

A Novel Biomarker for Monitoring Anaerobic Benzene Degradation



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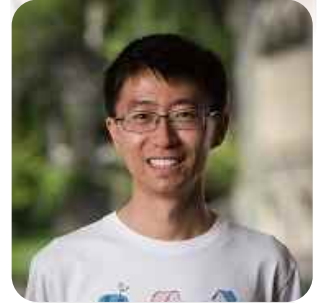
Co-Author Acknowledgements

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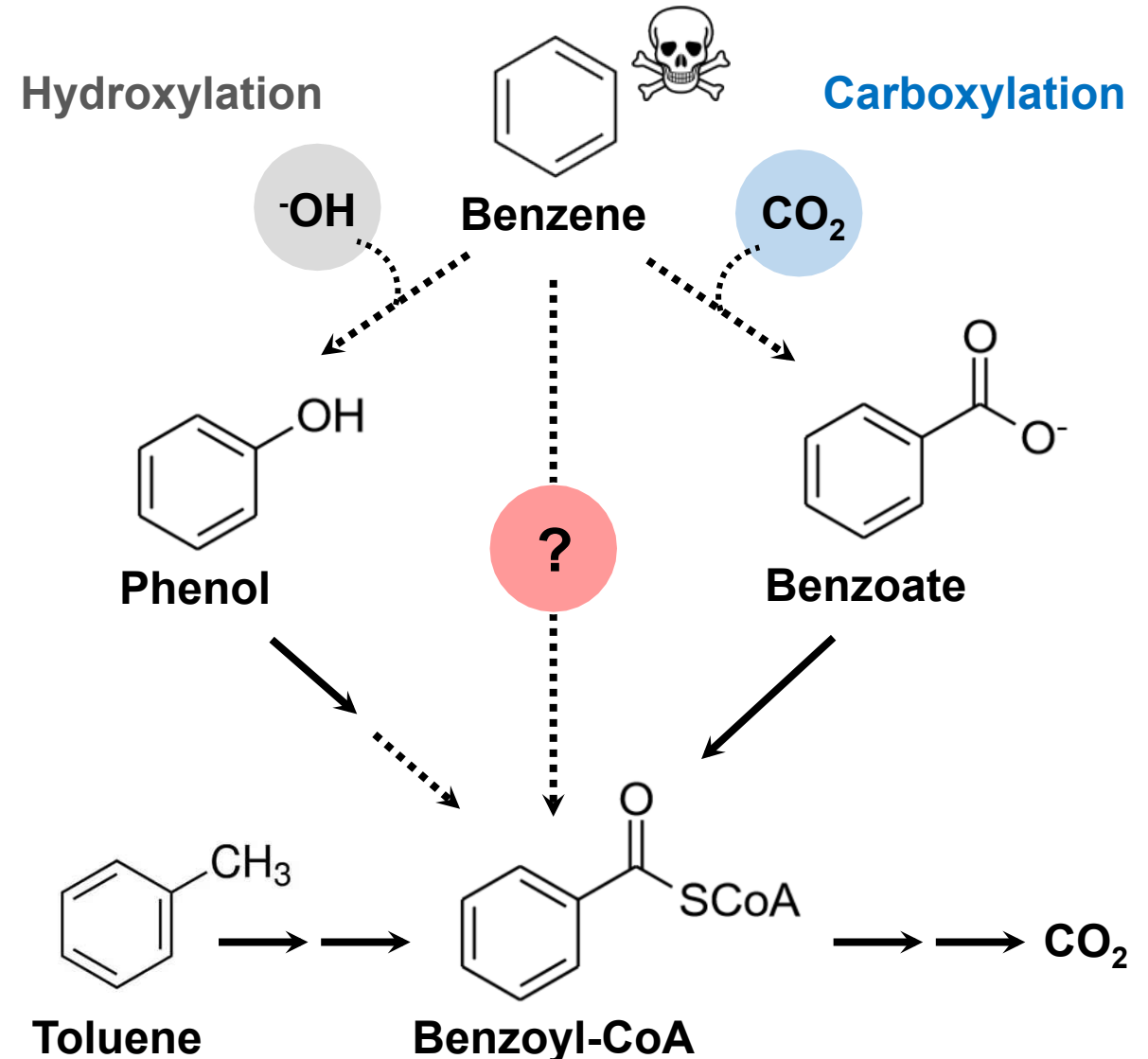
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Assessing the Feasibility & Performance of Anaerobic Benzene Bioremediation

Easier said than done...

- Benzene degrades slowly in the absence of O₂
- >1 metabolic pathway catalyze anaerobic benzene degradation, and most are not well understood
- No biomarker is 100% diagnostic of benzene degradation at all sites



