

# Use of Metagenomic and Metatranscriptomic Approaches to Elucidate Microbial Processes *in Situ*

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and Sustainable Environmental Technologies  
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Colorado State University

# Presentation overview

## Meta-omic tools for remediation biomarker development

- The challenges
- Meta-omic approach comparison for *o*-xylene
  - Metagenomics
  - Metatranscriptomics
  - cDNA Subtraction
- Thoughts regarding field applications



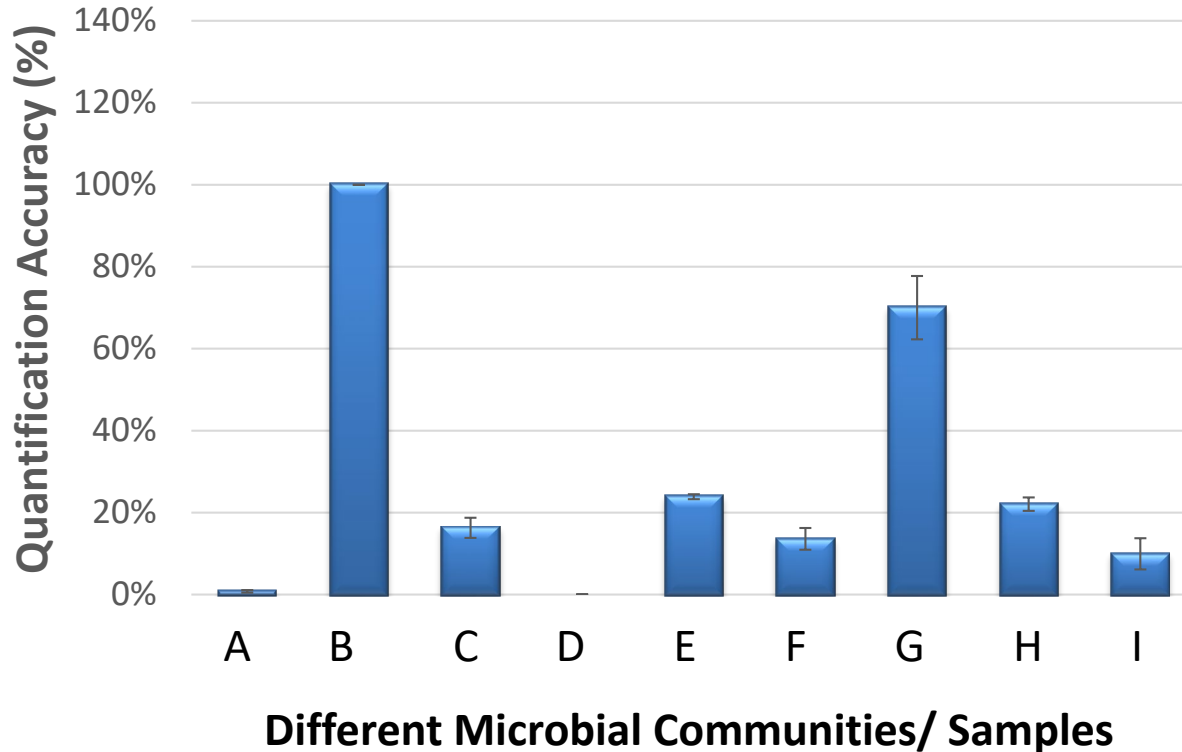
# Current challenges with molecular assays

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- “Menu” of available assays limited
  - For some contaminant(s)/redox conditions, none are available
- Available assays are sometimes inaccurate
  - Biomarker assays frequently developed originally based on pure cultures



# (RT-q)PCR accuracy depends on primer design



- Mismatches can reduce assay accuracy 2-3 orders of magnitude.
- **Non-detects may be misleading!**

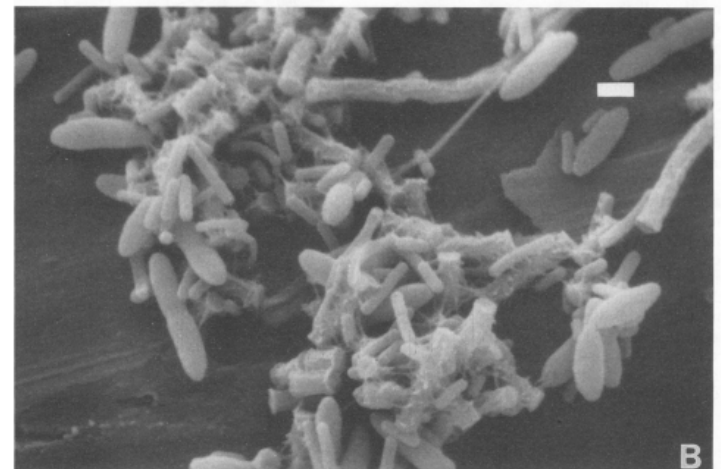
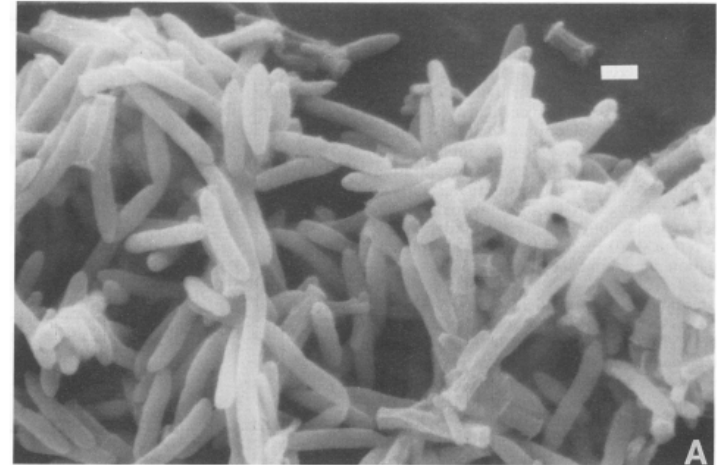
Ledeker BM, De Long SK. 2013. The effect of multiple primer-template mismatches on quantitative PCR accuracy and development of a multi-primer set assay for accurate quantification of *pcrA* gene sequence variants. *Journal of Microbiological Methods* 94:224-231.



