



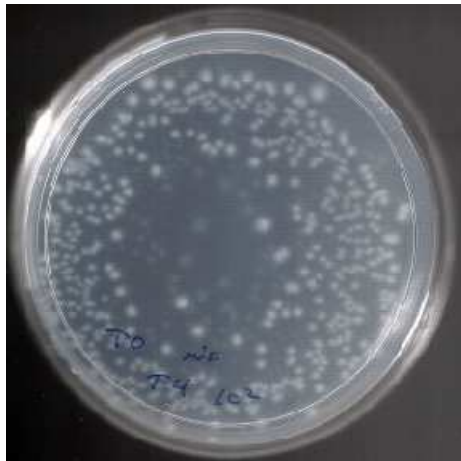
Molecular Biological Tools for Site Assessment & Remedy Selection

Microbial Insights, Inc.
www.microbe.com

Why do we need MBTs?

Contaminant concentrations and geochemistry don't always provide the complete picture.

Plate counts do not accurately reflect *in situ* microbial community



< 1 % of bacteria can be cultured in the laboratory

Questions that MBTs can answer

Is biodegradation occurring?

Stable Isotope Probing

Compound Specific Isotope Analysis

What is the concentration of contaminant degraders?

qPCR

QuantArray

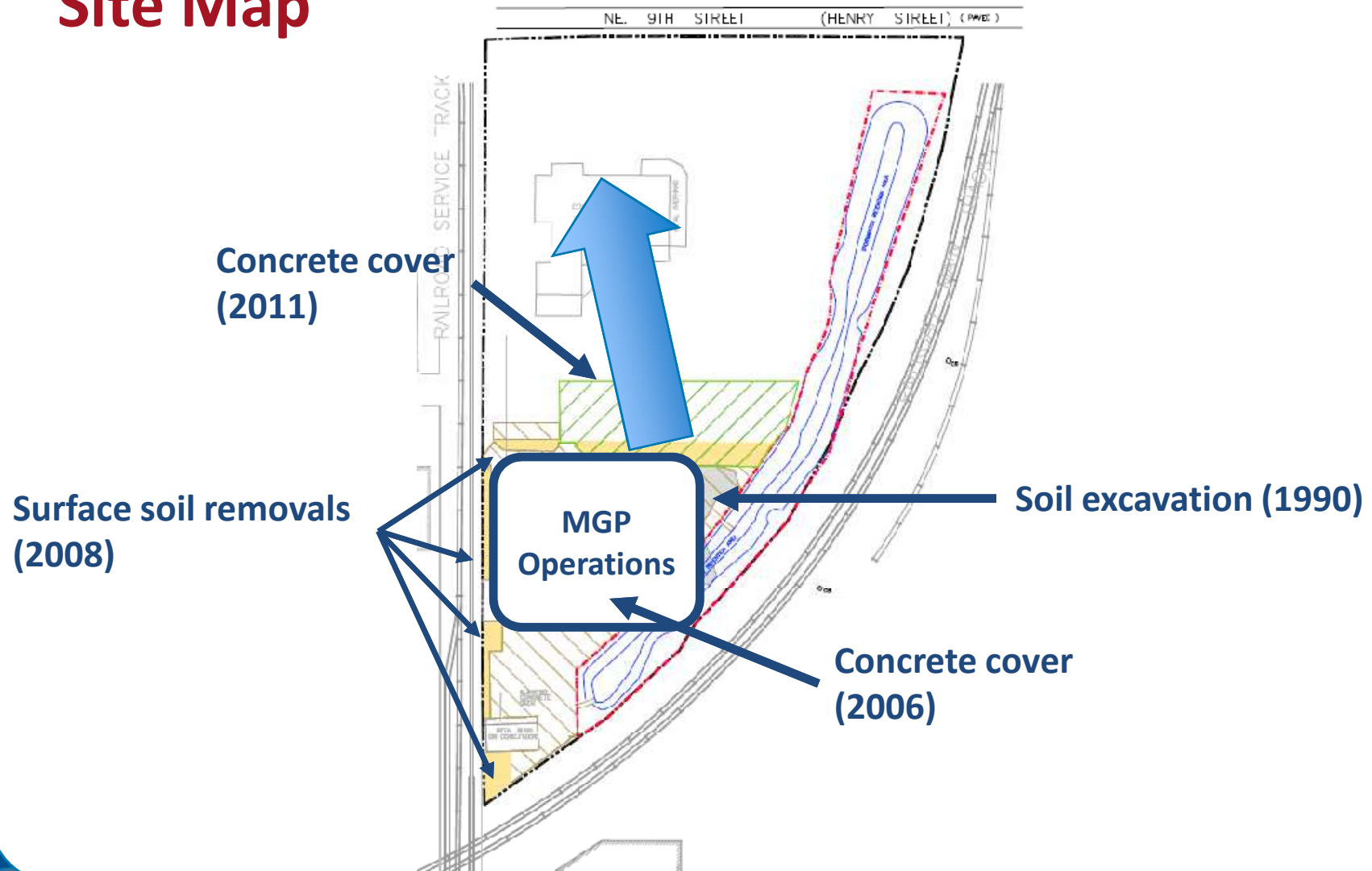
What microorganisms are present?

Next Generation Sequencing
(metagenomics)

Site Background

- Former manufactured gas plant (MGP)
- Operated from 1890s until 1953
- NAPL present
- Benzene, ethylbenzene
- Naphthalene, methylnaphthalenes, and other PAHs