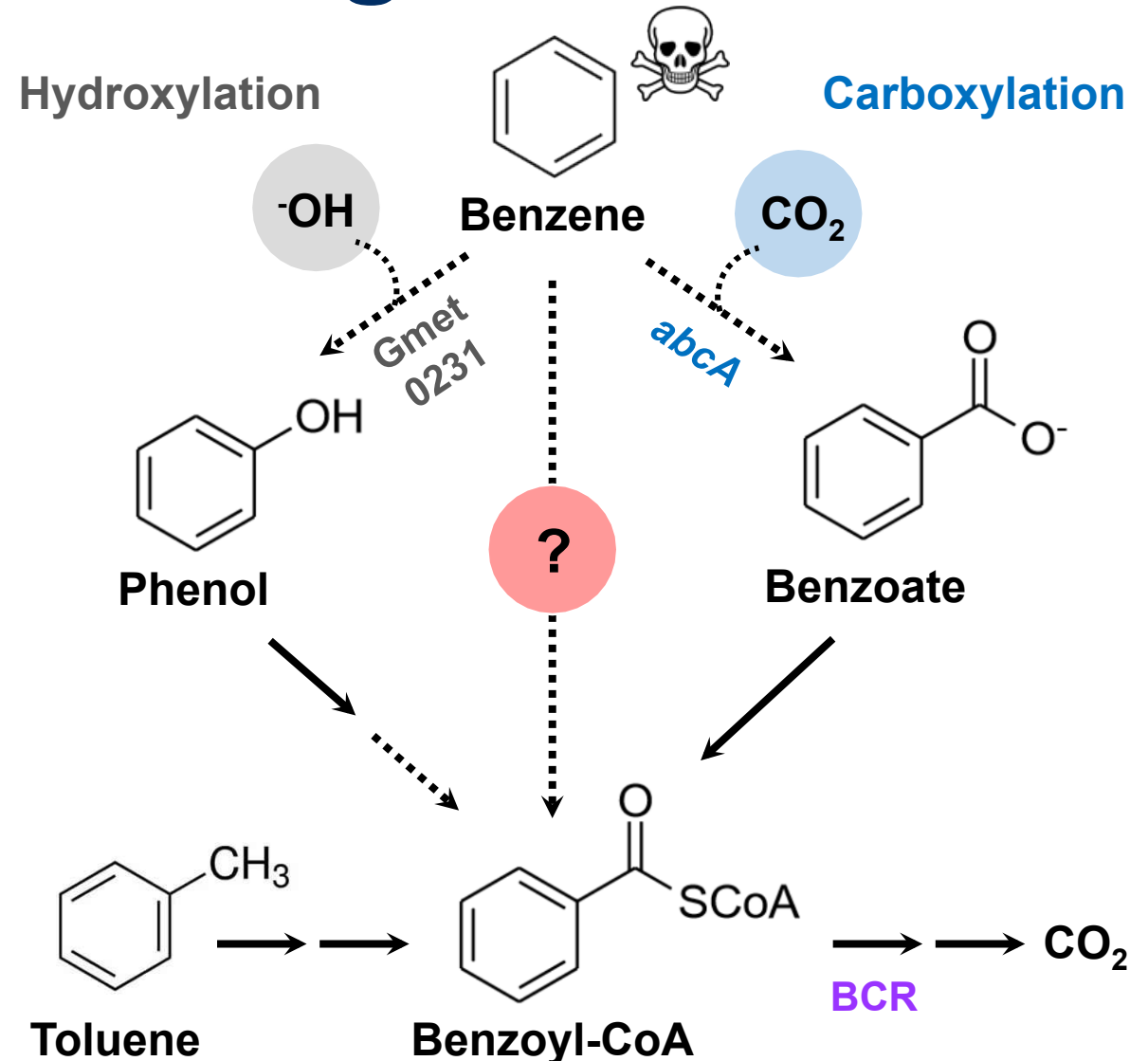
.....➤ = Unknown or unverified reaction

Example Biomarkers Used to Monitor Anaerobic Benzene Degradation

Functional Genes

- **abcA** (anaerobic benzene carboxylase, catalytic subunit)
- **Gmet 0231** (putative hydroxylation gene in *Geobacter metallireducens*)
- **bamA, bcrA** (benzoyl-CoA reductase, **BCR**)

.....➤ = Unknown or unverified reaction



Example Biomarkers Used to Monitor Anaerobic Benzene Degradation

Structural Genes (16S rRNA)