# Model system for anaerobic o-xylene degradation

## Field-derived methanogenic enrichment culture (Edwards *et al.* 1994)

- Degrades toluene and o-xylene
- Anaerobic o-xylene biodegradation *hypothesized* to be analogous to toluene

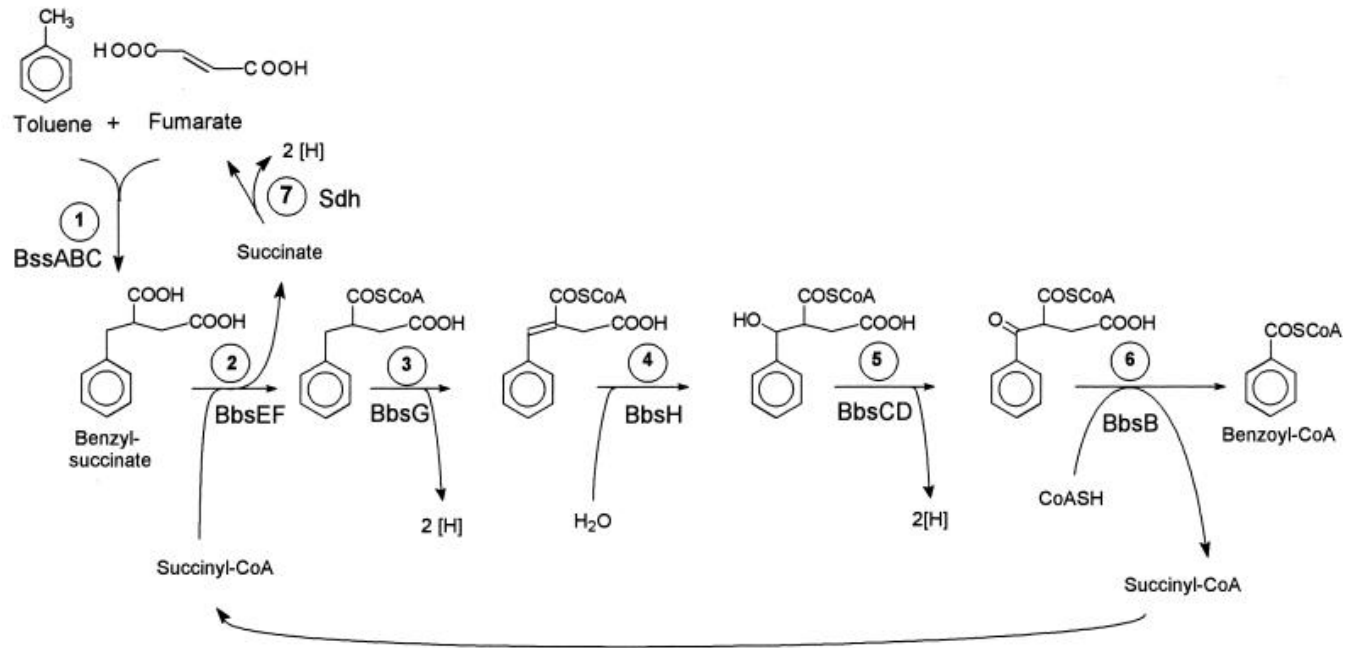


Edwards EA, Grbic-Galic D. 1994. Anaerobic degradation of toluene and o-xylene by a methanogenic consortium. *Applied and Environmental Microbiology* 60:313-322.



# Anaerobic biodegradation of toluene

- Initiated via fumarate addition by benzylsuccinate synthase (Bss)
  - Fumarate-adding enzyme (FAE)
- O-xylene??

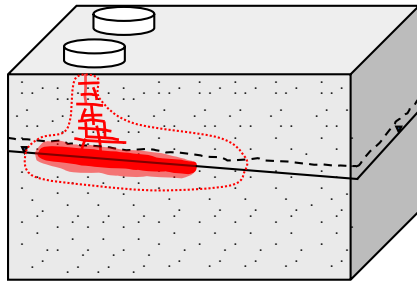


Leuthner B, Heider J. 2000. Anaerobic toluene catabolism of *Thauera aromatica*: the bbs operon codes for enzymes of beta oxidation of the intermediate benzylsuccinate. *Journal of Bacteriology* 182:272-277.