Site Map

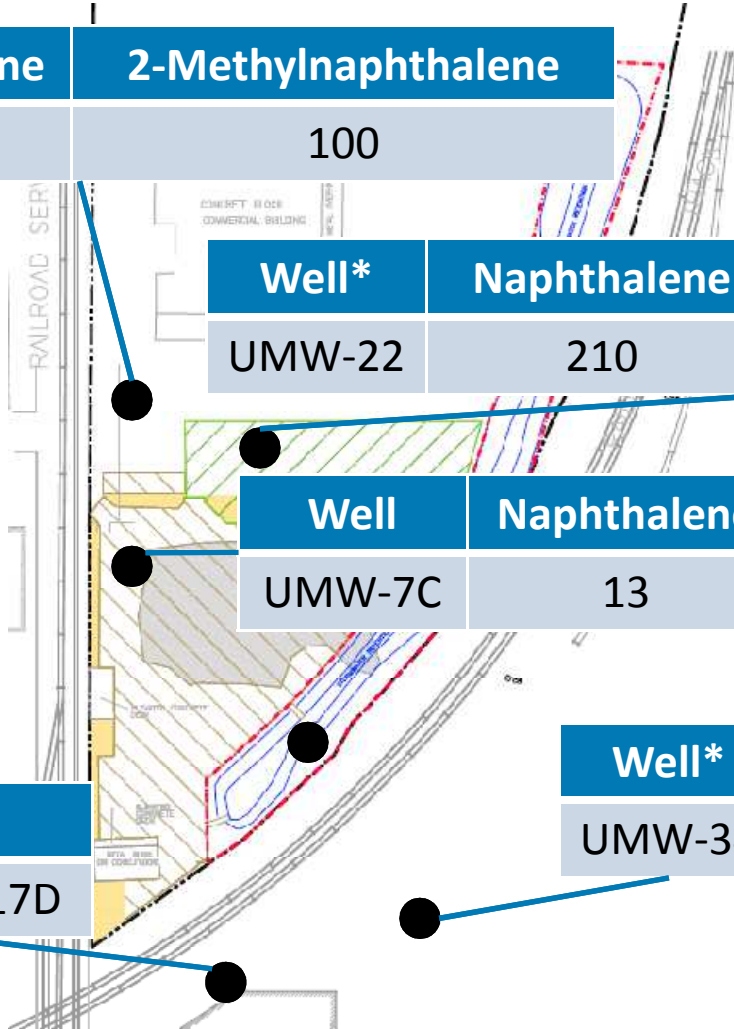


Weathered limestone unit - NAPL



Groundwater Impacts - PAHs

Well	Naphthalene	2-Methylnaphthalene
UMW-44	15	100



Well*	Naphthalene	2-Methylnaphthalene
UMW-22	210	1,700

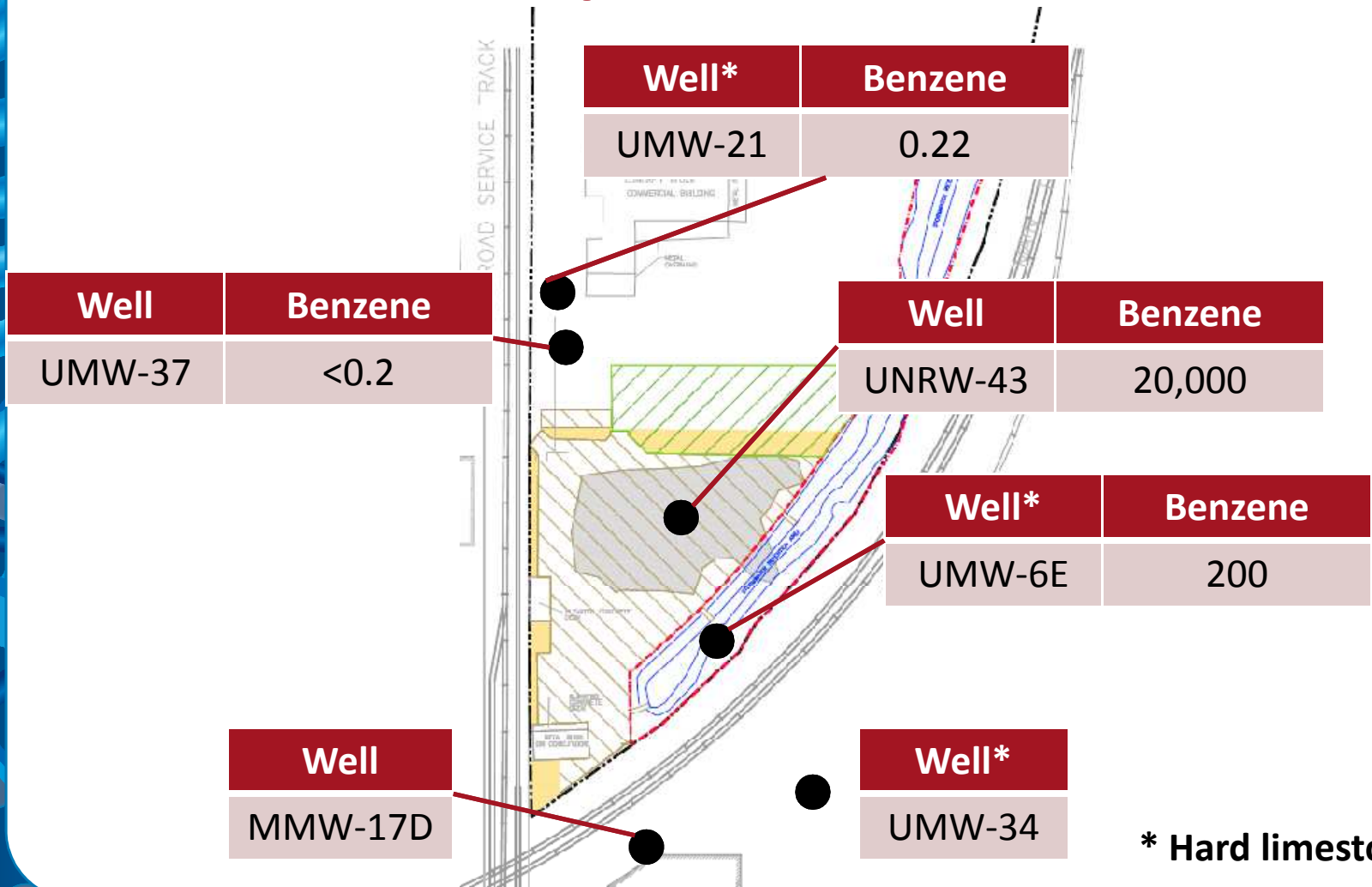
Well	Naphthalene	2-Methylnaphthalene
UMW-7C	13	1,000

Well
MMW-17D

Well*
UMW-34

* Hard limestone wells

Groundwater Impacts – Benzene



* Hard limestone wells

Contaminant Concentrations

Monitoring Well	Benzene Trend	Naphthalene Trend
UMW-7C	No Trend	Decreasing
UMW-44	No Trend	No Trend
UMW-37	No Trend	Near DL
UMW-6E	Decreasing	Decreasing
UMW-21	Decreasing	No Trend
UMW-22	No Trend	Decreasing

MNA Assessment

- ✓ Contaminant concentrations
- ✓ Geochemistry
- Molecular Biological Tools

Is biodegradation occurring?



Stable Isotope Probing
(SIP)

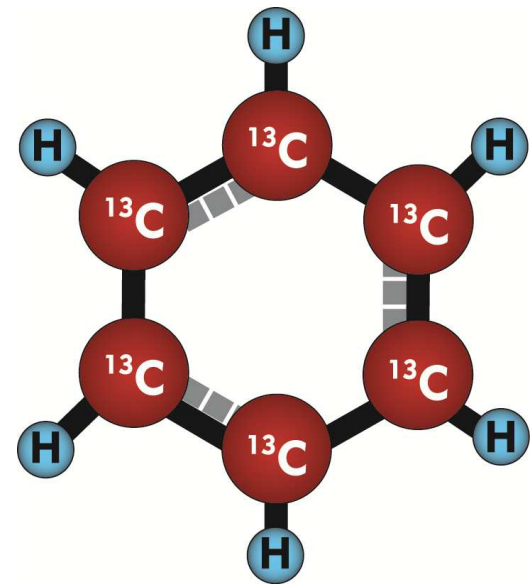
Concentrations of
contaminant degrading
microorganisms?



QuantArray & qPCR

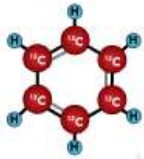
Stable Isotope Compounds

- Specially produced “heavy” compounds which are composed of 99+% ^{13}C
 - Natural compounds are 99% ^{12}C
 - Same characteristics as original compound
 - Behave similar to the natural compound
- Used as “tracers” to increase our understanding of contaminant fate



Overview of Bio-Trap SIP Approach

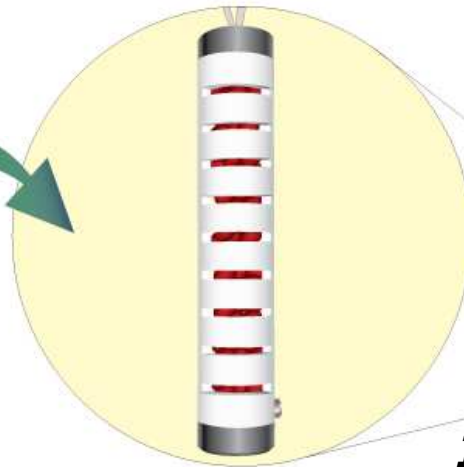
^{13}C labeled Benzene



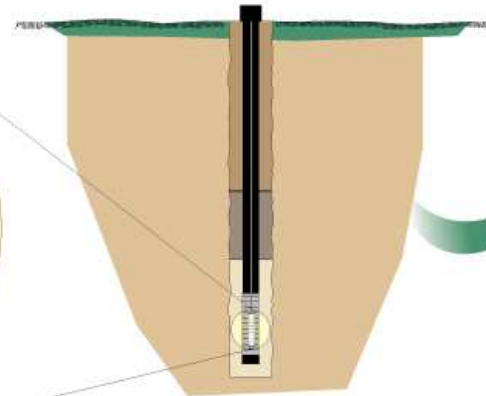
Beads loaded with ^{13}C compound



Bio-Trap with ^{13}C -benzene loaded beads



***In-Situ* deployment in monitoring well**



Beads analyzed following deployment



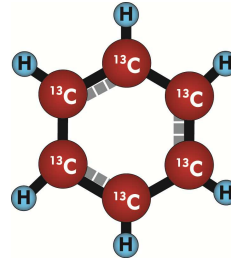
What Are Bio-Trap[®] Samplers?

