Omics:

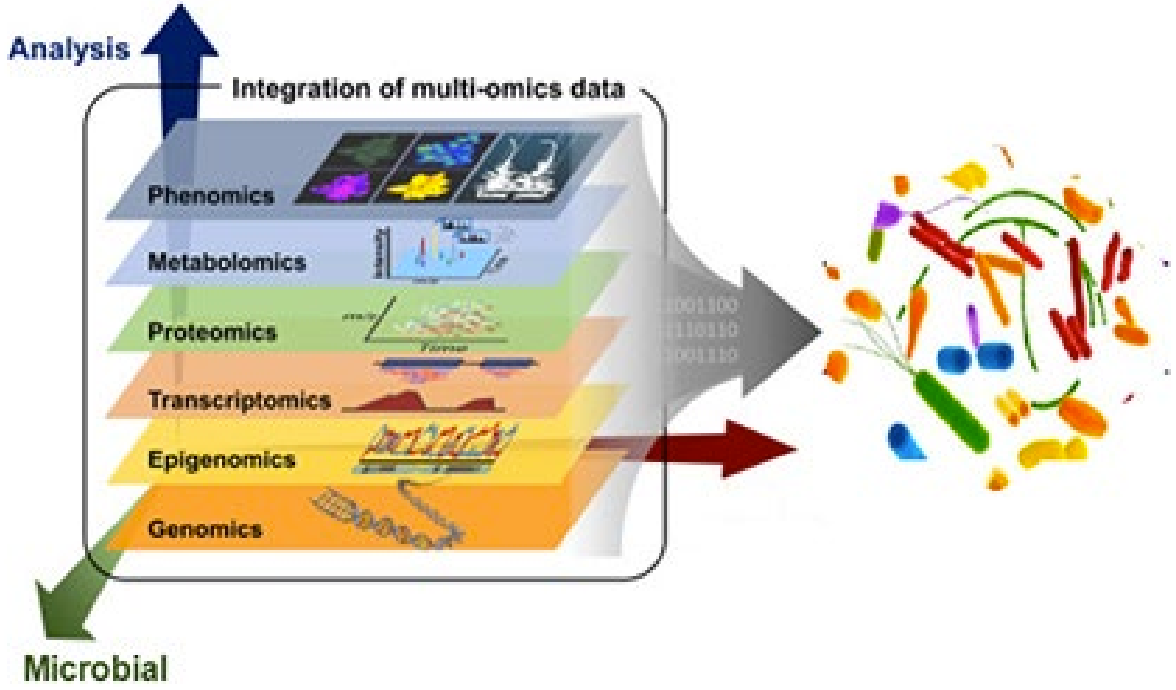
How Not to Make Your Site a Science Project

microbialinsights

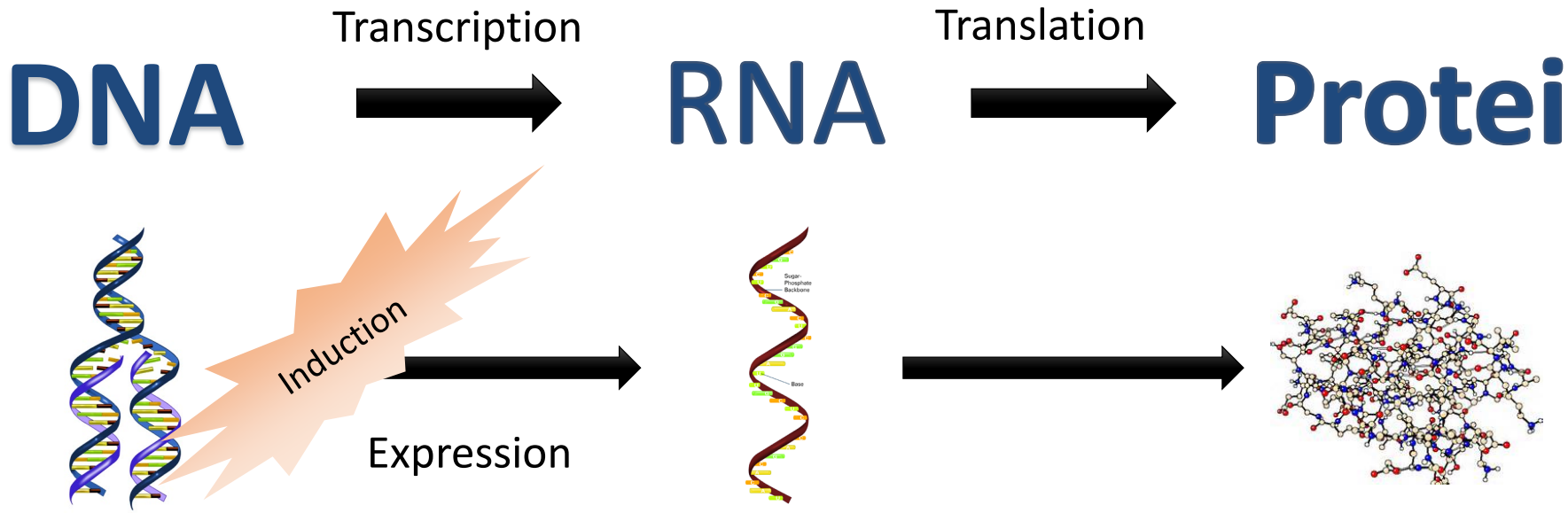
The Omics Revolution

Omics refers to the collective technologies used to characterize and quantify biological molecules and to explore their roles, relationships and actions in the cells of a living creature.

The ’omics’ suffix has been added to describe the use of these technologies to examine proteins (proteomics), the chemical processes involving metabolites (metabolomics) and RNA molecules (transcriptomics) in cells, as well as genomes.



Central Dogma of Molecular Biology



Gene

Potential Activity

mRNA Transcript

Gene Expression

Enzymes

Activity

Microbiology Questions & MBTs

- Who is there?
- Microbes of interest?
- Concentrations of degraders & genes of interest?

qPCR
Metagenomics

- Amplicon sequencing
- Whole genome sequencing

- Who is active?
- Is key organism active?
- What genes are transcribed?
- Degradation pathway transcribed?