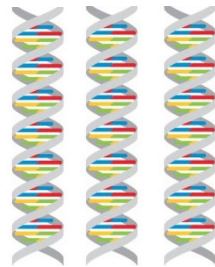




# Metagenomic workflow



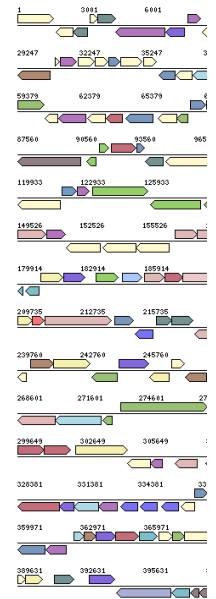
Hydrocarbon  
-degrading  
microbes



Extract  
nucleic acids



Next-generation  
sequencing

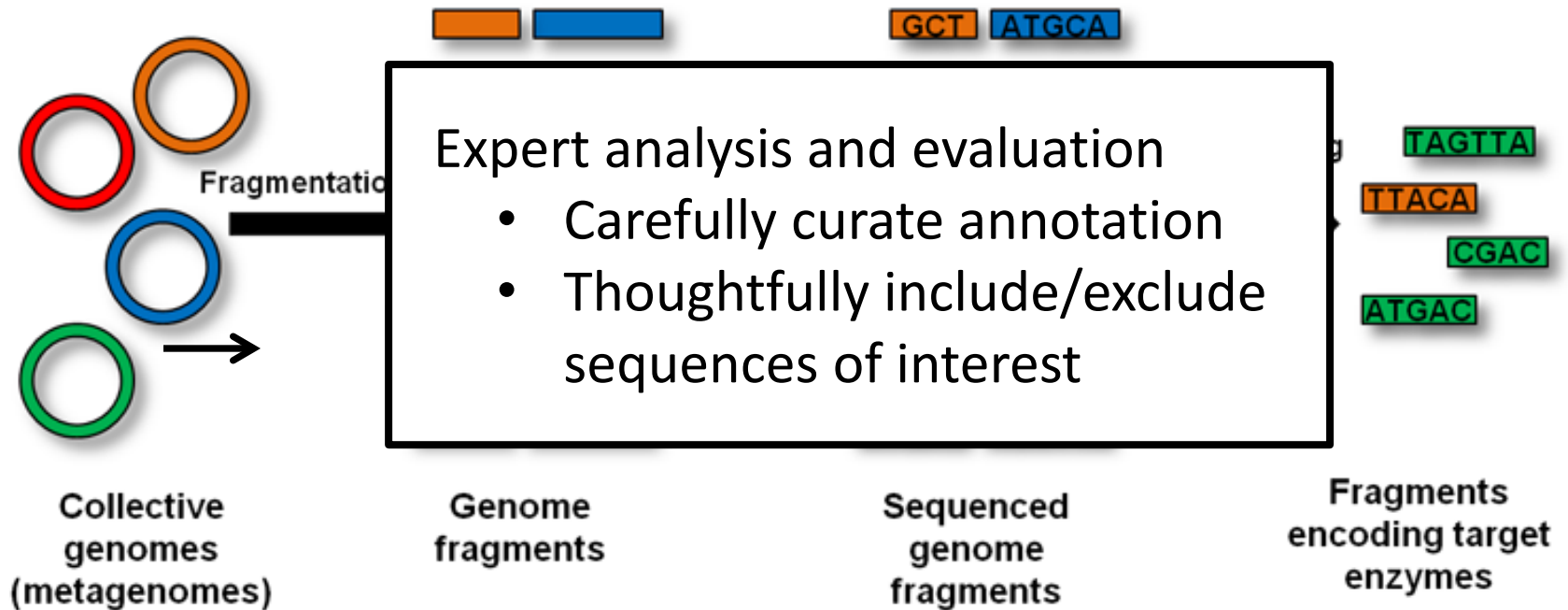


Bioinformatics  
to find new  
targets,  
sequences





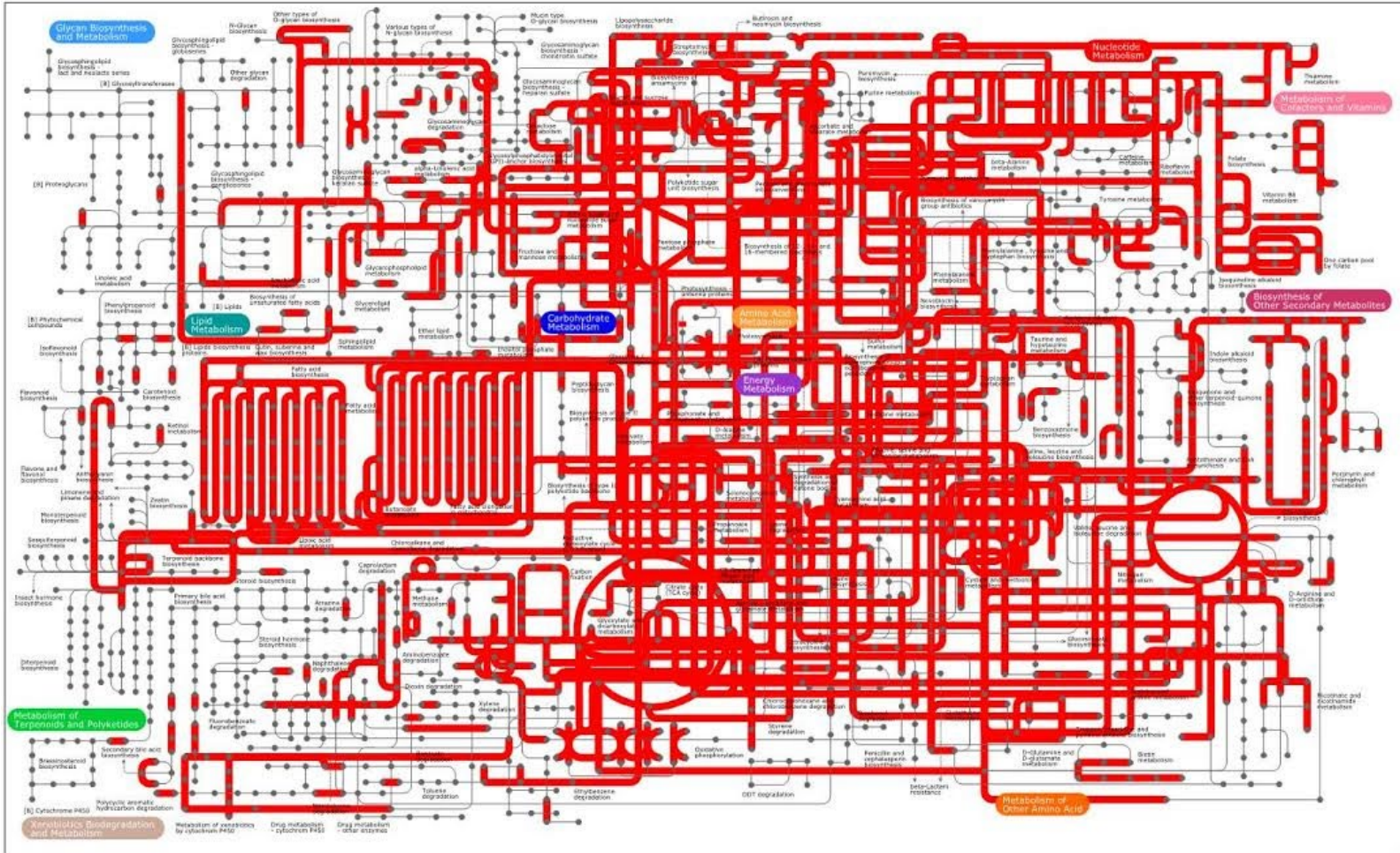
# De novo strategy for designing primers: Metagenomics



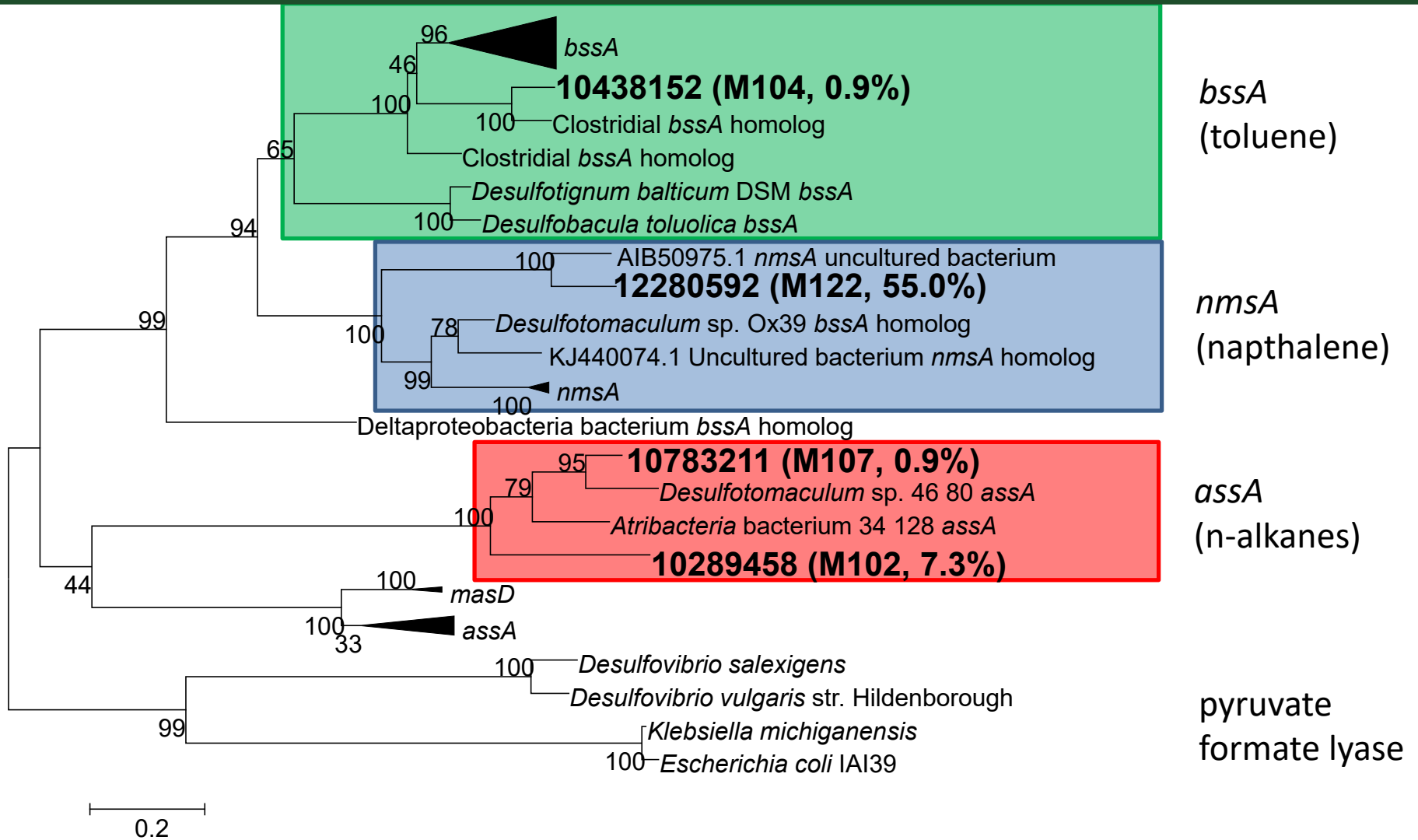
<http://www.cebitec.uni-bielefeld.de/clib-gc/index.php/alumni/20-martha-zakrzewski>