- Passive microbial sampling tool
- Colonized by active microbes
- 25% Nomex and 75% PAC
- Used in conjunction with
 - Stable isotope probing
 - qPCR and QuantArray
 - Other MBTs



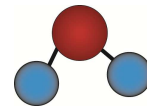
Bio-Trap SIP Analysis

Residual ^{13}C -Compound



Contaminant Loss

$^{13}\text{C}/^{12}\text{C}$ Dissolved Inorganic Carbon



Mineralization
(C for energy)

$^{13}\text{C}/^{12}\text{C}$ of Biomarkers



PLFA
DNA
RNA

Metabolism
(C for growth)

Unit of measure

Amount of ^{13}C relative to ^{12}C is expressed by the $\delta^{13}\text{C}$ notation

$$\delta^{13}\text{C} \text{ [‰]} = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{Sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{Standard}}} - 1 \right) \cdot 1000$$

The standard is a specific carbon-containing mineral from a specific location: Pee Dee Belimnite (PDB)

Units of $\delta^{13}\text{C}$ are ‰ or “per mill”

MNA Assessment

- ✓ Contaminant concentrations
- ✓ Geochemistry
- Molecular Biological Tools

Is biodegradation occurring?



Stable Isotope Probing
(SIP)

Concentrations of
contaminant degrading
microorganisms?

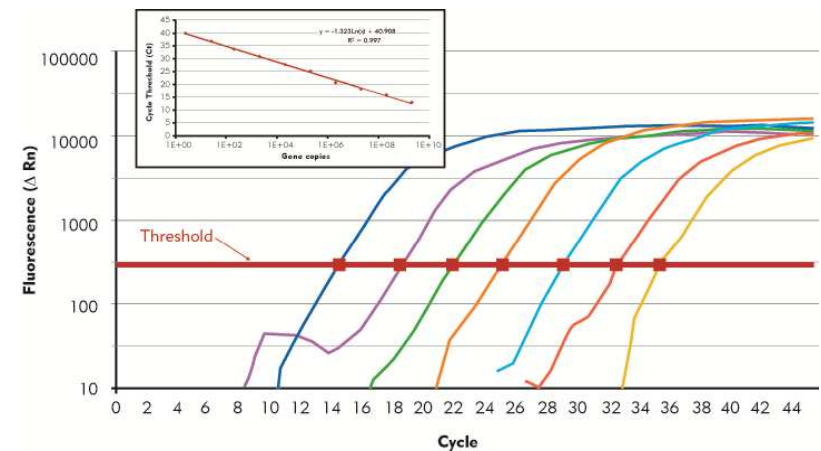


QuantArray & qPCR

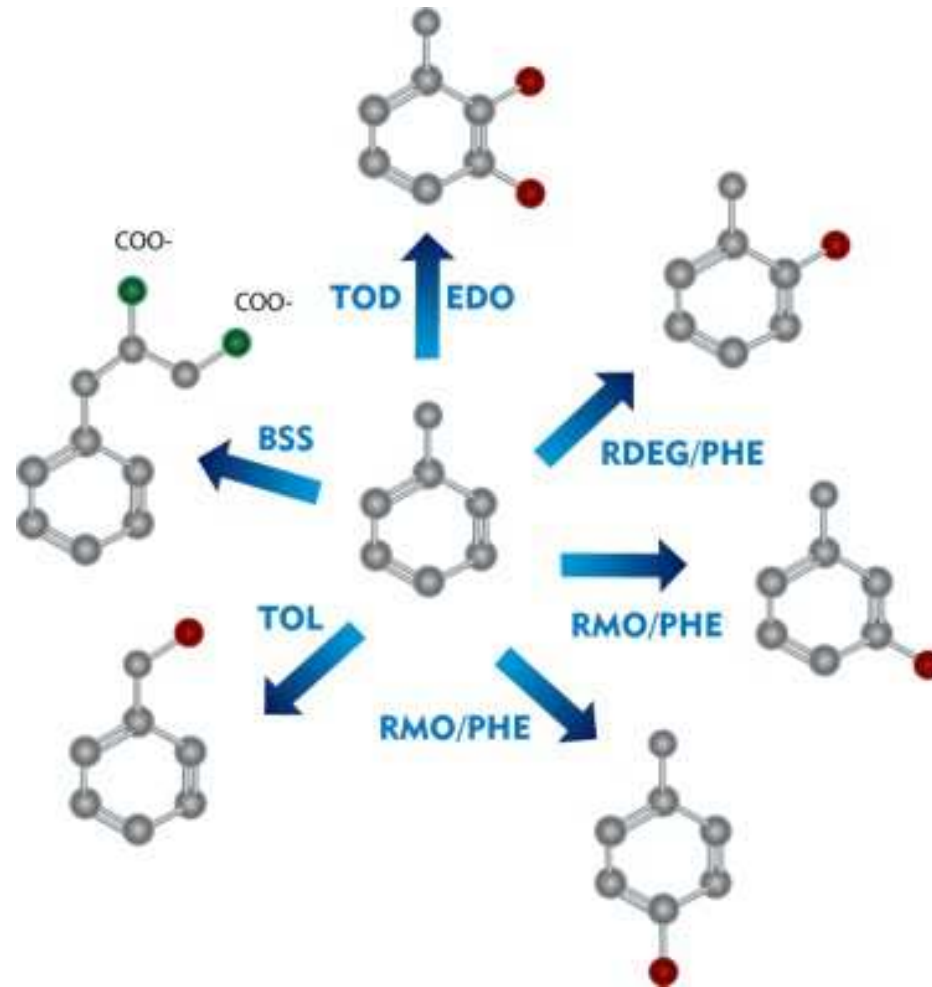
What is CENSUS (qPCR)?

Rapidly detect and quantify a target gene or microbial population

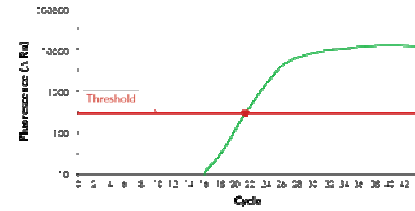
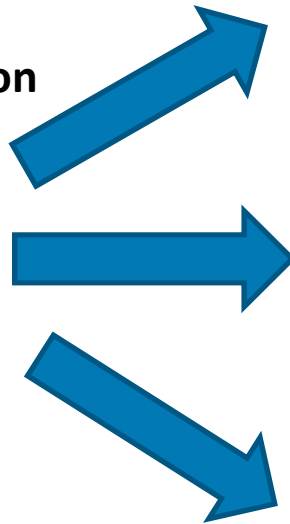
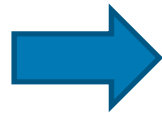
- qPCR Amplification
 - Primers & probe bind to target gene
 - Fluorescence signal increase proportional to concentration
- Two main types of target genes
 - Taxonomic
 - Functional