Substrate

● Benzene

▲ PAH

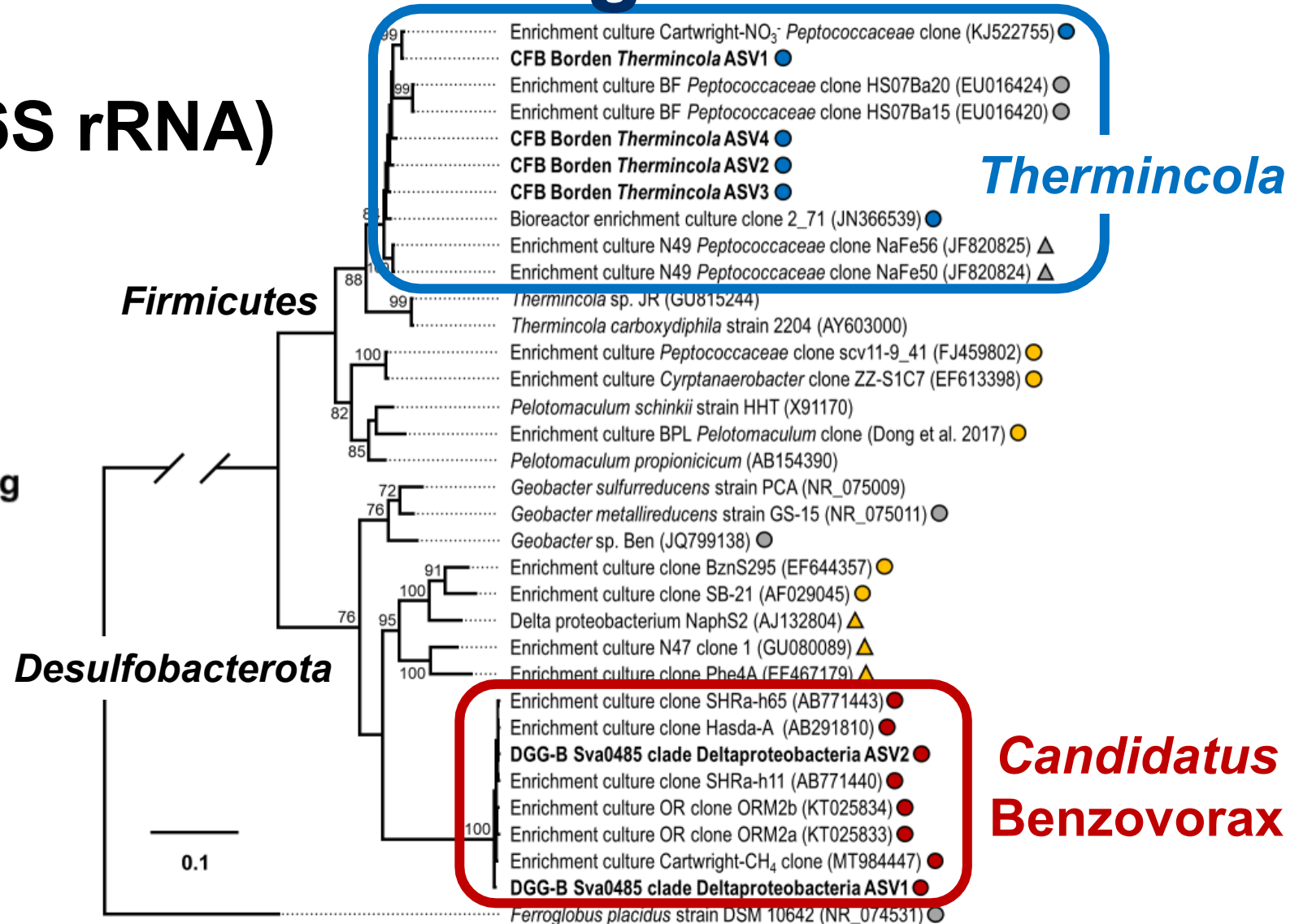
Electron-accepting growth condition

○ Fe³⁺

● NO₃⁻

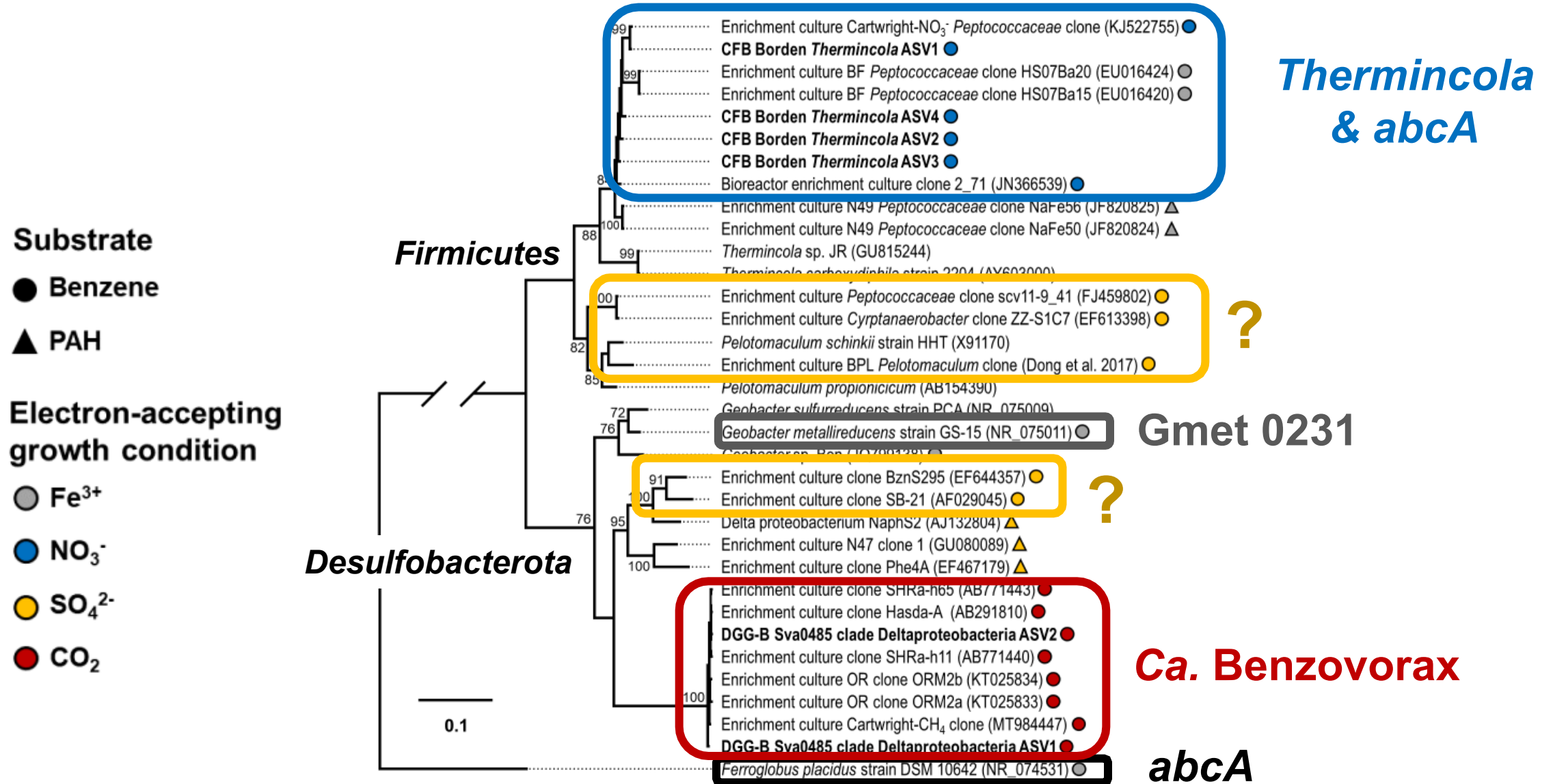
● SO₄²⁻