# Functional potential of metagenome



# Fumarate-adding enzyme genes in metagenome

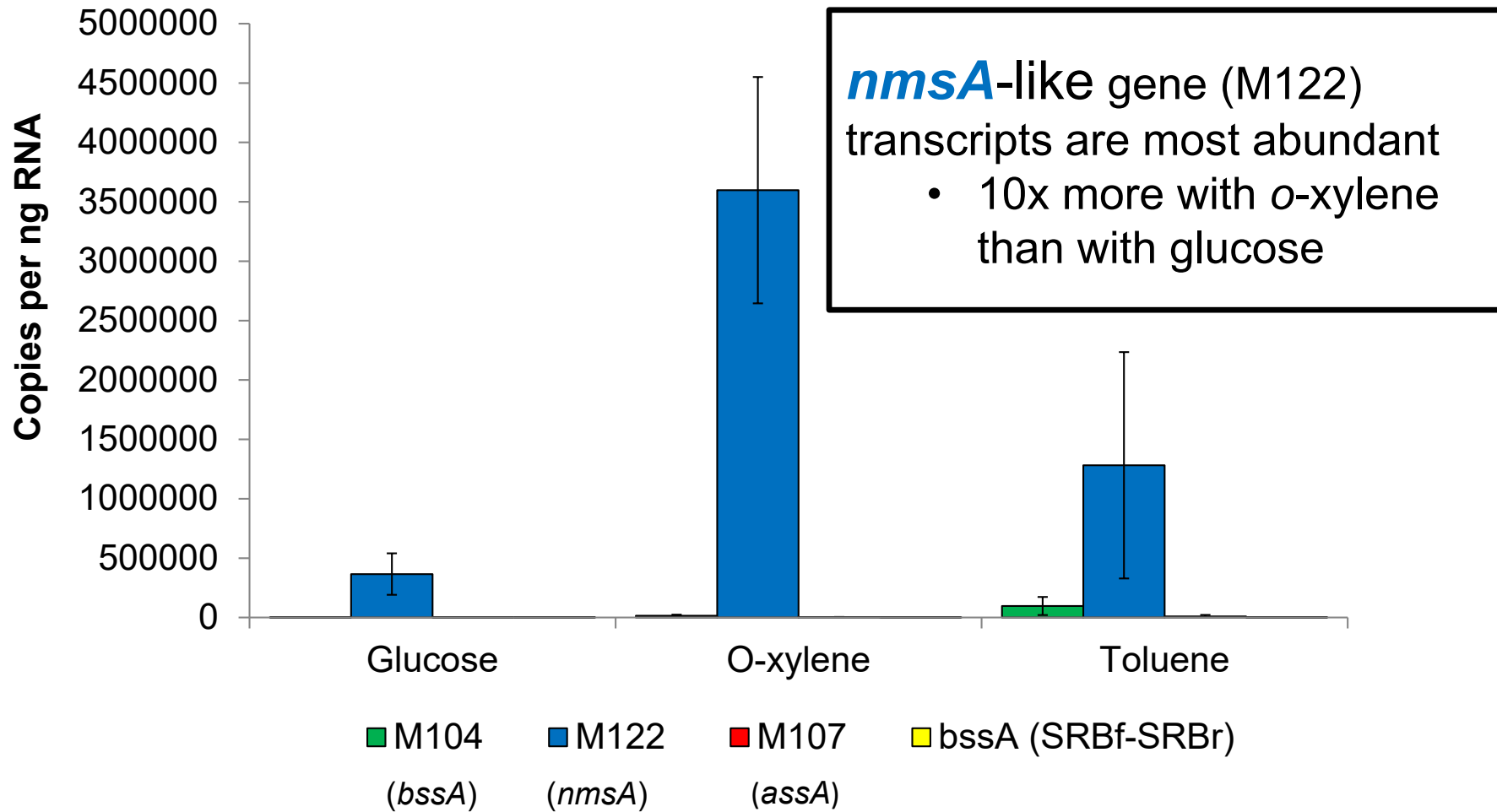


# Sequences compared to previously published *bssA* primers

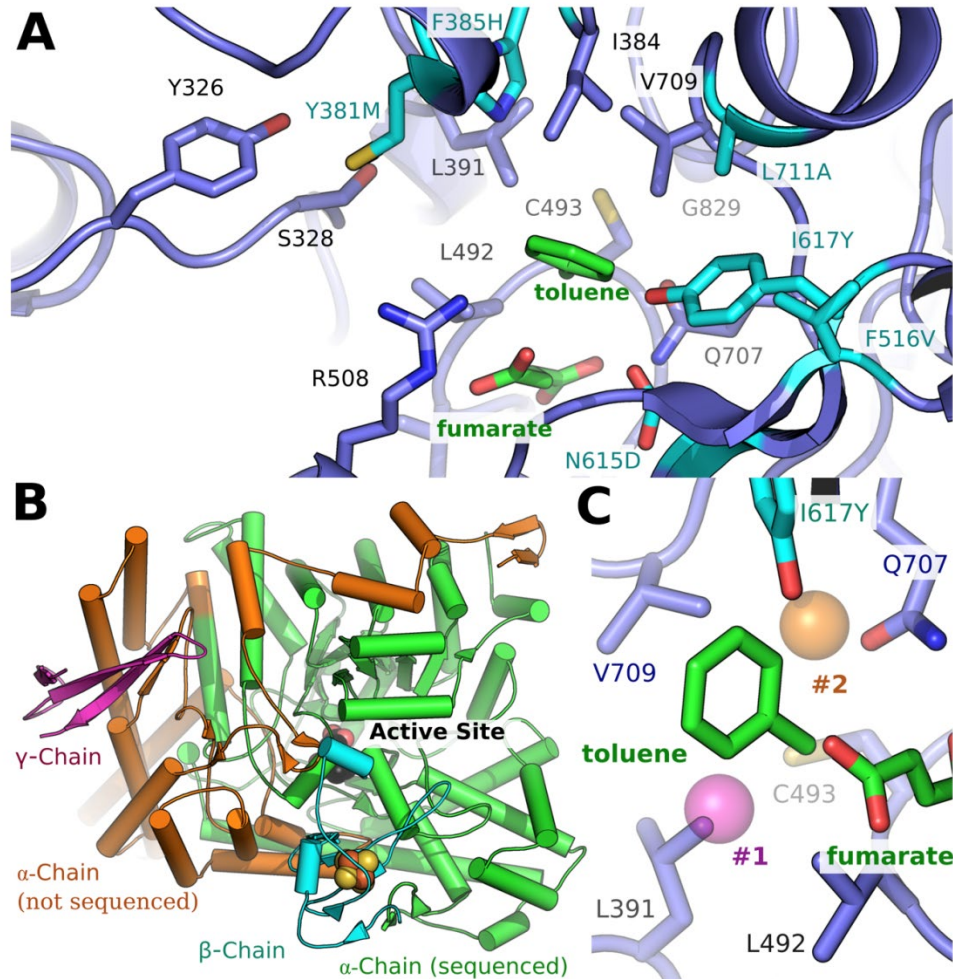
Reference	Primer name	Mismatches	Mismatches	Mismatches	Mismatches
		against M102 (F, R)	against M104 (F, R)	against M107 (F, R)	against M122 (F, R)
Beller <i>et al.</i> , 2002	BellerF BellerR	<b>7, 5</b>	<b>2, 2</b>	<b>7, 4</b>	<b>8, 8</b>
Beller <i>et al.</i> , 2008	SRBf SRBr	<b>4,</b>			
Staats <i>et al.</i> , 2011	bssA3f bssAr	<b>7,</b>			
Fowler <i>et al.</i> , 2014	MbssA1F MbssA1R	<b>6, 6</b>	<b>4, 6</b>	<b>7, 6</b>	<b>6, 5</b>