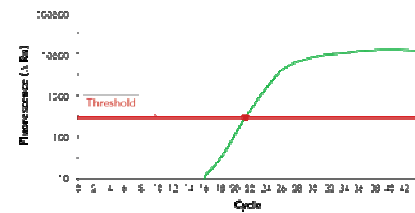
Multiple Pathways



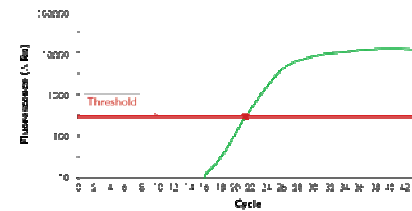
CENSUS qPCR Approach



TOD

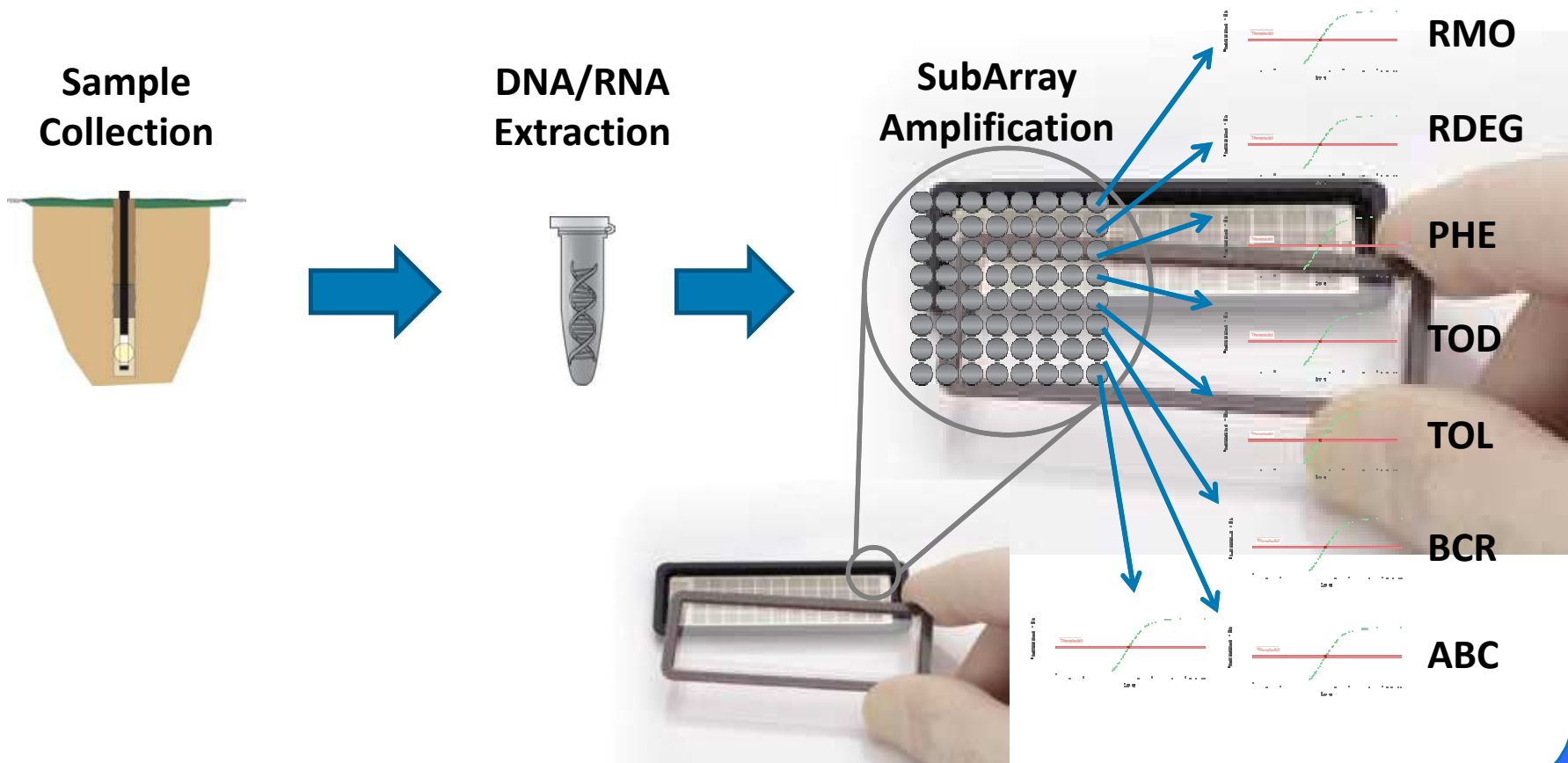


PHE



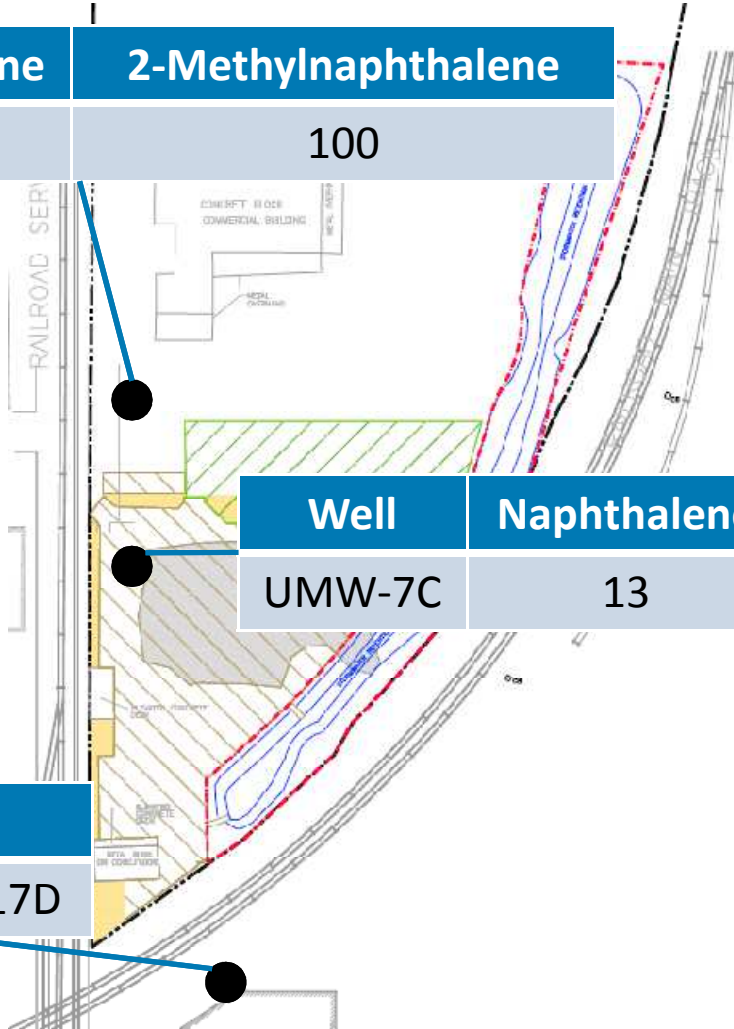
BSS

QuantArray[®] Approach



Study Wells – Weathered Limestone

Well	Naphthalene	2-Methylnaphthalene
UMW-44	15	100

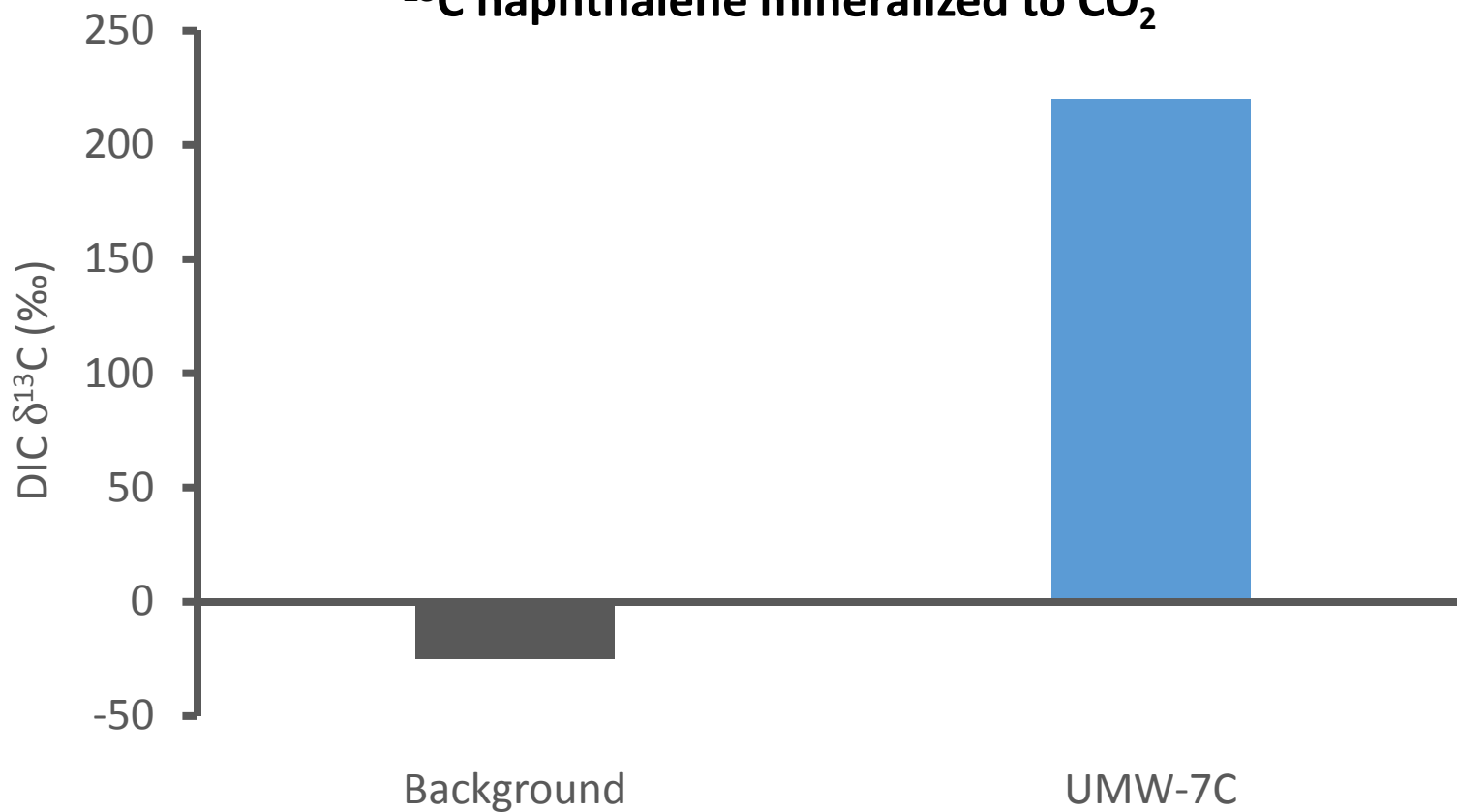


Well	Naphthalene	2-Methylnaphthalene
UMW-7C	13	1,000

Well
MMW-17D

Is naphthalene biodegradation occurring?