● CO₂

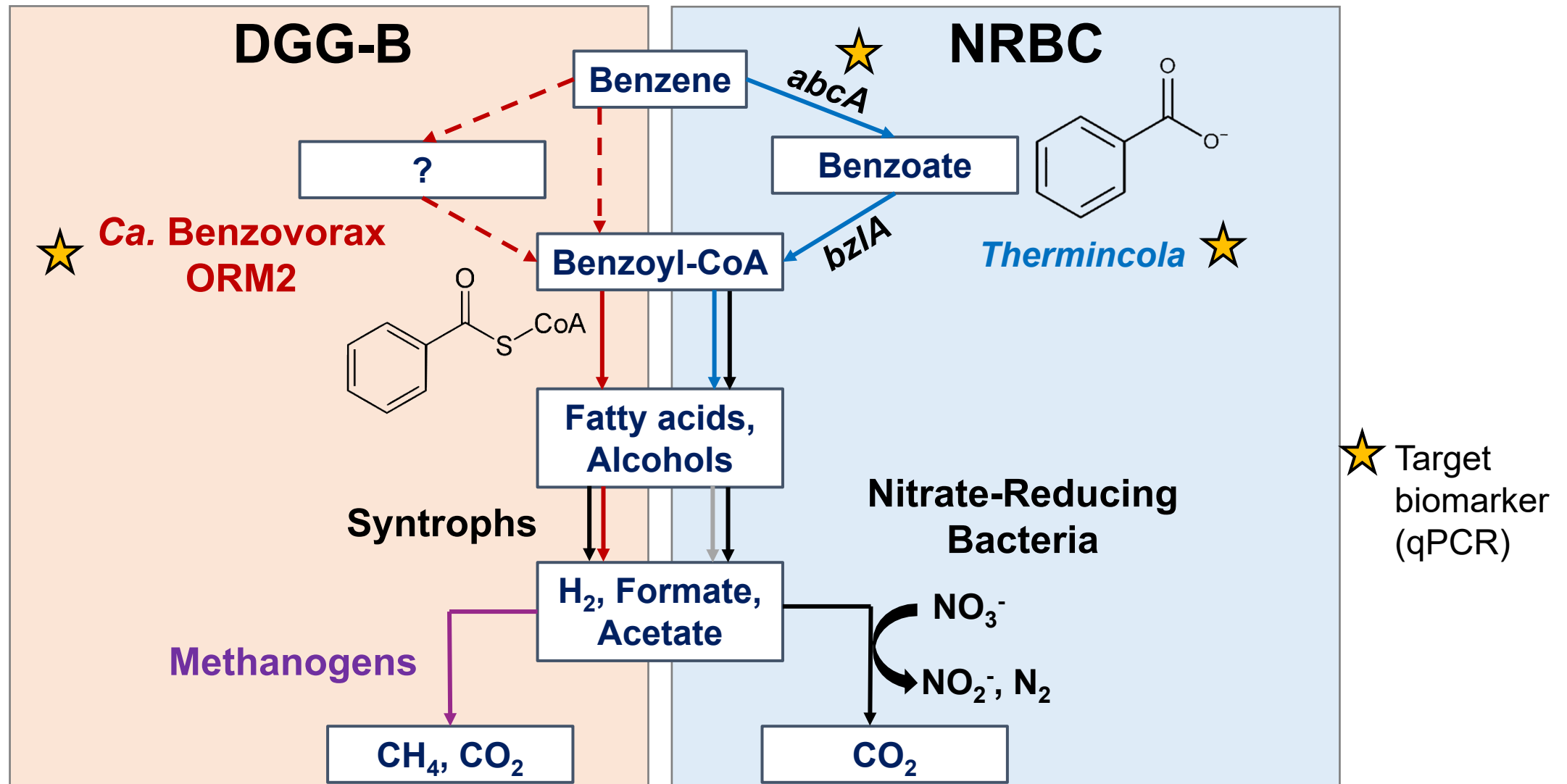


Gaps in Biomarker Coverage

A better understanding of benzene activation reactions may help us close these gaps