All will be inaccurate for quantification/detection of the sequences from the metagenome!

# Expression of fumarate adding enzyme genes



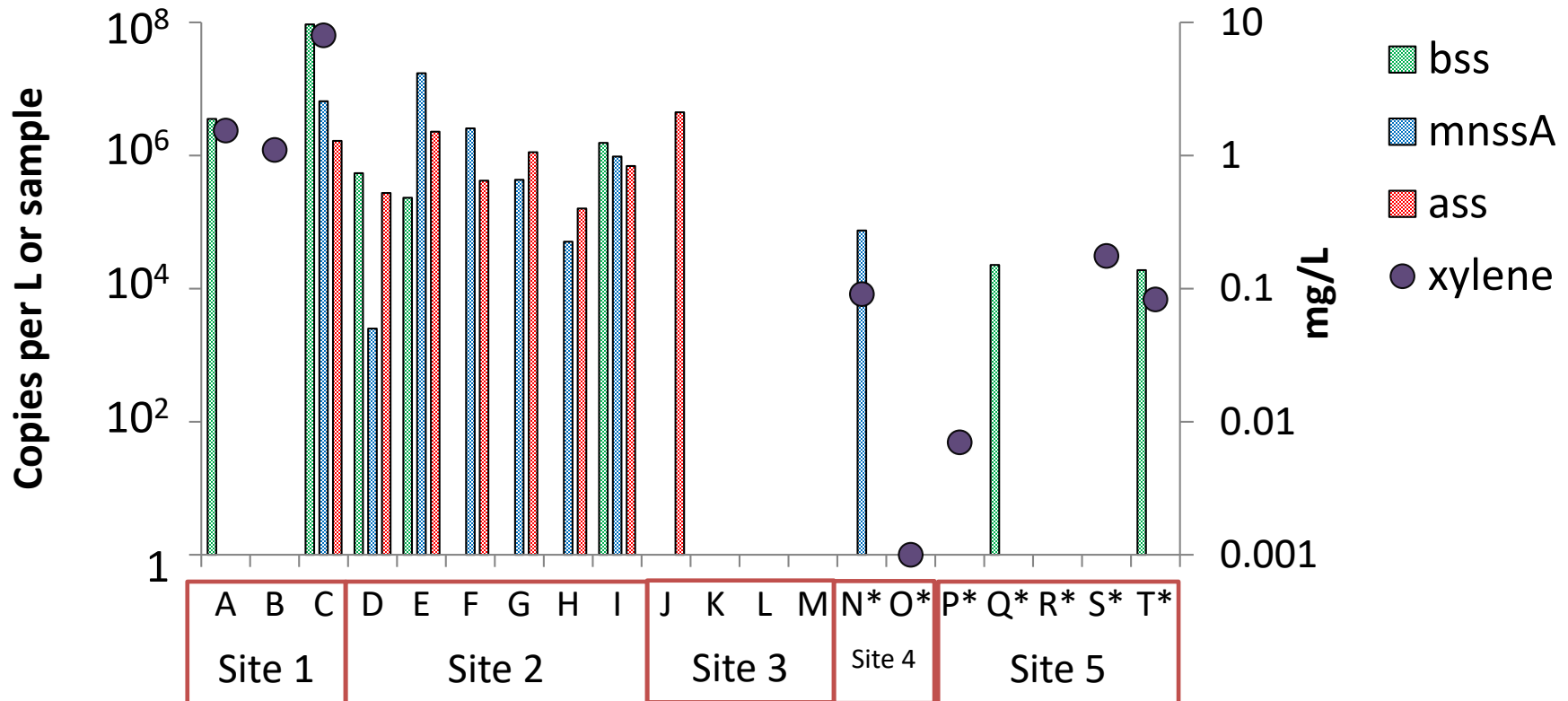
# Predicting novel enzyme catalysis



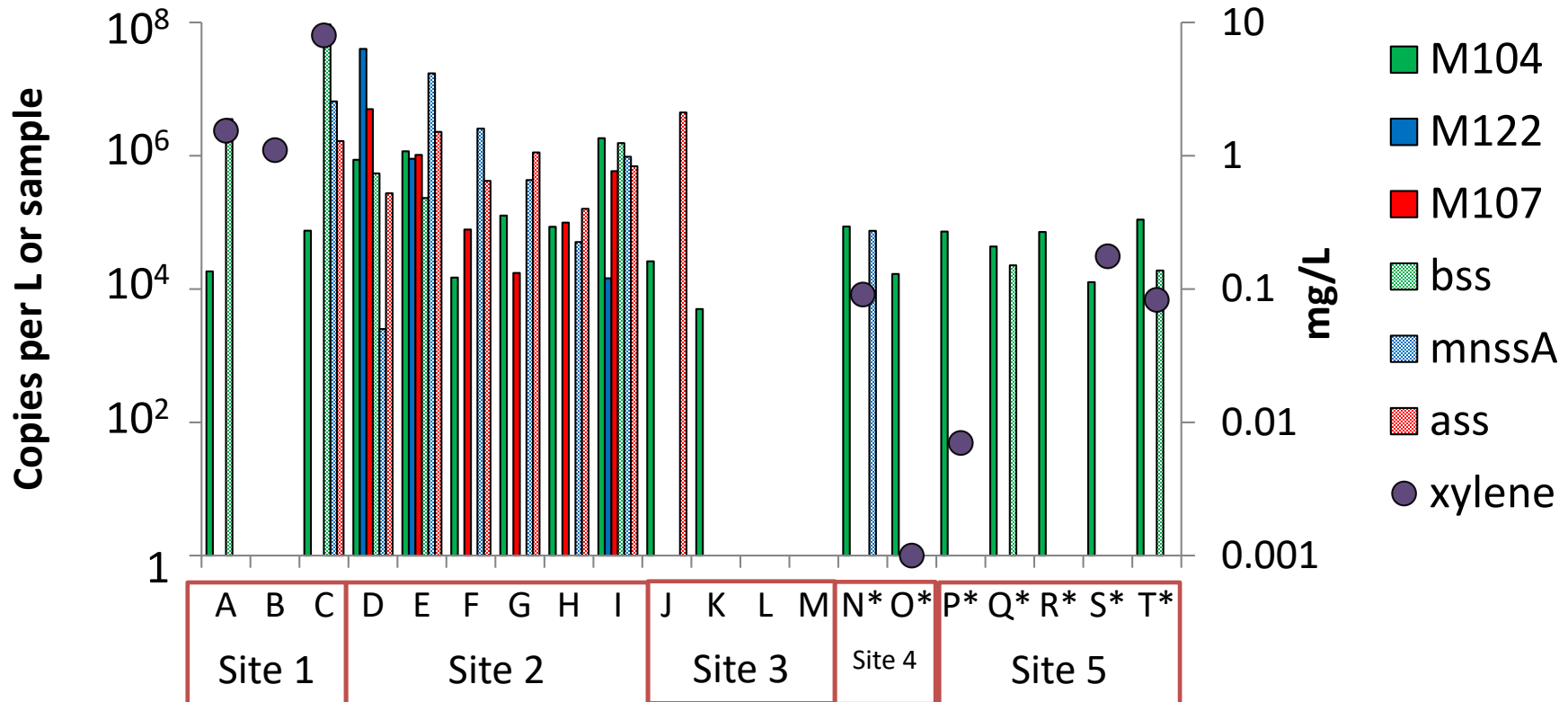
- *In silico* protein structure modeling suggest M122 is a fumarate adding enzyme with larger active site.
- Fits larger substrates like o-xylene?