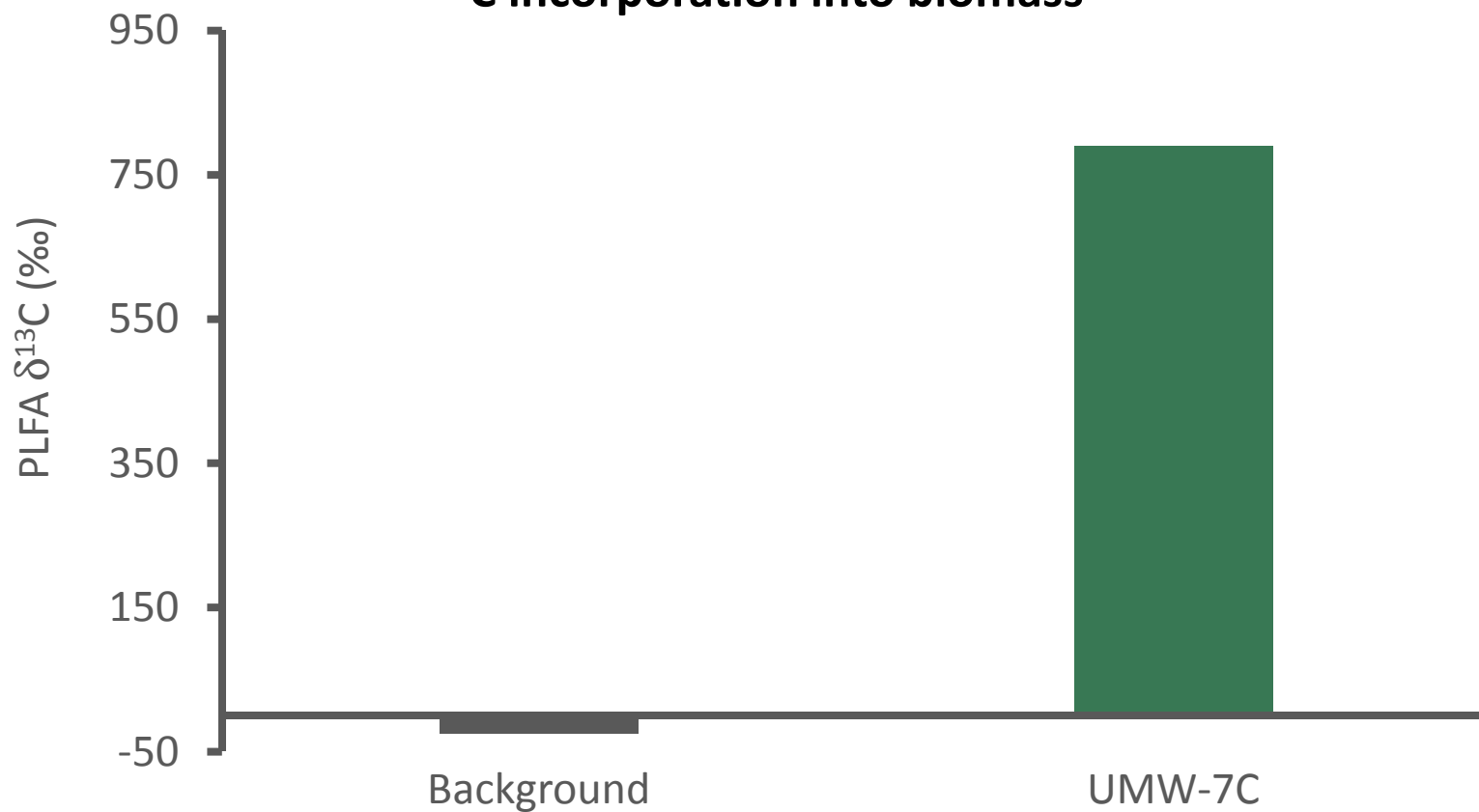
^{13}C naphthalene mineralized to CO_2



UMW-7C

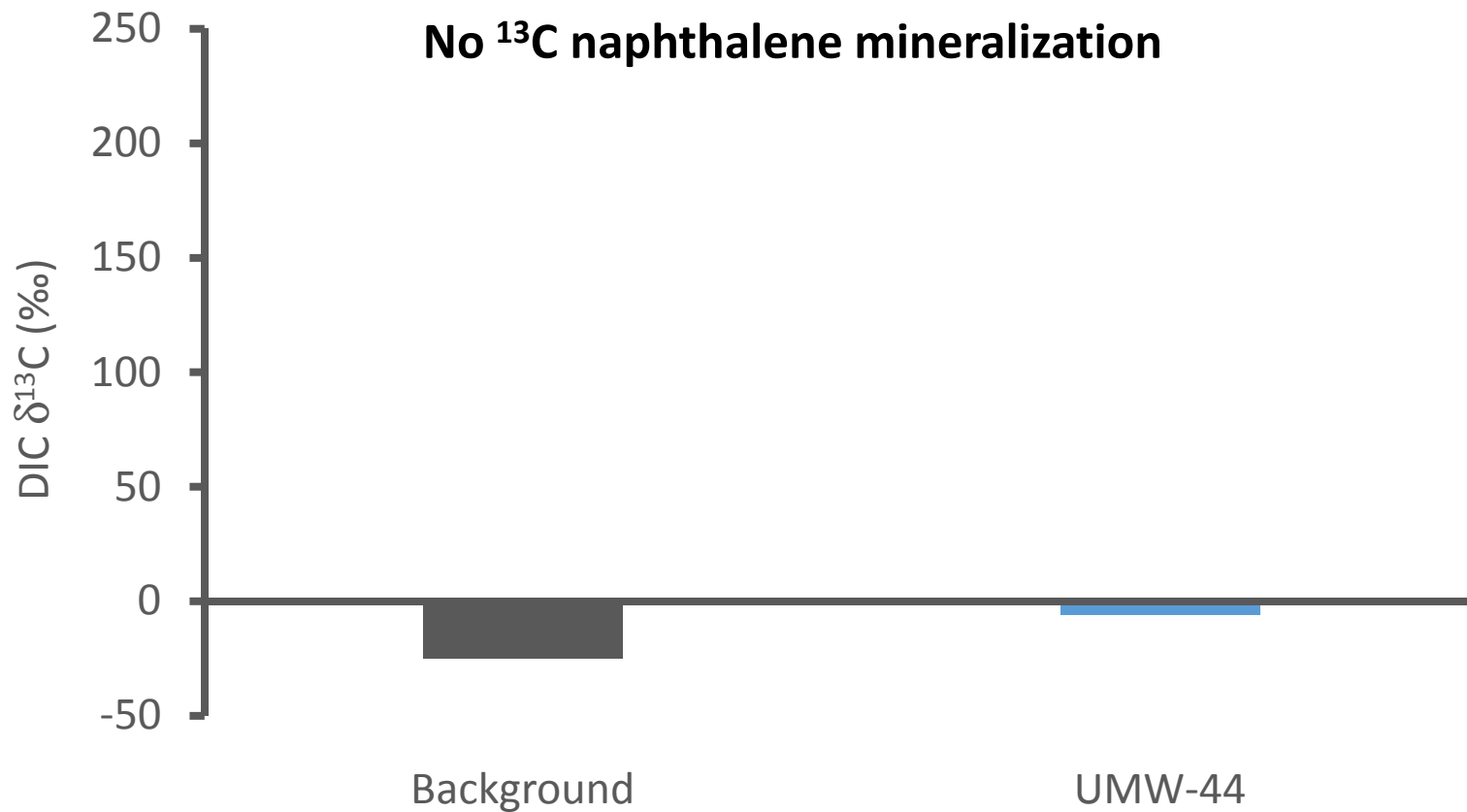