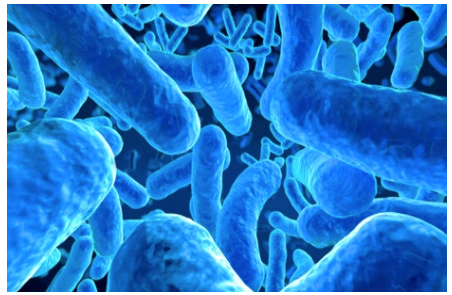
RT-qPCR
Transcriptomics

- What organisms & functions are active?

Proteomics

Metagenomics Terminology

Microorganisms from sample



Mixed Genomic DNA

Shotgun Metagenomics

NGS

Genetic potential of microbial community

Computationally analyze sequences

Who is there?

Amplification with primers targeting gene of interest (e.g. 16S rRNA)

Amplicon Sequencing

PCR Products (amplicons)

Computationally analyze amplicon sequences

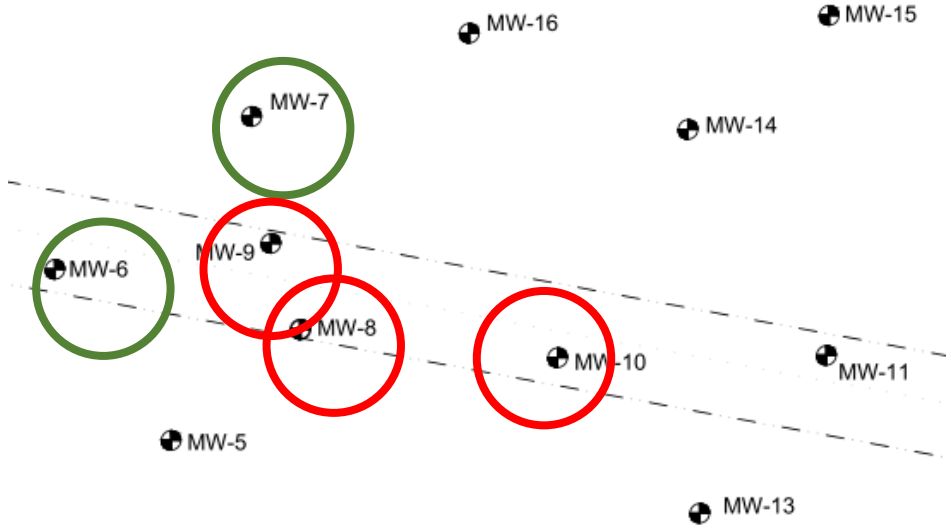
NGS

Amplicon Sequences

(Adapted from Frank Löffler)

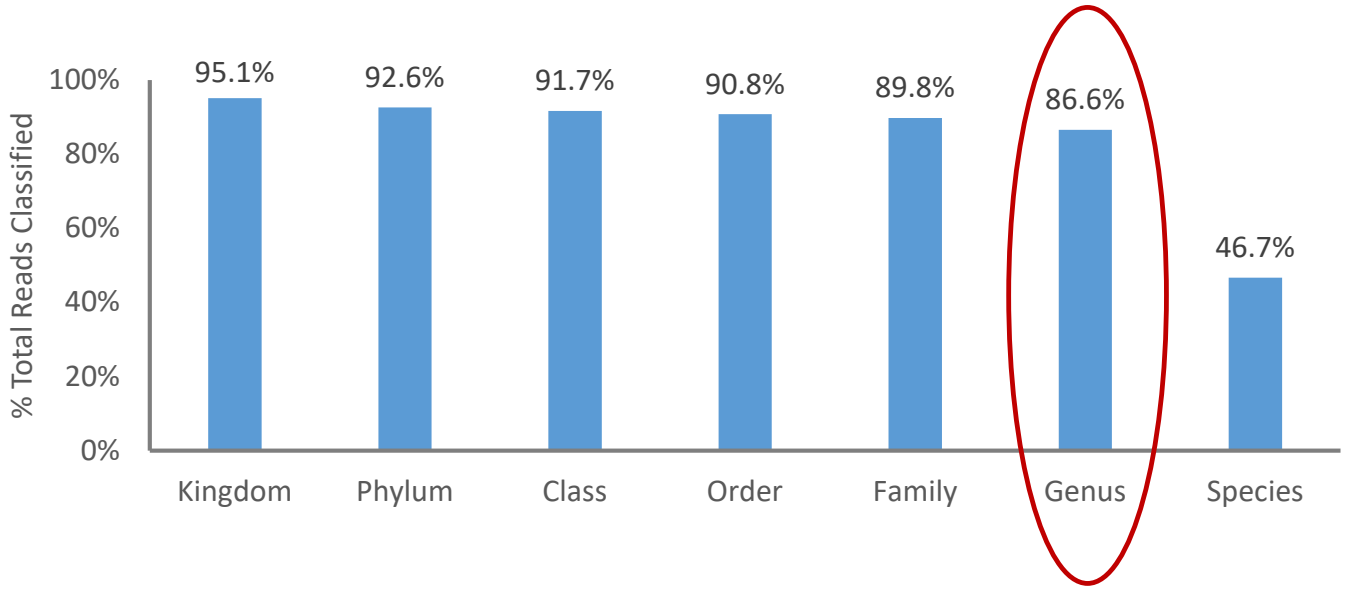
Pipeline Release – Evaluating MNA

- BTEX
  - Stable to decreasing concentrations
- Geochemistry
  - Electron acceptor consumption
  - Predominantly anaerobic redox conditions
- Microbiology
  - Who is there? (NGS)
  - Microbial community changes? (NGS)
  - Concentrations of anaerobic BTEX degraders? (qPCR)