# Field applications of new FAE assays



# Field applications of new FAE assays

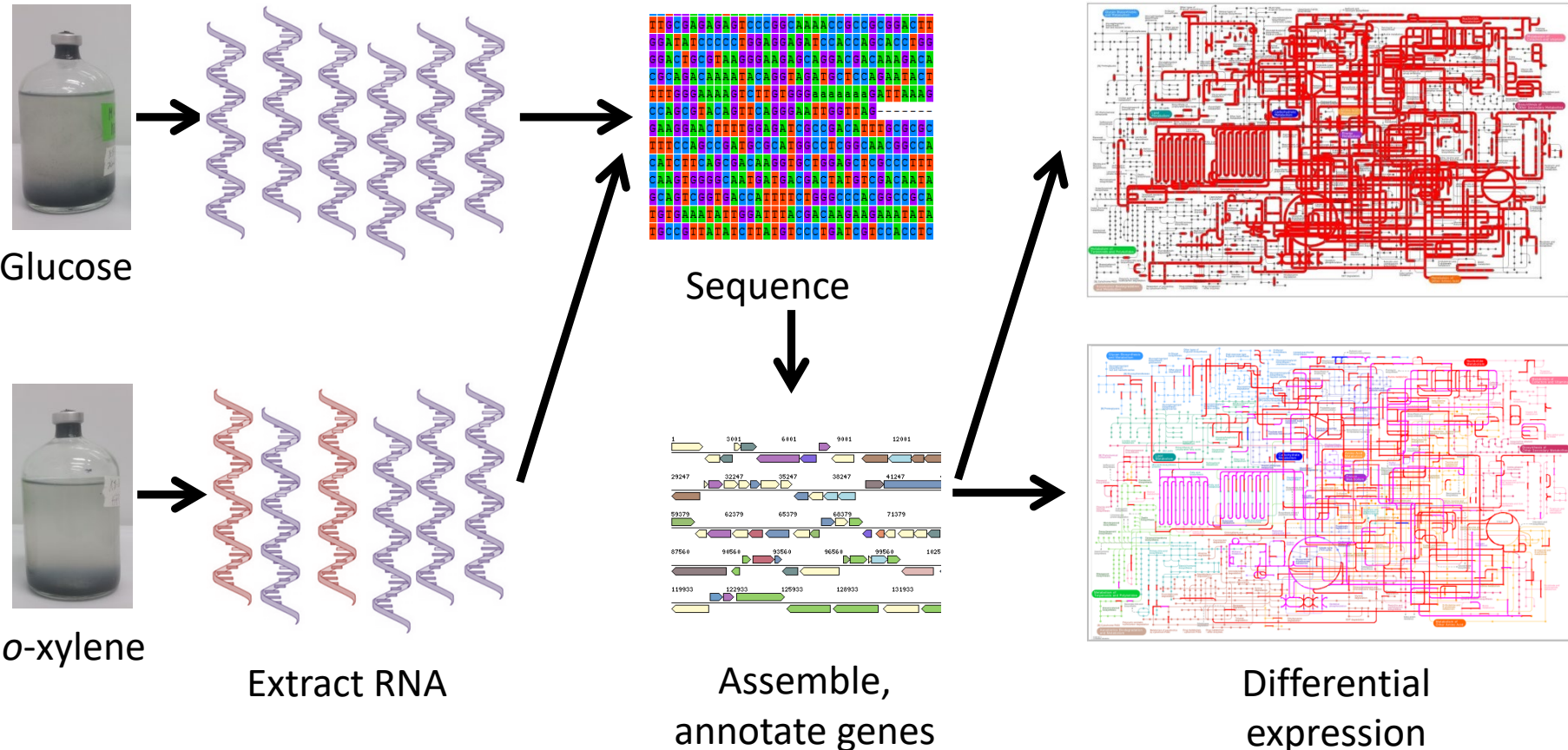


- New assays increased the number of fumarate-adding enzyme detects and copies

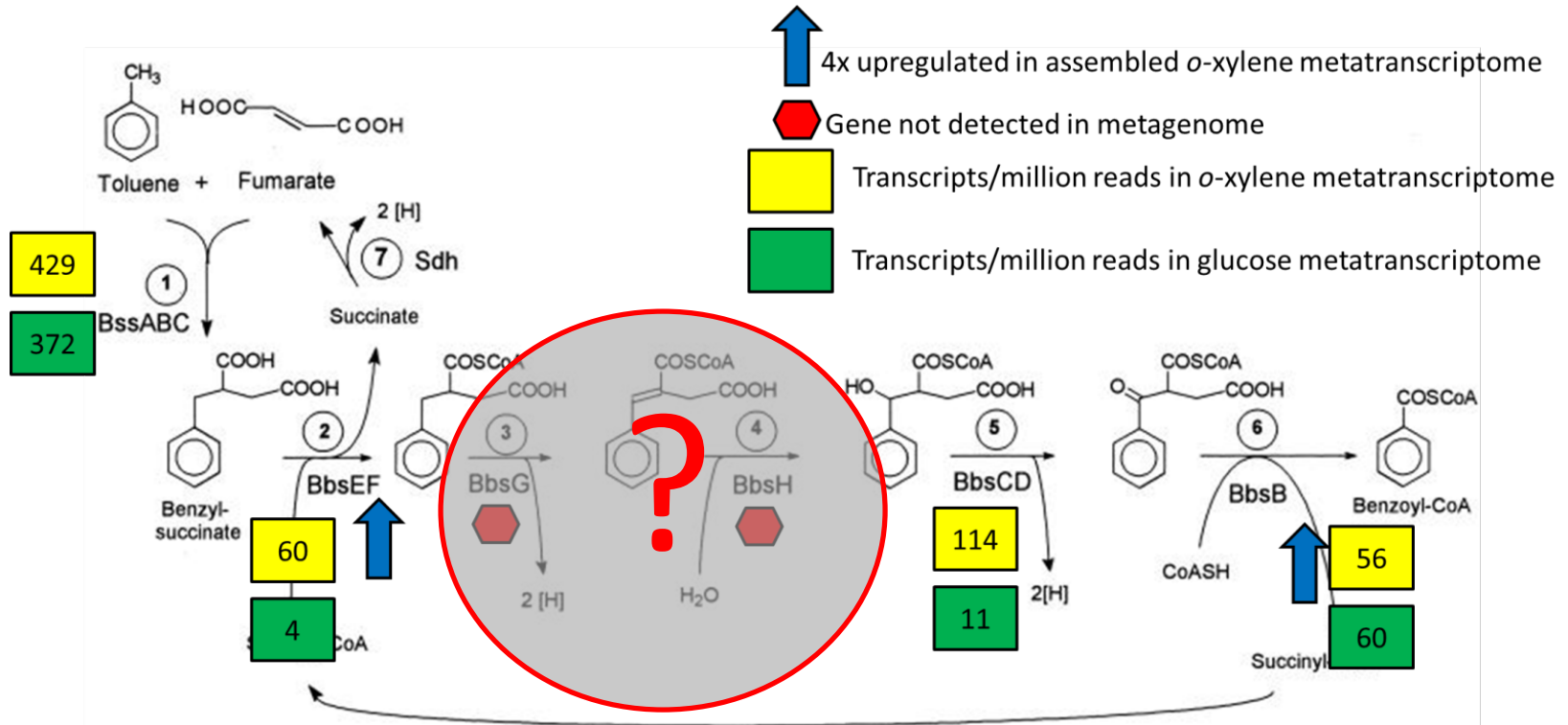




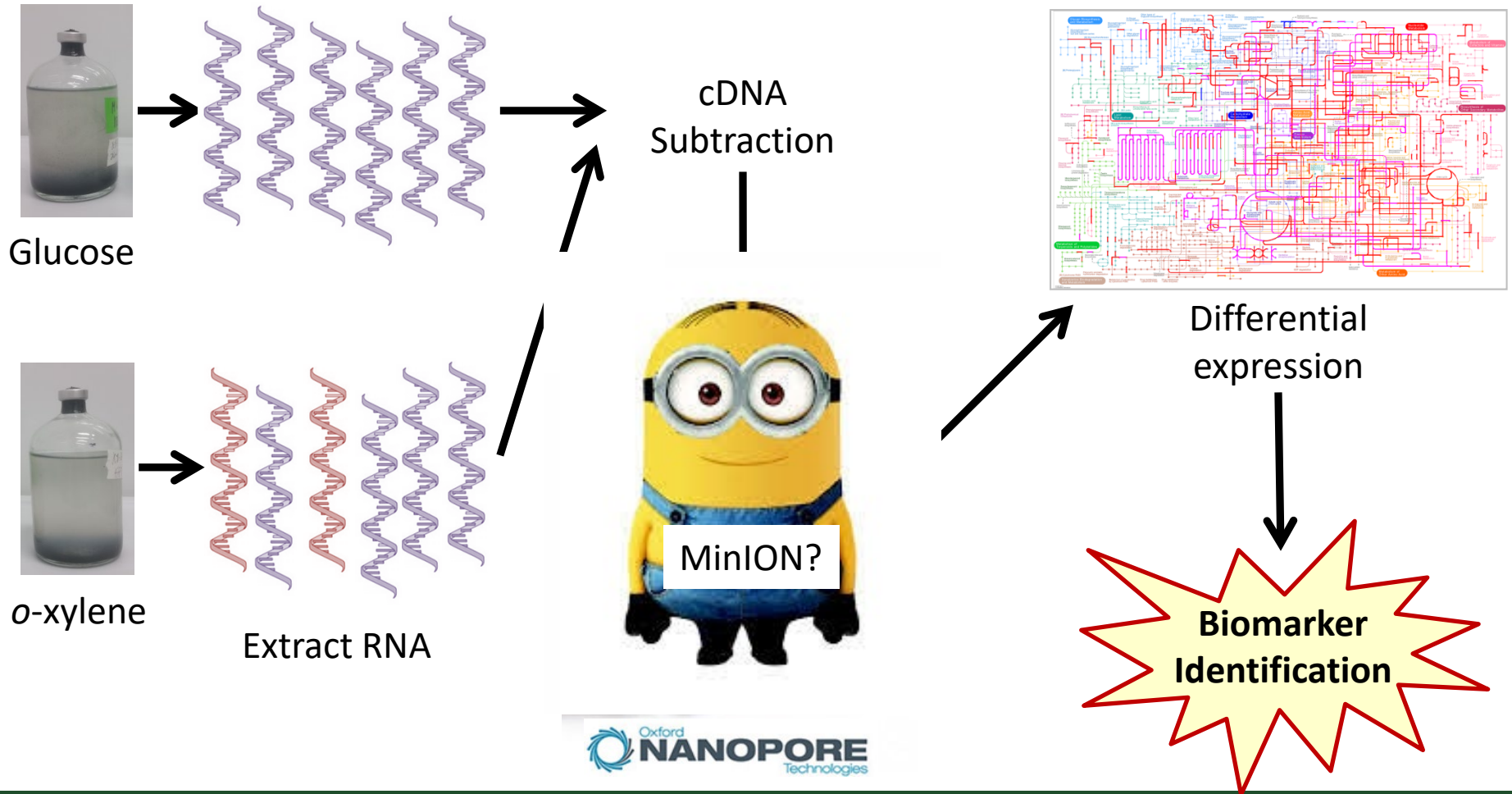
# Metatranscriptomics – differential expression




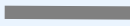
# Metatranscriptomics – differential expression



# cDNA Subtraction – differential expression



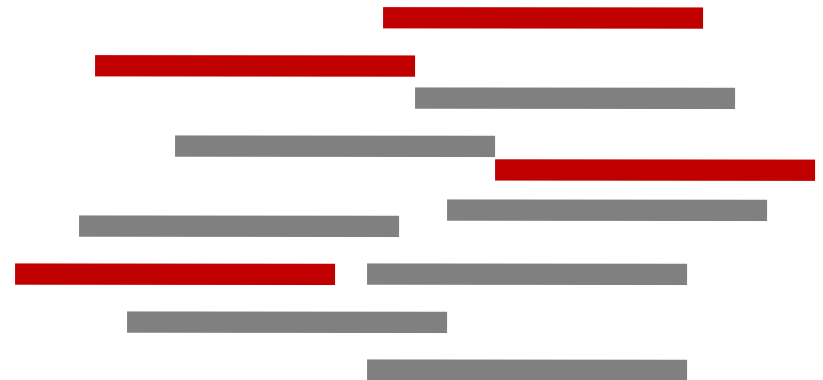
# Finding the needle in the haystack: cDNA Subtraction