Anaerobic Benzene Cultures Under Investigation

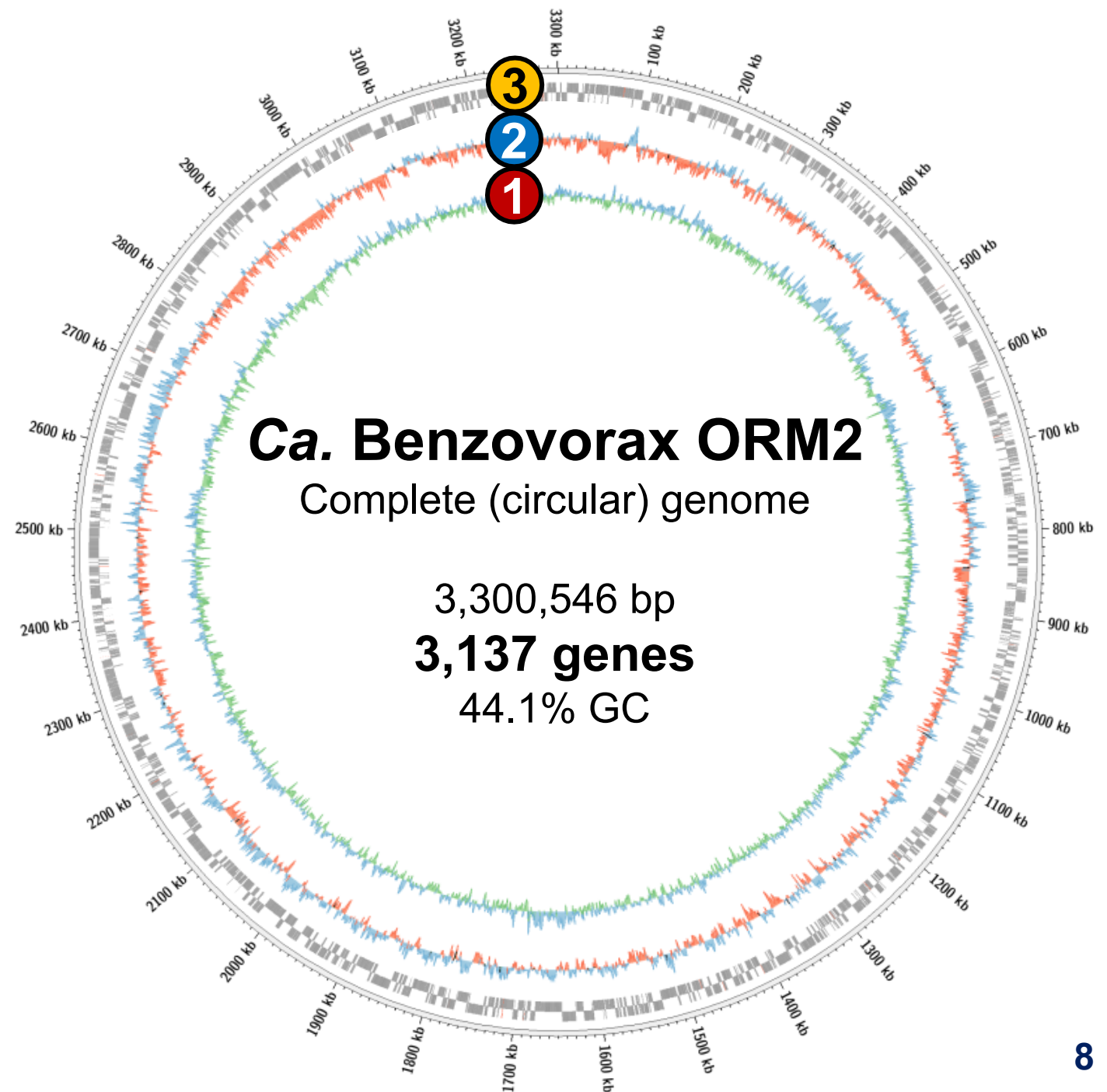


abcA = Anaerobic benzene carboxylase gene
bzIA = Benzoyl-CoA ligase gene

Searching for a Needle in a Haystack

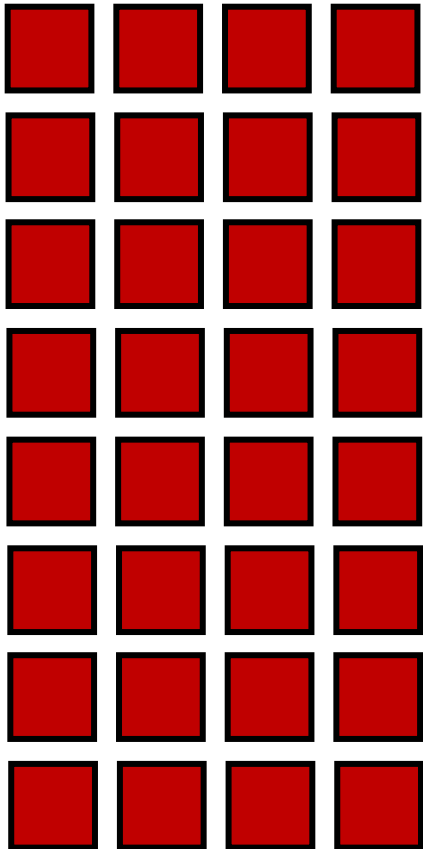
- Metagenomic sequences of DGG-B were used to reconstruct ORM2 genome
- No *abcA*, Gmet 0231, or any other known hydrocarbon activation genes

- ① = GC content (↑ avg., ↓ avg.)
- ② = GC skew (+, -)
- ③ = Genes identified



Proteomics enabled us to close in on ORM2 protein-encoding genes that may be involved in anaerobic benzene activation

ORM2 genes = 3173
(1 box = 100 genes)



ORM2 proteins detected
during benzene
degradation = 278



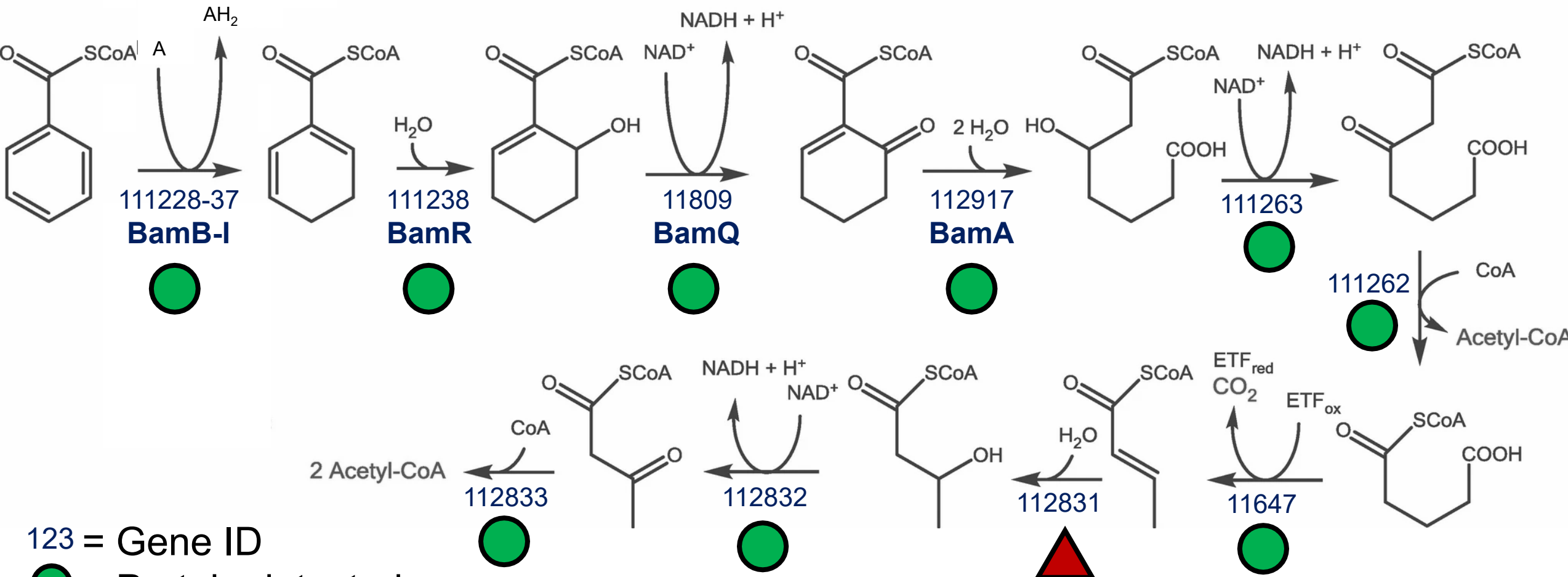
ORM2 proteins that are also
found in other methanogenic,
sulfate-reducing benzene
cultures = 38