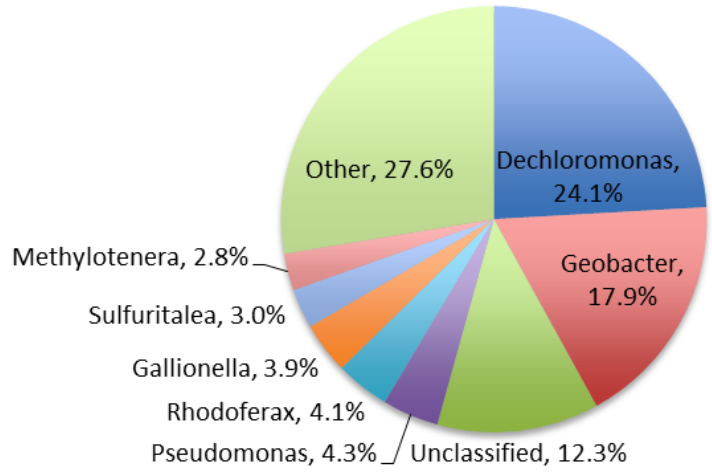
Next Generation Sequencing Results

Sample ID	Reads Passing Quality Filtering	% Reads Classified to Genus	Shannon	Simpson	Chao1 Predicted Genera	Total Genera Observed
MW6	215,515	86.6%	3.4	0.89	570	533
MW7	227,887	80.0%	3.9	0.95	600	530
MW8	308,636	84.1%	3.6	0.92	620	570
MW9	392,036	79.7%	3.8	0.93	650	579
MW10	452,956	84.2%	4.2	0.96	710	663



Top Genus Classification Results

Classification	Number of Reads	% Total Reads	Description
Dechloromonas	146,290	24.1%	Facultative anaerobic bacteria (uses oxygen as electron acceptor when available). Some strains utilize nitrate as an electron acceptor and some can reduce perchlorate and chlorate.
Geobacter	108,799	17.9%	Anaerobic, gram-negative, iron reducing bacteria. Some species can also reduce sulfur.
Unclassified at Genus Level	74,511	12.3%	
Pseudomonas	26,248	4.3%	Pseudomonas is a metabolically diverse genus of aerobic organisms. Some species can also denitrify. Some strains use common hydrocarbons as carbon sources.
Rhodoferax	25,011	4.1%	anaerobic genus that oxidizes acetate with the reduction of Fe (III).
Gallionella	23,727	3.9%	Aerobic, iron oxidizing bacteria
Sulfuritalea	18,234	3.0%	Genus of facultative anaerobes bacteria (uses oxygen as electron acceptor when available) that also reduce nitrate. Grows chemolithoautotrophically by oxidation of reduced sulfur compounds and hydrogen under anoxic conditions. Heterotrophic growth on organic acids.
Methylotenera	16,927	2.8%	Facultative methylotrophs that utilize methylamine. Some may utilize methanol, ethanol and pyruvate.



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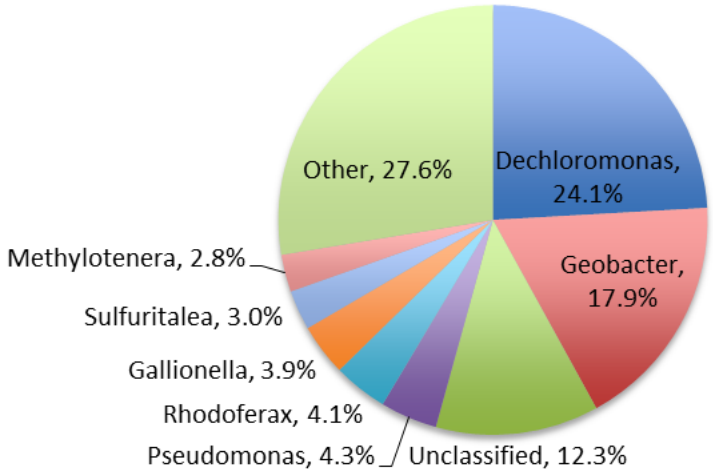
Genus descriptions are not provided by all laboratories



Less relatively abundant microorganisms?

You may have to search summary data files

Summary Files

1
2
3

Kingdom	Phylum	Class	Order	Family	Genus	Species	hit	%hit
Unclassified							32920	5.416
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Dechloromonas	aromatica	130259	21.43
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter		95259	15.673
Bacteria	Proteobacteria	Betaproteobacteria	Gallionellales	Gallionellaceae	Gallionella		21151	3.48
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Sulfuritalea		18234	3.00
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax		14142	2.327
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas		11130	1.831
Bacteria							10708	1.762
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Dechloromonas		10489	1.726
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	ferrireducens	9393	1.545
Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylotenera		9273	1.526
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum		8402	1.382
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium		6732	1.108
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	psychrophilus	6557	1.079
Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylobacillus	glycogenes	6139	1.01
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Oxalobacter	vibrioformis	5618	0.924
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Propionivibrio		5611	0.923
Bacteria	Proteobacteria	Betaproteobacteria					5481	0.902
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Thiorhodococcus	pfennigii	1[illegible]8	0.024