-  Genes for target compound degradation
-  Basic function genes

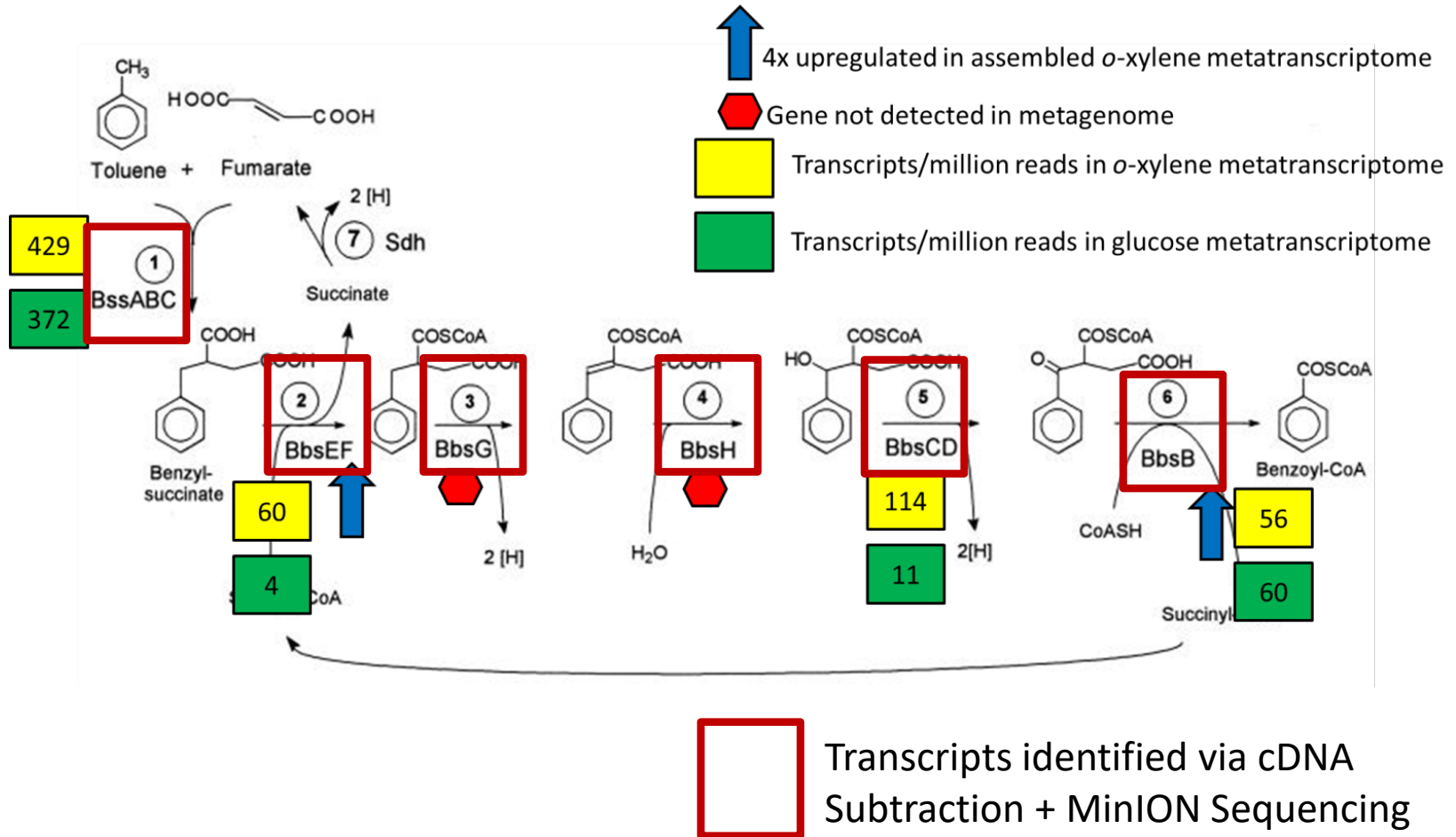
**cDNA:  
Not degrading compound**



**cDNA:  
Degrading compound**



# Metatranscriptomics – differential expression



# Use of meta-omics for biomarker assay development

- Metagenomics: **improved, hypothesis-driven** biomarker assay development
- Metatranscriptomics: **hypothesis-independent** biomarkers discovery
  - Folate biosynthesis genes
- cDNA Subtraction: **better pathway coverage** with less sequencing
- Meta-omics can be applied to mixed microbial communities, including field sites
  - New assays, biomarkers are *more* **field-relevant**

Rossmassler, K, Snow, C, Taggart, De Long, S.K, (2019) Advancing biomarkers for anaerobic *o*-xylene biodegradation via metagenomic analysis of a methanogenic consortium. *Applied Microbiology and Biotechnology*

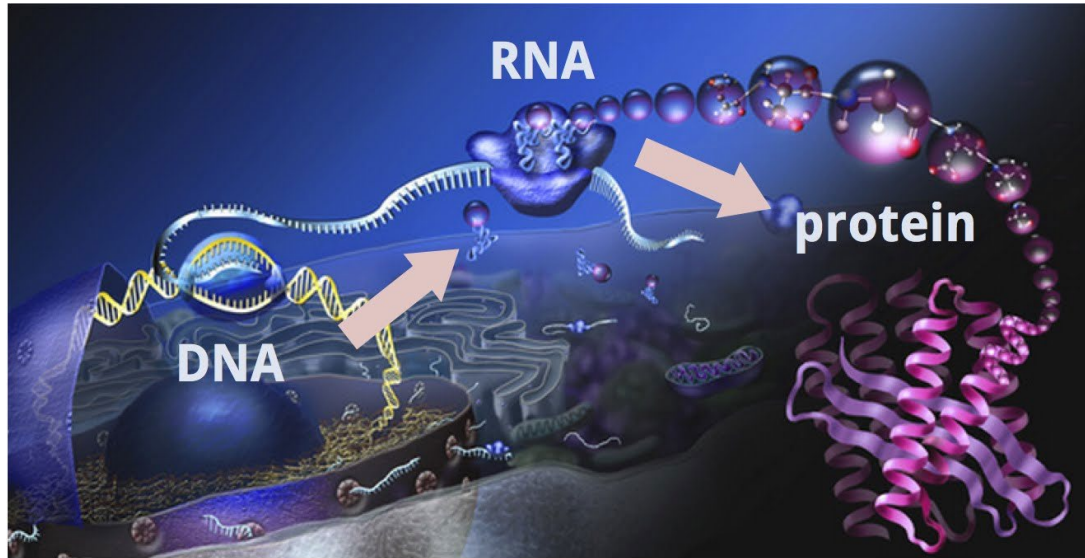


# Acknowledgements

- Dr. Elizabeth Edwards
- Dr. Fei Luo
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- Funding - NSF



# Questions ?



<https://i.ytimg.com/vi/ISqUDu4zb5k/maxresdefault.jpg>

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