Is naphthalene biodegradation occurring?

¹³C incorporation into biomass



UMW-7C

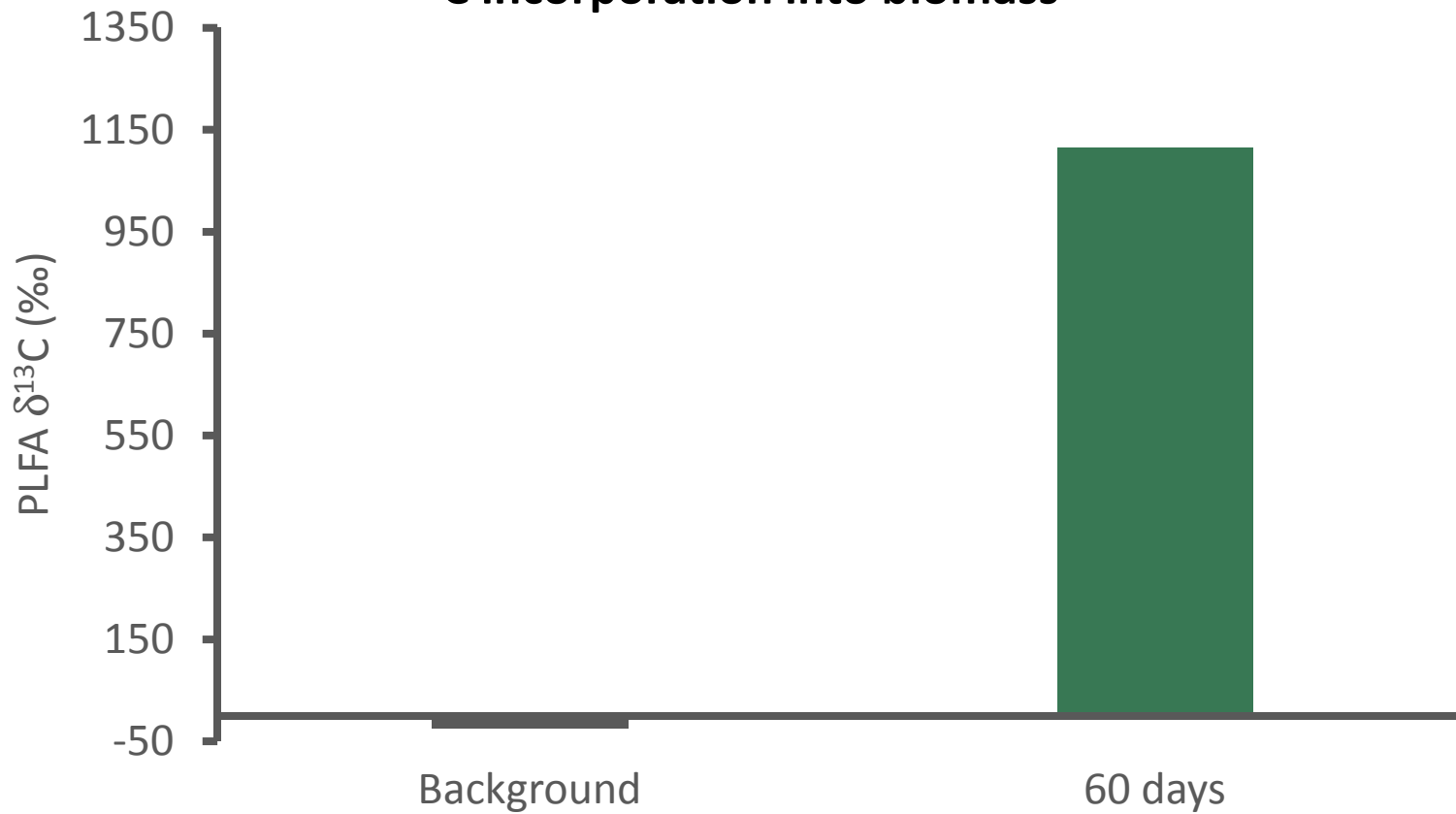
Naphthalene biodegradation downgradient?