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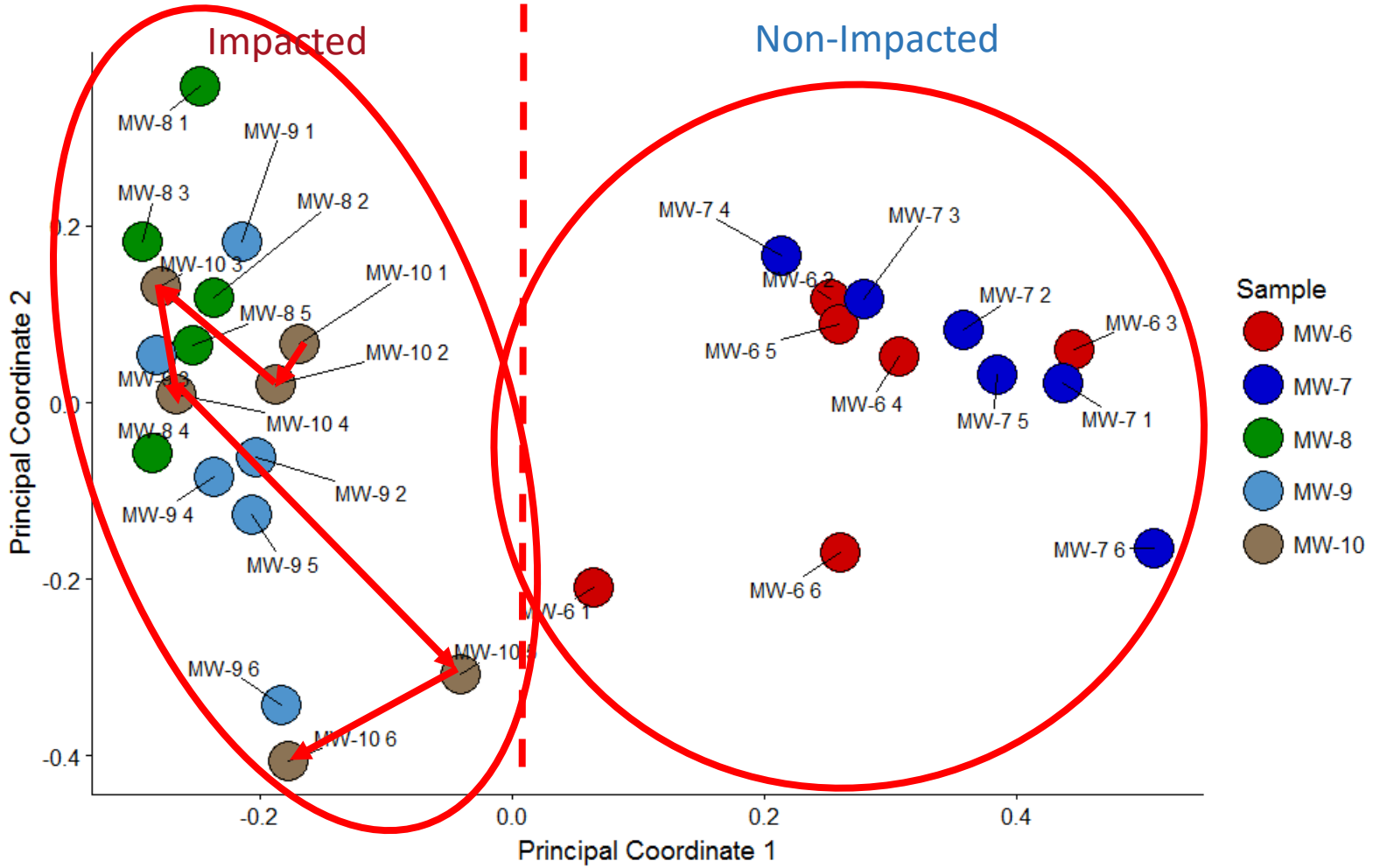
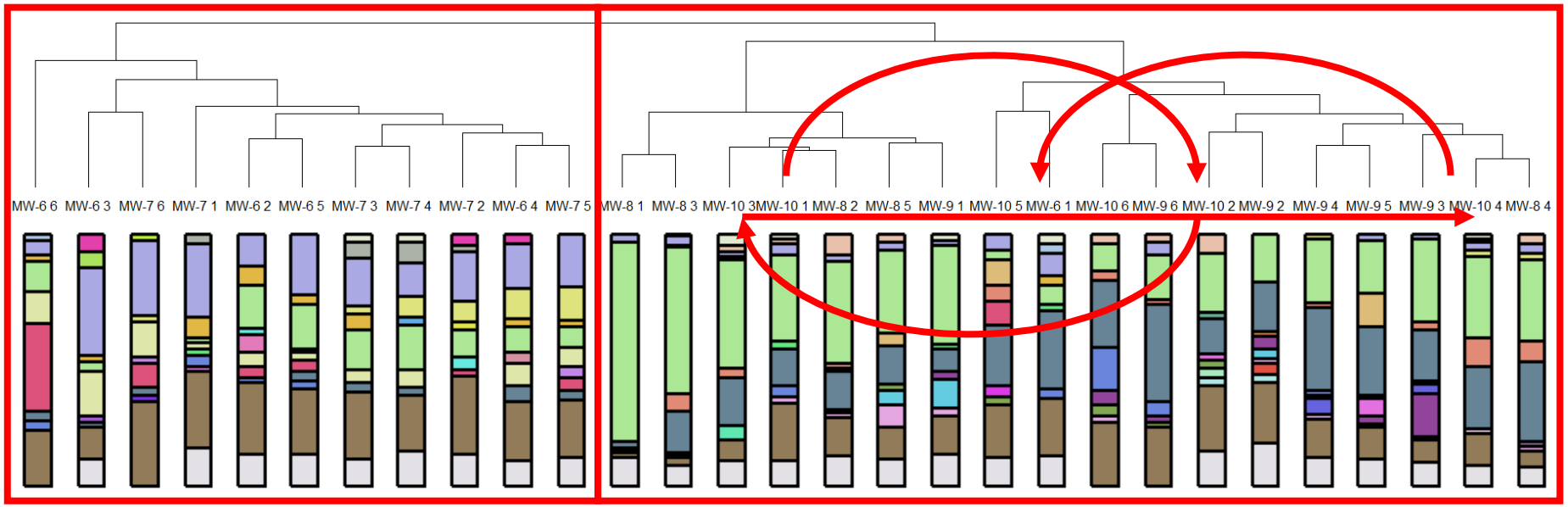
Between Sample Comparisons

- **Individual sample results**
  - Number and percent of reads classified to specific taxonomic levels
  - Alpha diversity measures
  - Lists of classified genera
  - Relative abundances
- **Comparisons between samples**
  - Statistics can help with comparisons – identify key differences and similarities
  - Principal coordinates analysis (PCoA)
  - Hierarchical clustering analysis and dendrograms (HCD)

Principal Coordinate Analysis (PCoA)