ORM2 proteins with a
known function = 27



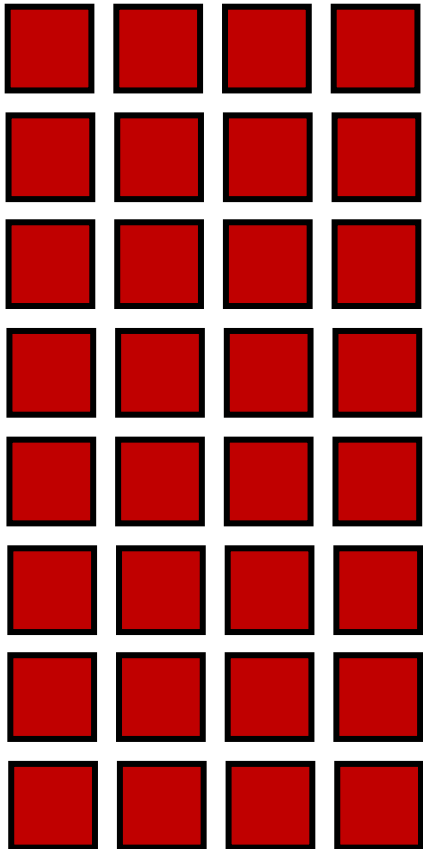
A complete benzoyl-CoA degradation pathway (Bam) is encoded in the ORM2 genome. Corresponding proteins were IDed for most genes.



123 = Gene ID
 ● = Protein detected
 ▲ = Protein not detected

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ORM2 proteins that are also found in other methanogenic, sulfate-reducing benzene cultures = 38

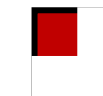


ORM2 proteins with no known function = 11



The fun stuff!

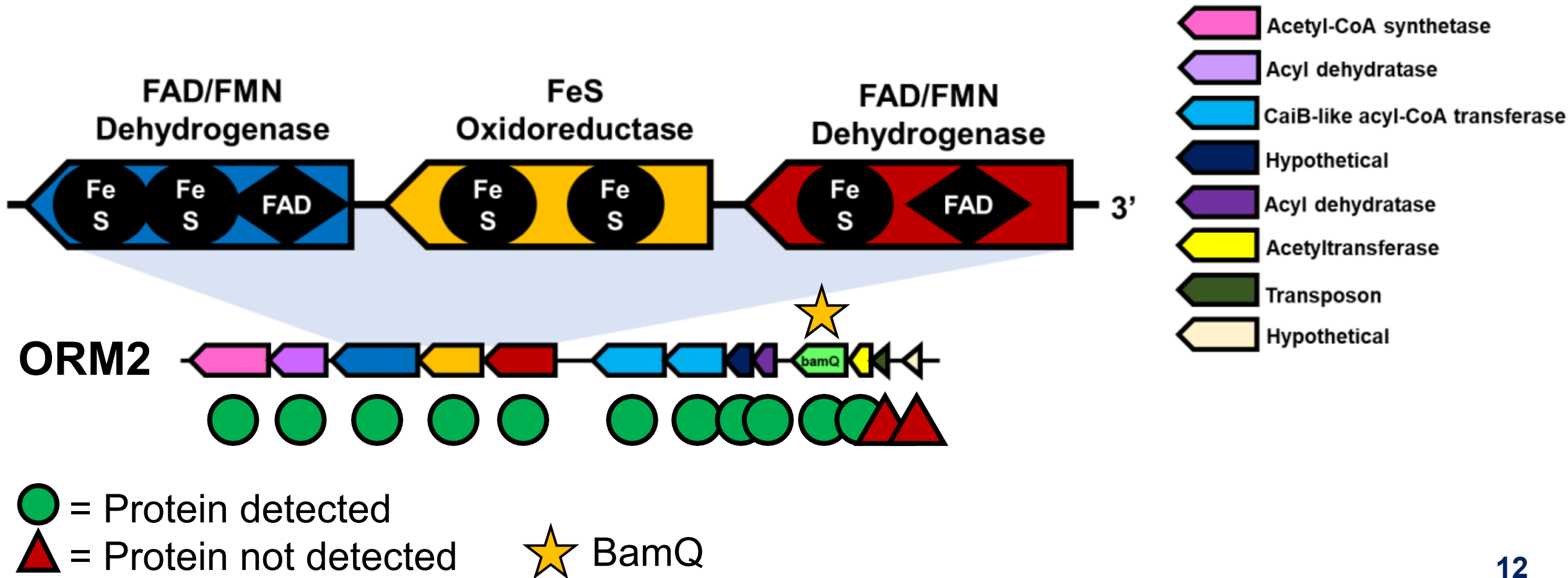
ORM2 proteins with a known function = 27



