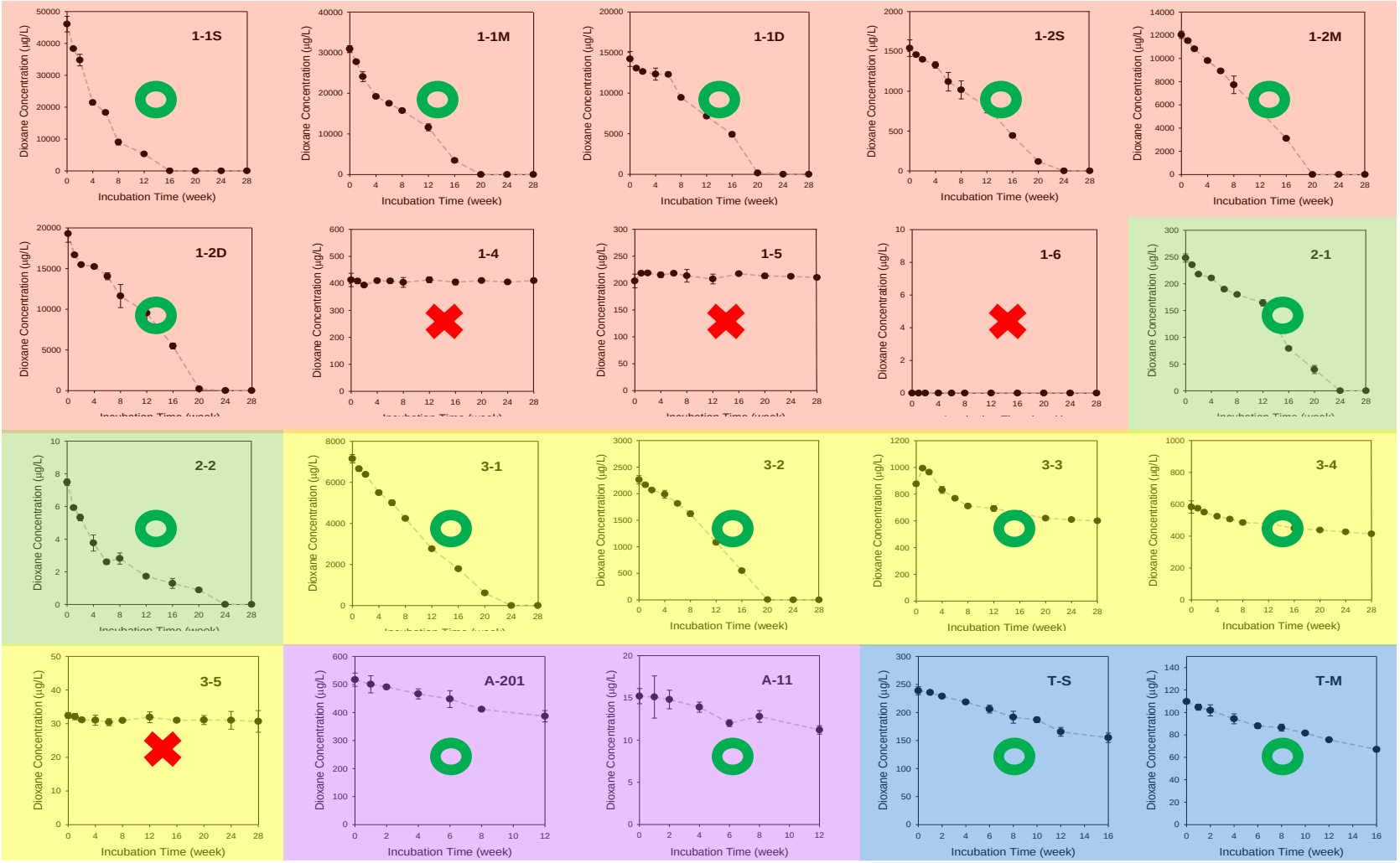


Probe Design

Sampling Sites in US

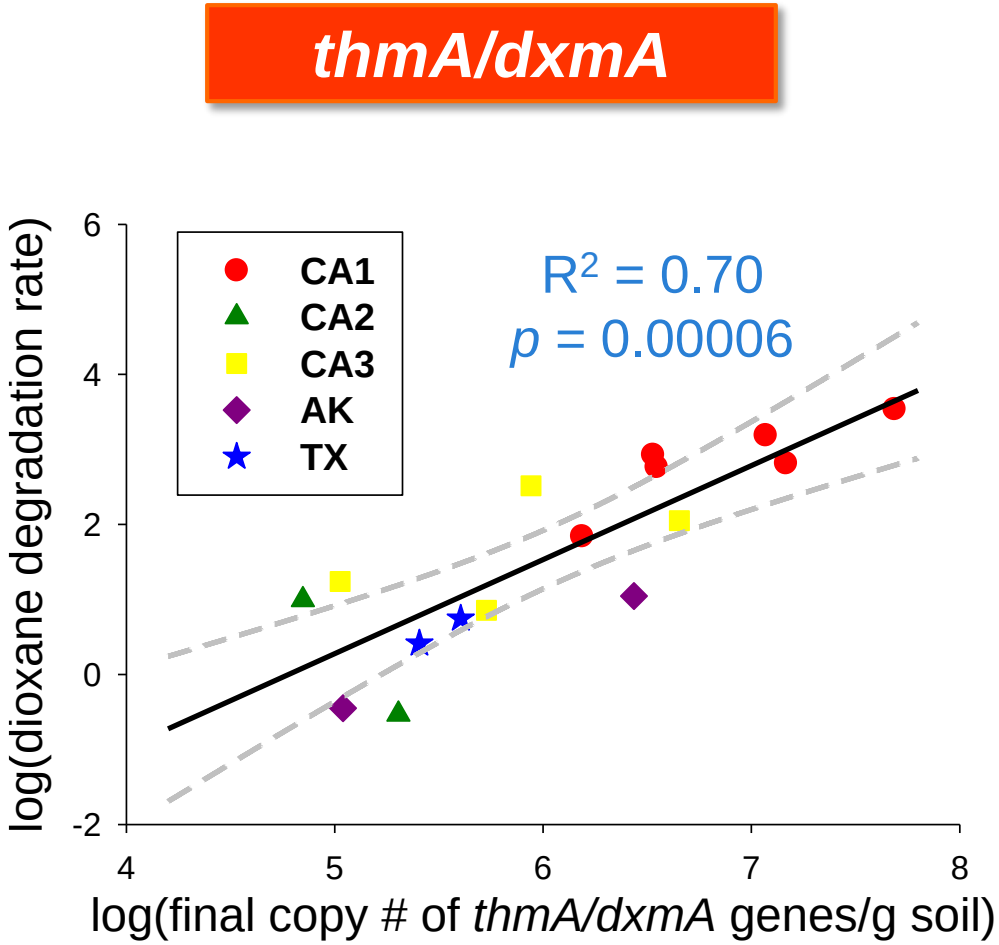


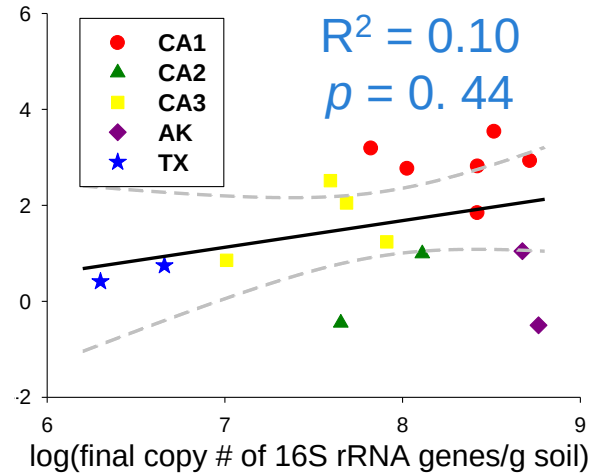
20 Microcosm Sets Mimicking Dioxane Natural Attenuation (80% +)



Li *et al.*, ES&T Letters, 2014; Li *et al.*, Biodegradation, 2014

Degradation Activity Is Correlated with the Abundance of *thmA/dxmA* Genes



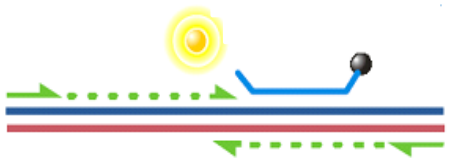


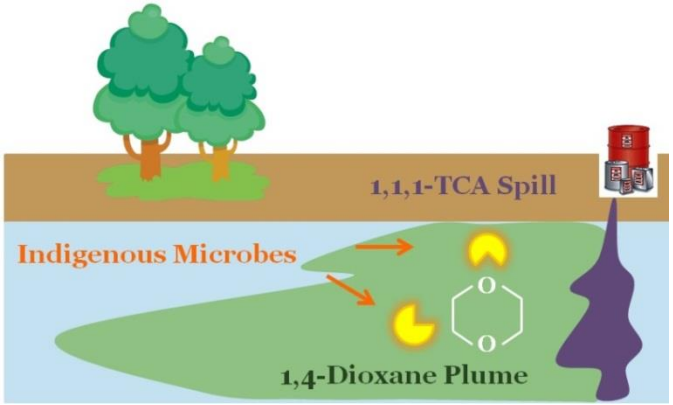
Li *et al.*, ES&T Letters, 2014

Concluding Remarks

- MNA is likely a feasible and cost-efficient treatment alternative for some dilute dioxane plumes. However, *improved forensic tools to ensure MNA is occurring are needed.*
- Catabolic biomarkers of high selectivity and sensitivity were developed to target THF/dioxane MO genes using *Taqman* chemistry.
- Dioxane degradation rates, averaged over large spatial and temporal scales that are relevant to MNA, are correlated to the abundance of *thmA/dxmA genes*, suggesting them as reliable indicators of dioxane biodegradation activity.

Implications of Biomarker Technique in Environmental Remediation





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Acknowledgements

Stephanie Fiorenza
Michael McAnulty

Jim Hatton
Bill DiGuiseppi

David DeVries
Zhong Xiong
Patrick Hsieh

Rob Hinchee

Anita Erickson
Melissa Pike

Ramona Darlington

Any Questions?

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