UMW-44

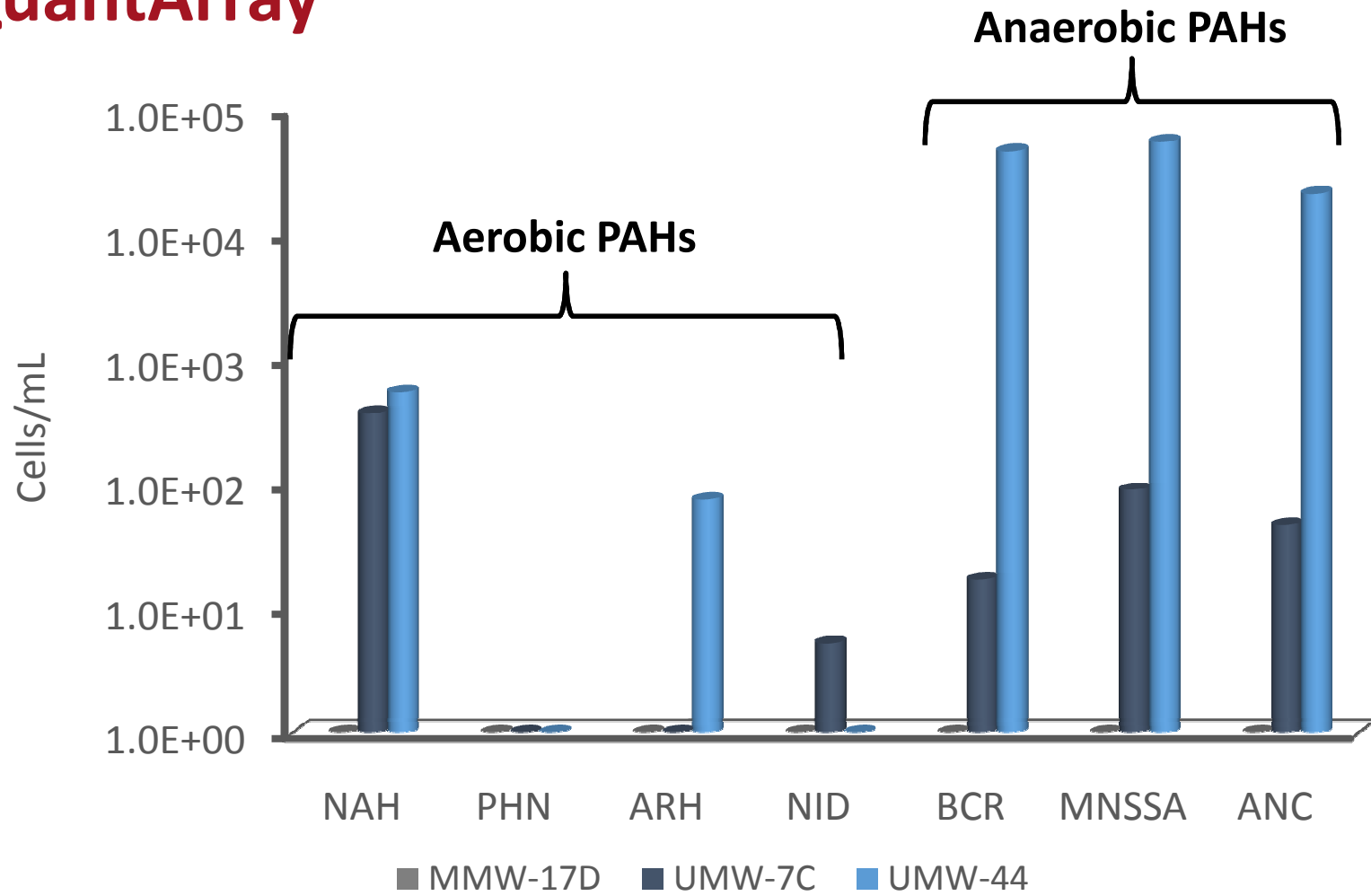
Naphthalene biodegradation downgradient?

¹³C incorporation into biomass






UMW-44

QuantArray



MNA Assessment

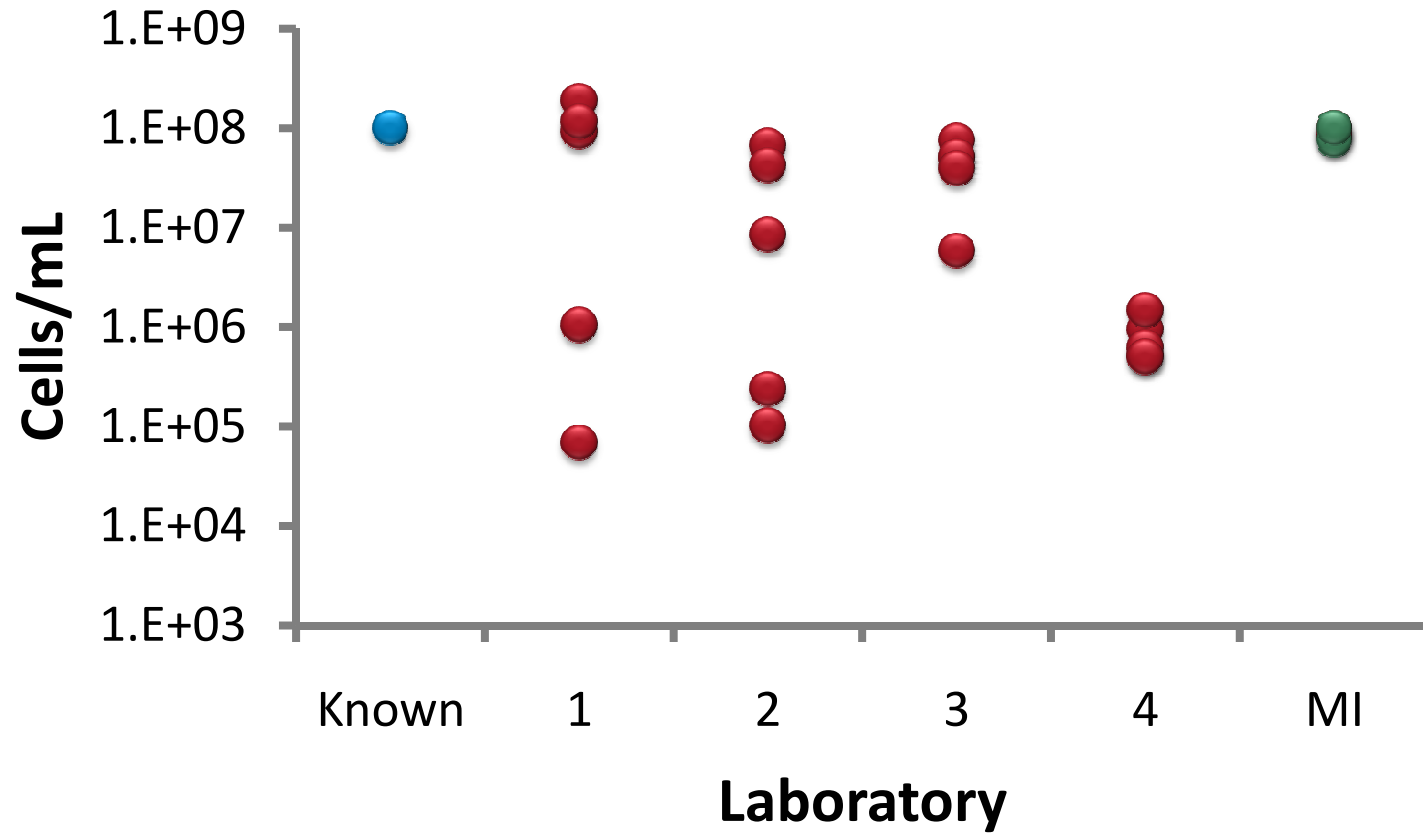
	Chemical	Microbiological	
	Decreasing contaminant concentration?	<u>Stable Isotope Probing</u> Did biodegradation occur?	<u>QuantArray</u> Concentrations of contaminant degraders?
Naphthalene			
Benzene			

A little info about Microbial Insights

Founded in 1992 as a technology transfer company based on the research of Dr. D.C. White at the University of Tennessee