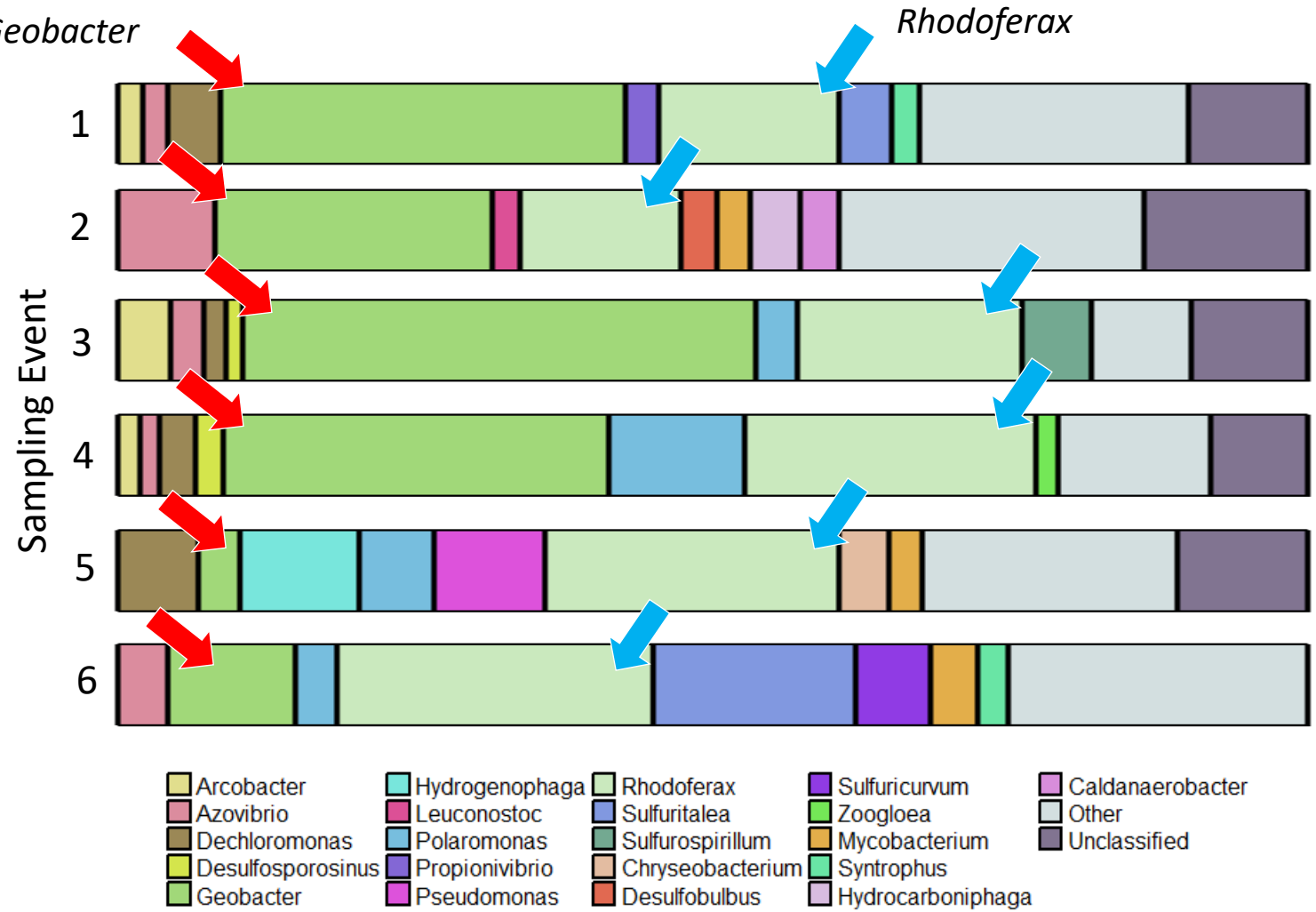


Hierarchical Clustering Dendrogram



- Acinetobacter
- Arcobacter
- Azoarcus
- Azospirillum
- Azovibrio
- Candidatus Amoebophilus
- Crenothrix
- Dechloromonas
- Desulfosporosinus
- Desulfotalea
- Flavobacterium
- Gallionella
- Geobacter
- Hydrogenophaga
- Janthinobacterium
- Leuconostoc
- Limnohabitans
- Methylobacillus
- Methylotenera
- Oxalobacter
- Polaromonas
- Propionivibrio
- Pseudomonas
- Rhodobacter
- Rhodoferax
- Sulfuritalea
- Sulfurospirillum
- Treponema
- Chryseobacterium
- Desulfobulbus
- Desulfuromonas
- Sulfuricurvum
- Zoogloea
- Mycobacterium
- Natronincola
- Pelotomaculum
- Syntrophus
- Hydrocarboniphaga
- Methylomicrobium
- Veillonella
- Caldanaerobacter
- Streptomyces
- Other
- Unclassified

Changes Over Time (MW-10)



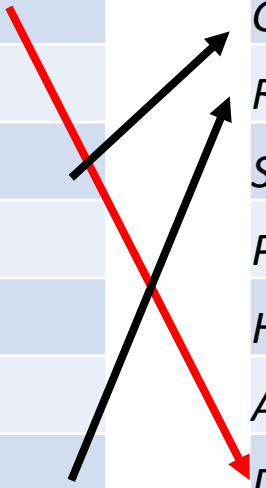
Background vs Impacted

Background (MW-7)

Genus	% Reads
Dechloromonas	21.10
Desulfosporosinus	13.10
Geobacter	8.10
Methylotenera	7.60
Pseudomonas	5.10
Oxalobacter	4.40
Rhodoferax	3.90

Impacted (MW-8)

Genus	% Reads
Geobacter	33.10
Rhodoferax	15.00
Syntrophus	8.70
Pelotomaculum	5.70
Hydrogenophaga	5.10
Azovibrio	3.60
Dechloromonas	2.90



Species Level Identification (Geobacter)

Background

Geobacter	% Reads
unclassified	8.558
psychrophilus	0.411
uraniireducens	0.137
pickeringii	0.132
grbiciae	0.07
toluenoxydans	0.026
hydrogenophilus	0.015
argillaceus	0.013
lovleyi	0.003