The (Putative) Needle in the Haystack

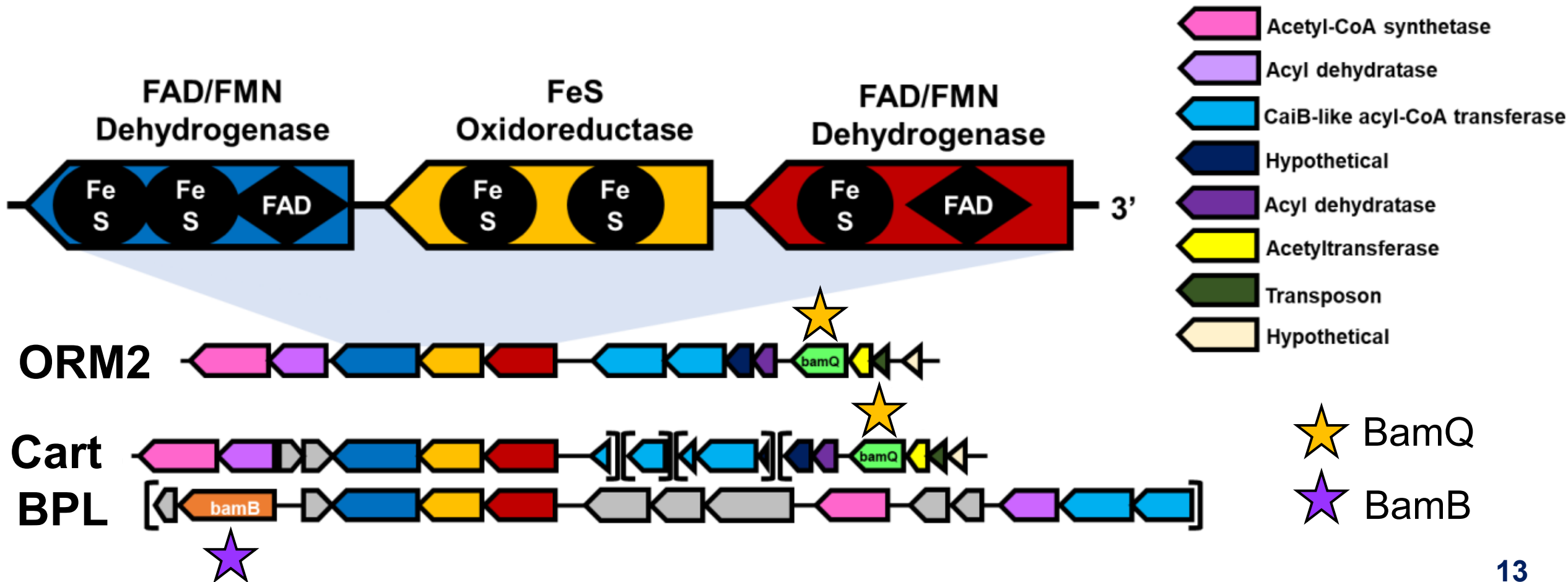
The most abundantly expressed gene cluster in ORM2.

The presence of BamQ hints at a possible role in benzene activation.

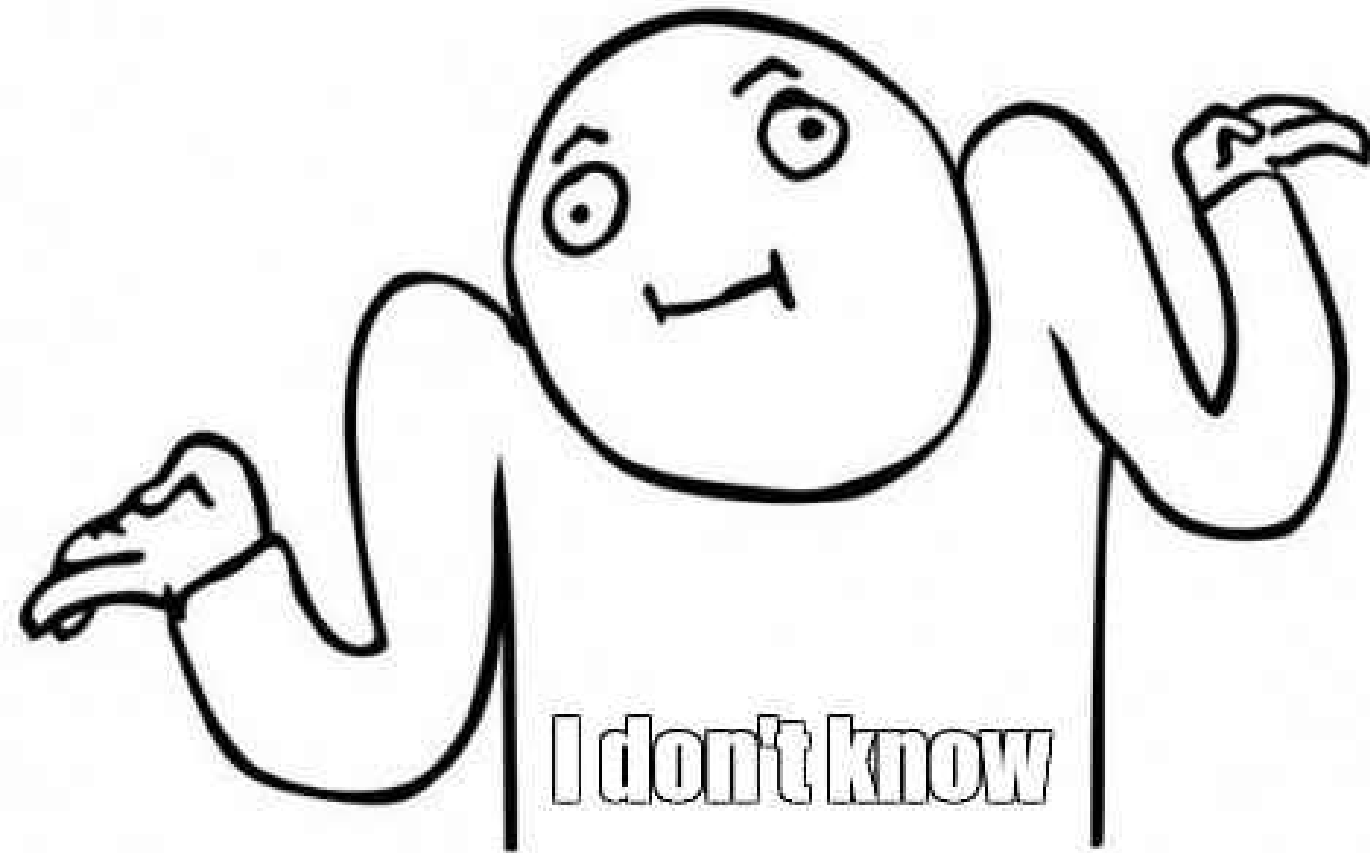


Homologous gene clusters (and proteins) are present in other anaerobic benzene-degrading enrichment cultures.