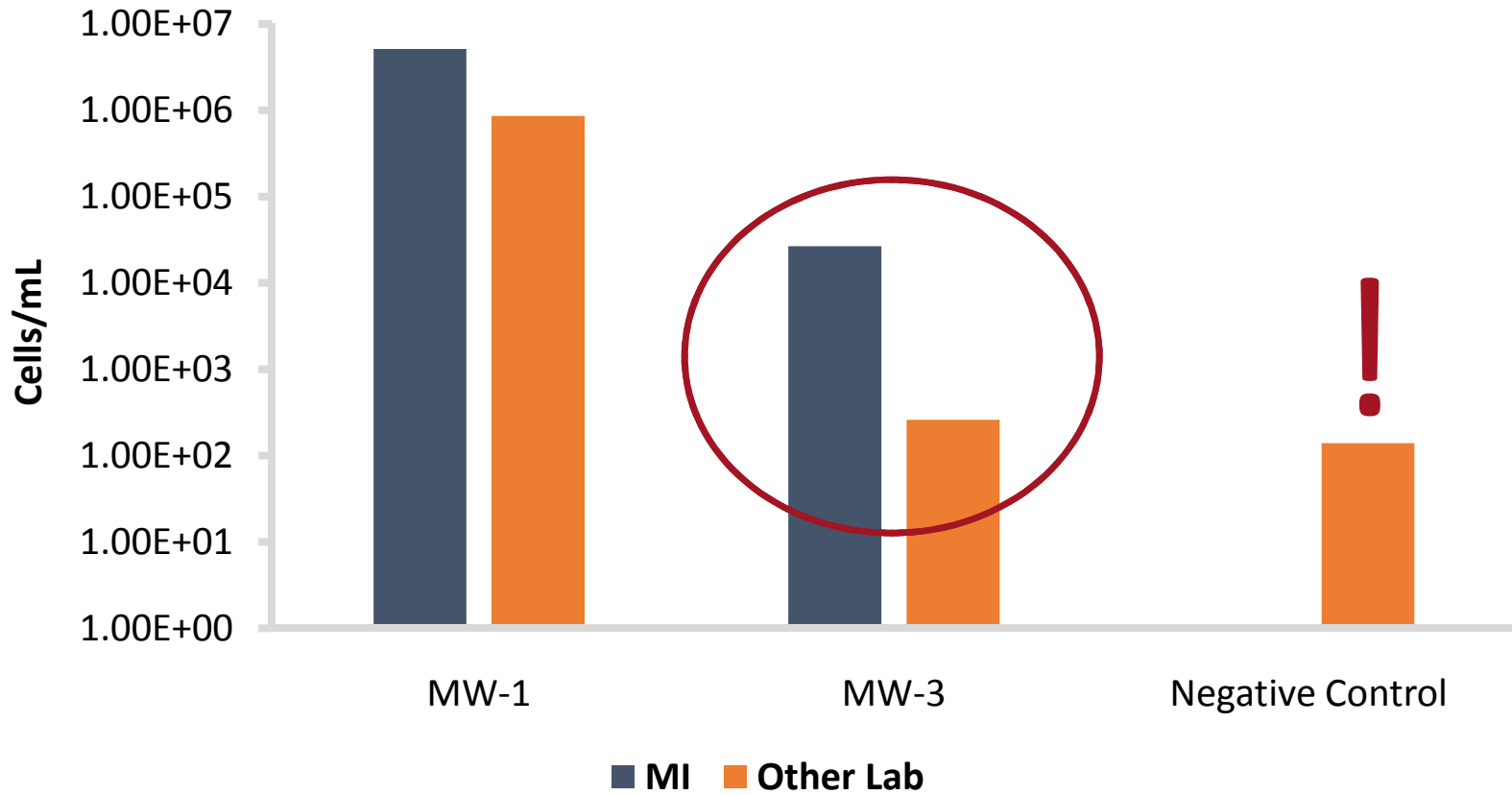


Comparison



Comparison with new laboratory

Dehalococcoides Quantification Comparison



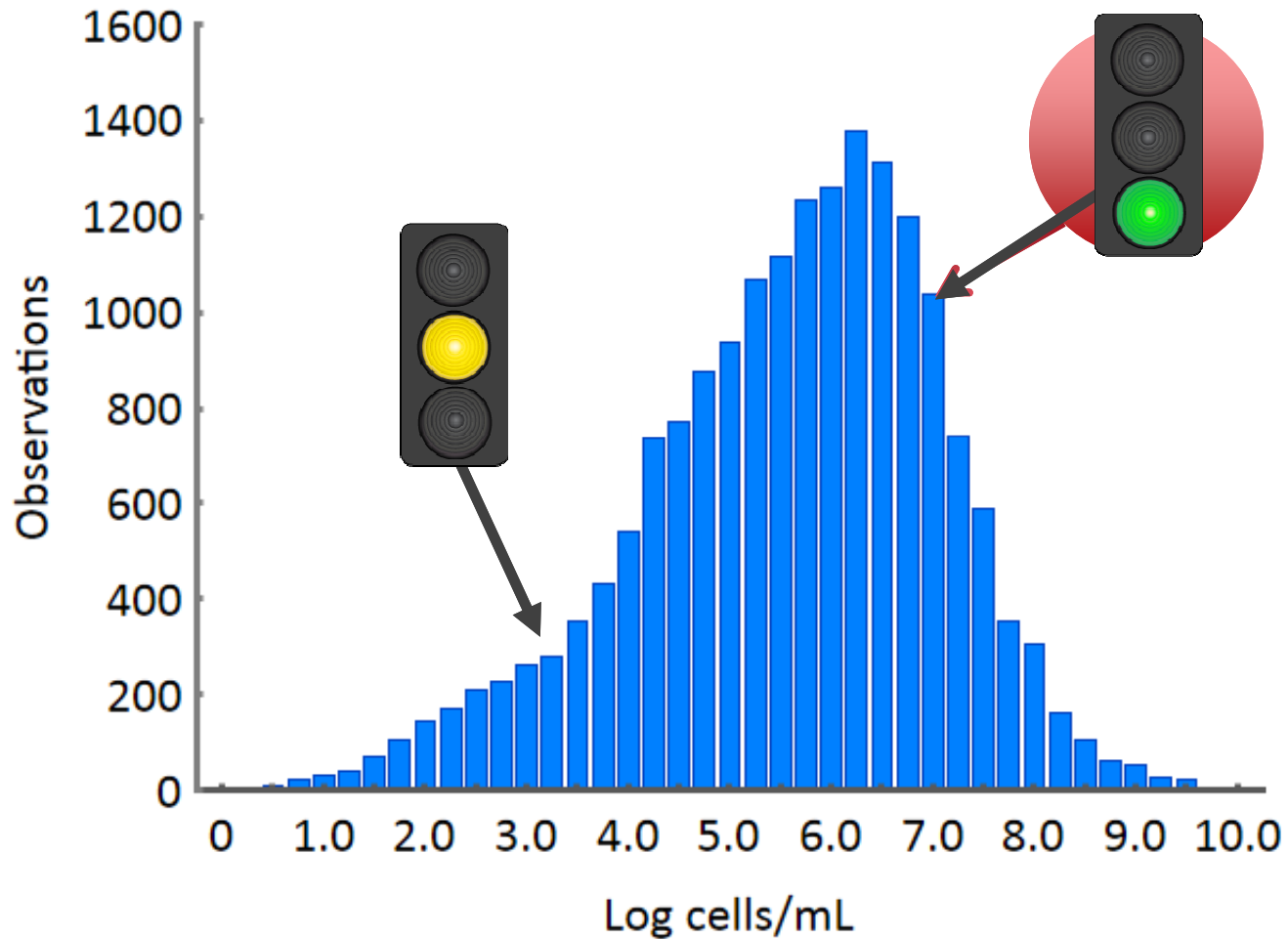
A little more about Microbial Insights

- Experience
- Accuracy, precision and quality control
- Innovation
 - Comprehensive suite of MBT analyses
 - Microbial Insights Database
 - QuantArray & continuous assay development
 - Next Generation Sequencing (Metagenomics)
- Customer Service

Microbial Database

- 20 years of data
- Over 120 unique organisms and gene targets
- Over 43,000 field samples
- All 50 states
- And 34 countries on 6 continents

Is that a low, medium or high concentration?



Resources

Microbial Insights

- Kate Clark (kclark@microbe.com)
- Casey Brown (cbrown@microbe.com)
- (865) 573-8188

Online

- MI webpage (www.microbe.com)
- Remediapedia (www.environmentalrestoration.wiki)
- EPA CSIA Guide (www.nepis.epa.gov)



SIP



CSIA



qPCR



QuantArray



Sequencing

www.microbe.com

ER
WIKI