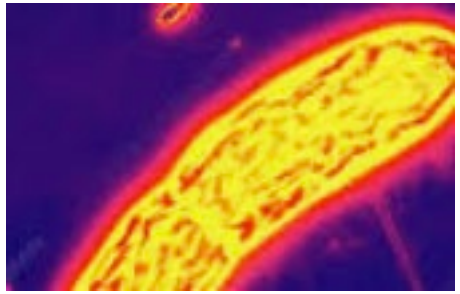
Impacted

Geobacter	% Reads
unclassified	11.216
psychrophilus	0.477
uraniireducens	0.326
pickeringii	0.184
grbiciae	0.109
toluenoxydans	0.059
hydrogenophilus	0.03
argillaceus	0.004
lovleyi	4.222

Note: *Geobacter lovleyi* SZ does not utilize toluene

NGS Conclusions

- Background vs Impacted wells
  - Decrease in microbial diversity within plume
  - Higher relative abundance of anaerobes within plume (e.g. *Geobacter*)
- Changes Over Time
  - Shifts in microbial community composition in some impacted wells
  - Competition between microbial groups (e.g. *Geobacter* vs *Rhodoferax*)
  - Do changes correspond to variability in nutrient availability?
- BTEX Biodegradation Potential?
- Species associated with BTEX biodegradation were detected in background & impacted wells

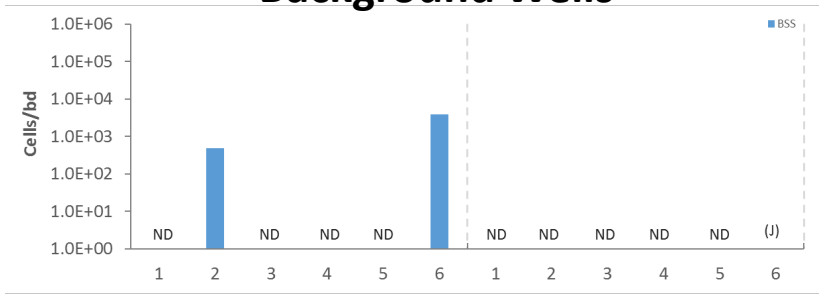


But what are degrader concentrations? → qPCR

Are degrader concentrations high, medium, or low? → Database

qPCR Results & Database Rankings

Background Wells



abcA

bssA

bssA percentiles from MI Database

Impacted Wells

MW-7

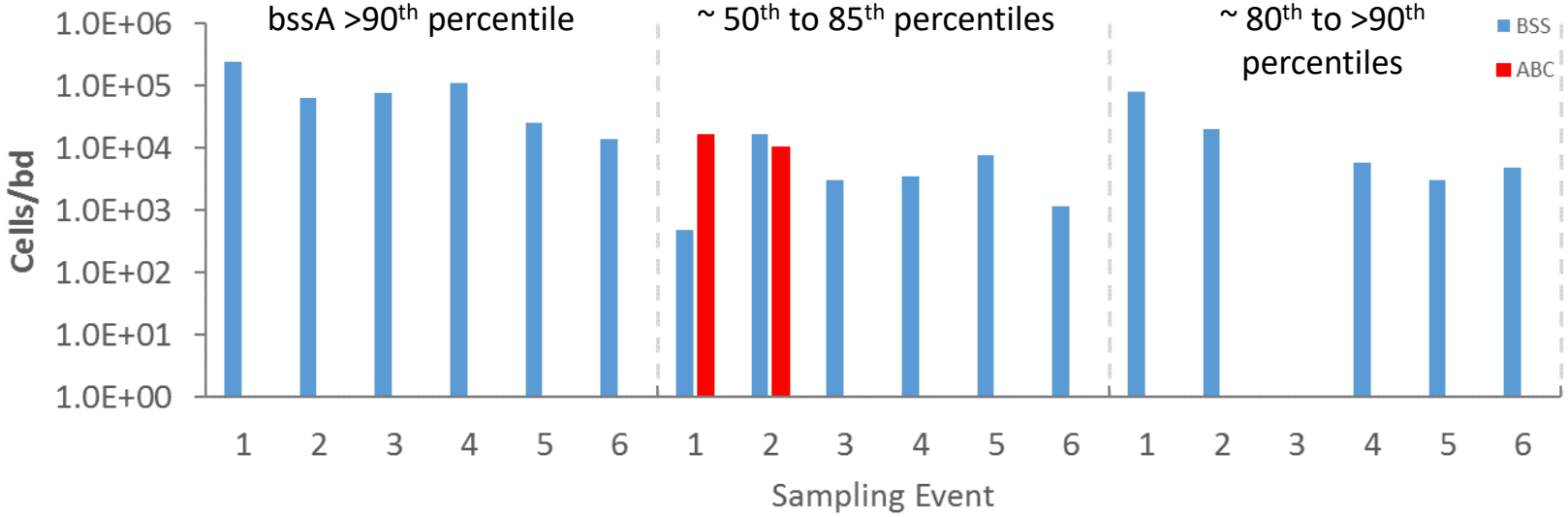
bssA >90th percentile

MW-8

~ 50th to 85th percentiles

MW-9

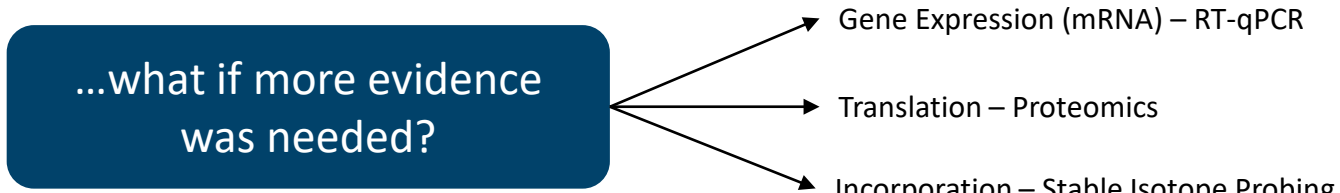
~ 80th to >90th percentiles



qPCR Conclusions

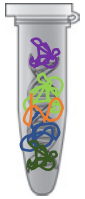
- **BTEX Biodegradation Potential**
  - Concentrations of functional genes responsible for initiating BTEX biodegradation were substantially greater in impacted wells than in background wells
  - High concentrations of bssA in impacted wells compared to other sites (~80th to 90th percentiles)
  - abcA also detected during some sampling events
  - Stable to decreasing BTEX concentrations & electron acceptor utilization