- **Cart** (methanogenic, contains *Ca. Benzovorax*)
- **BPL** (sulfate-reducing, contains a benzene-degrading firmcute, *Pelotomaculum*)



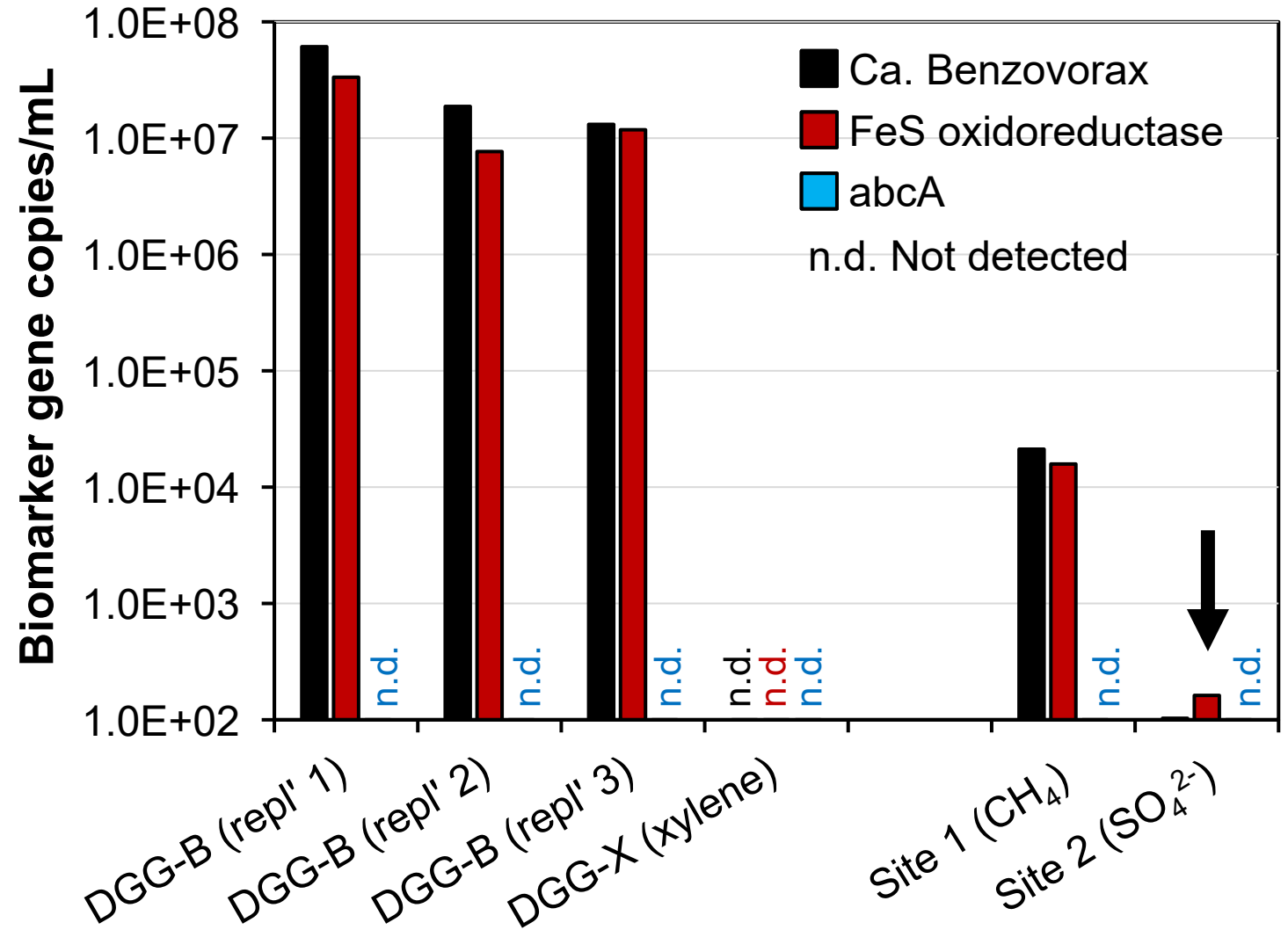
OK, But What's the Benzene Activation Mechanism?



- The biochemistry is complicated
- Machine Learning tools have offered few insights into gene / protein functions
- We're now trying enzyme assays to ID genes / proteins (stay tuned...)
- **Any ideas? Want to help us out? Please email Courtney!**

Biomarker Testing

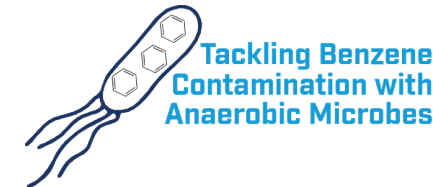
- Despite not knowing its exact function, the **FeS oxidoreductase** is a compelling biomarker
- Development of a qPCR assay is underway
- Preliminary qPCR tests indicate this assay can detect **methanogenic (CH₄)** and **SO₄²⁻-reducing benzene degraders in situ**



Take Home Messages

1. There is no single biomarker that will detect anaerobic benzene degradation at all sites
 - ***abcA*, *Thermincola*** = iron- and nitrate-reducing sites
 - ***Ca. Benzovorax*** = methanogenic sites
2. A putative benzene activation gene cluster has been identified in methanogenic & sulfate-reducing, benzene-degrading cultures
3. Testing of a qPCR functional gene assay is underway
 - **FeS oxidoreductase** = methanogenic and sulfate-reducing sites

Thank You for Your Attention



Contact Us!



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