MNA was accepted based on these lines of evidence but…



Global Proteomics

Sample Collection



Sample Processing

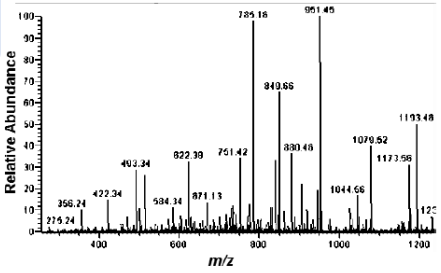
Cell lysis, protein extraction, digestion, and cleanup

HPLC and MS/MS

Peptide separation and identification by MS/MS

Locus ID	Length	Mol Wt	Spectral counts	Best Match	Organism
gi|270154553	519	57534	136	Vinyl chloride reductase	Dehalococcoides sp. VS
gi|146270437	516	57405	4	Reductive dehalogenase	Dehalococcoides sp. BAV1

Raw MS/MS spectra



Metagenomics Results

Match raw MS/MS spectra with predicted spectra

Filtering & assembly

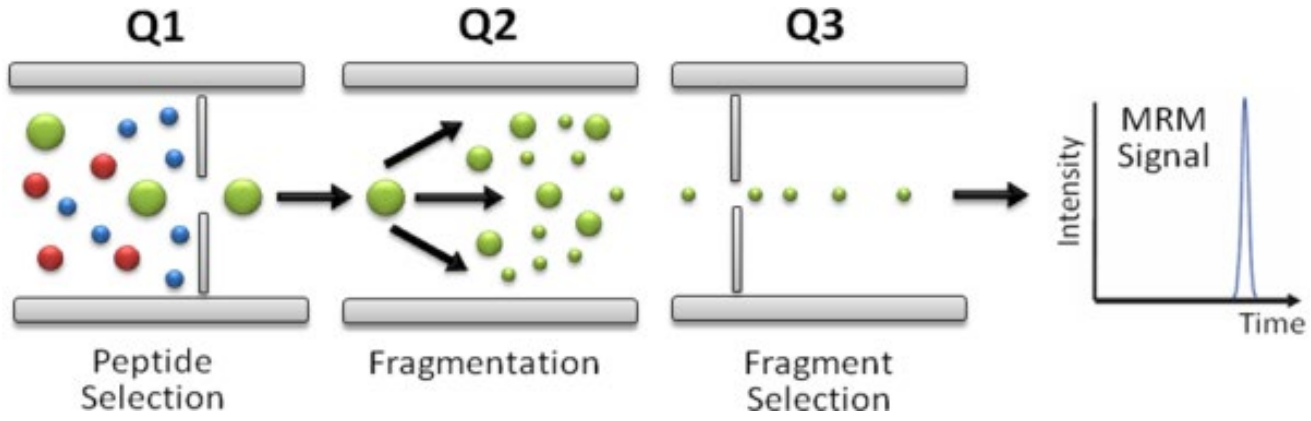
Protein identification

Computational Analysis

(Adapted from Frank Löffler)

Targeted Proteomics

- Triple quadrupole MS
- Q1 and Q3 isolate a peptide ion and a corresponding fragment ion (mass filters)
- The signal of the fragment ion is monitored over time
- With a standard, quantitative results for the proteins of interest



http://www.mrmatlas.org/mrmassay.php

Research - Investing in Proteomics

- Advanced Environmental Molecular Diagnostics to Assess, Monitor, and Predict Microbial Activities at Complicated Chlorinated Solvent Sites (ER-2312, Löffler et al)
- Validation of Advanced Molecular Biological Tools to Monitor Chlorinated Solvent Bioremediation and Estimate CVOC Degradation Rates (ER-201726, Michalsen et al.)
- Assessment of Post Remediation Performance of a Biobarrier Oxygen Injection System at an MTBE Contaminated Site (ER-201588, Neil et al)

Environmental Proteomics

Global Proteomics

- All proteins
- High end instrumentation
- Computational challenges
- Relative quantification
- Open approach
- Site specific database

Metagenomics

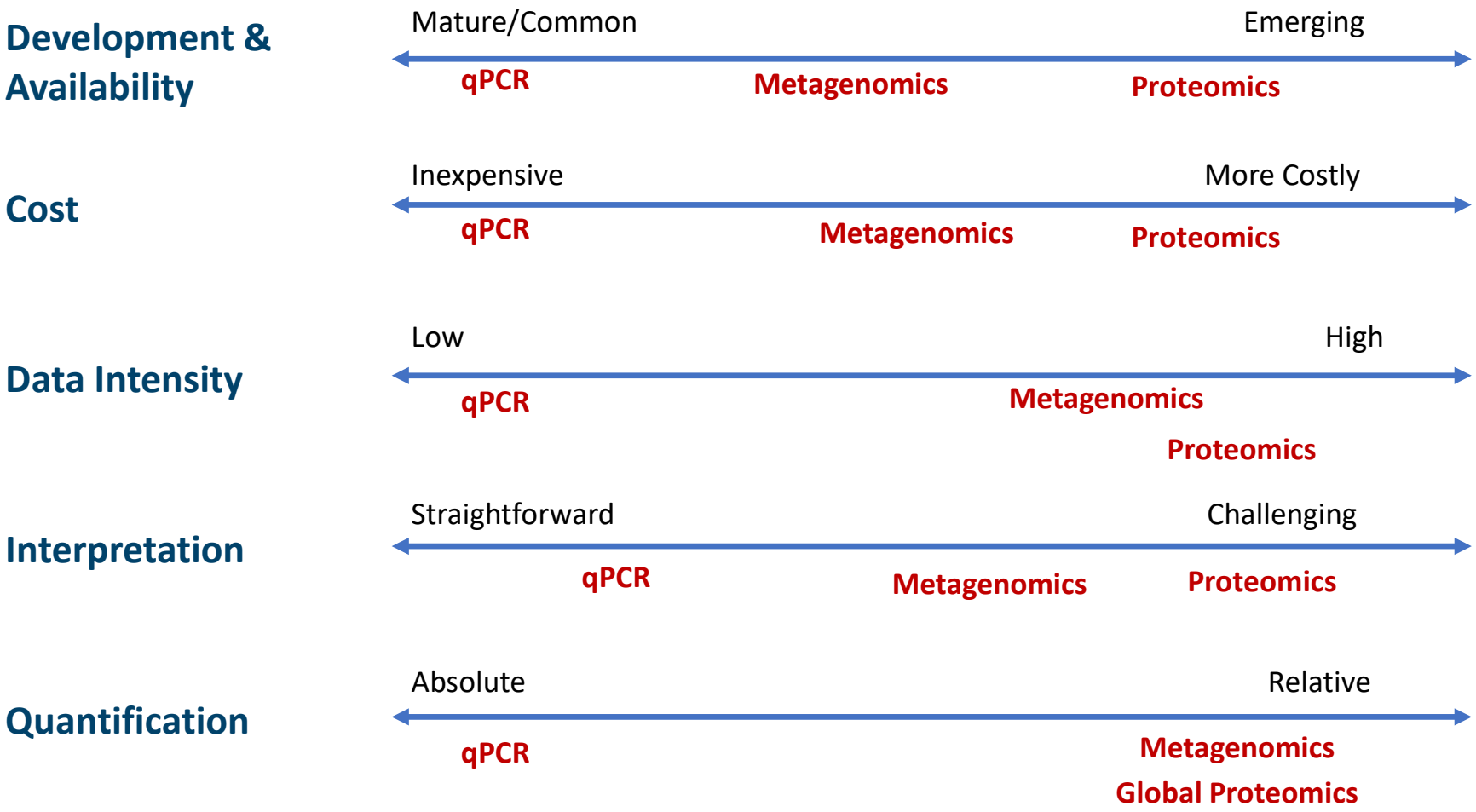
To identify proteins, the peptide sequences must be matched to genes sequences

Targeted Proteomics

- Protein of interest
- Simpler instrumentation
- Computationally easier
- Absolute quantification
- Closed approach
- Must have knowledge of allelic variation to avoid false negatives

Sequence variability but same function

MBT Considerations



Simple Sites

Common Contaminants

Well-Known Biodegradation Pathways

Lower Risk

Routine Site Management Questions

- Are degraders present?
- What are degrader concentrations?
- Did degrader concentrations increase?

Absolute Quantification
(Targeted & Specific)

Start with
qPCR
or QuantArray

No additional MBTs may be necessary

Additional MBTs - NGS

Emerging Contaminants

Biodegradation Pathways are Unknown

qPCR assays are not available

Higher Risk & More Complex

- Contaminant mixtures
- Challenging environmental conditions

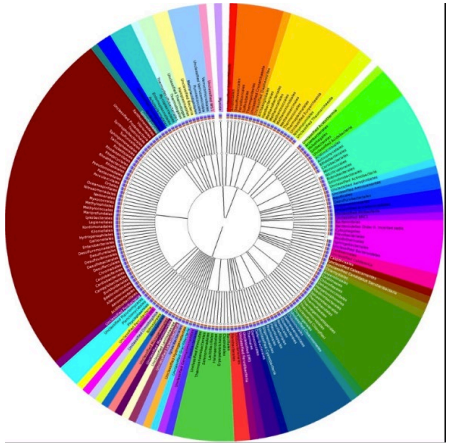
Consider Next Generation Sequencing

More General Questions

- Who is present?
- How has the microbial community changed?
- How is microbial community different?

Relative Abundance

- Overall Profile



Other analyses are inconclusive

Additional MBTs - Proteomics

Common Contaminants

Known Biodegradation Pathways

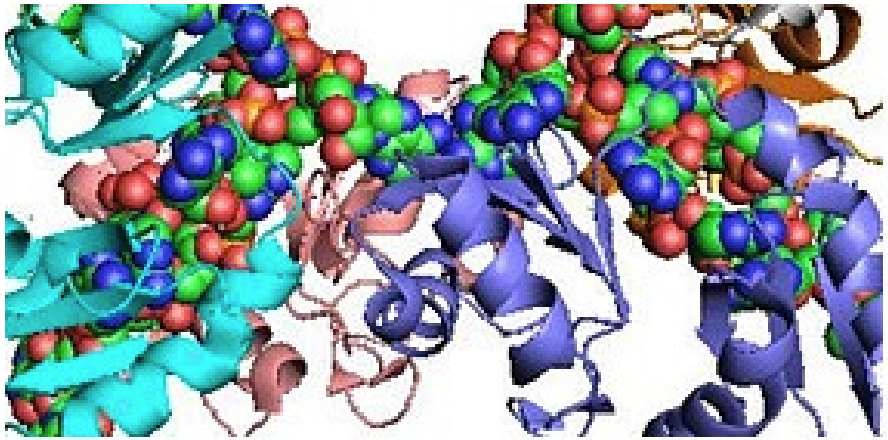
High Risk

Demonstrating Activity is Paramount

- Are degraders active?
- Are pathway enzymes being produced?
- Is pathway active?

Other analyses are inconclusive

Consider Proteomics tools or
Stable Isotope Probing (SIP) or
Compound Specific Isotope Analysis (CSIA)



Final Thoughts

- Every MBT can provide useful information
- Different MBTs answer different questions
- Select MBTs to answer most important site questions
- Know what questions each MBT can answer
- Know the limitations of each MBT, especially less established analyses
- Use a tiered approach to MBT selection
- Start simple whenever possible
- Increase the analytical “degree of difficulty” as or if needed

Most sites don’t need to become a “science project”

Questions?

microbialinsights