Meta-omics Enabled Approaches for Identifying Biomarkers Directly from Mixed Microbial Communities

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Colorado State University

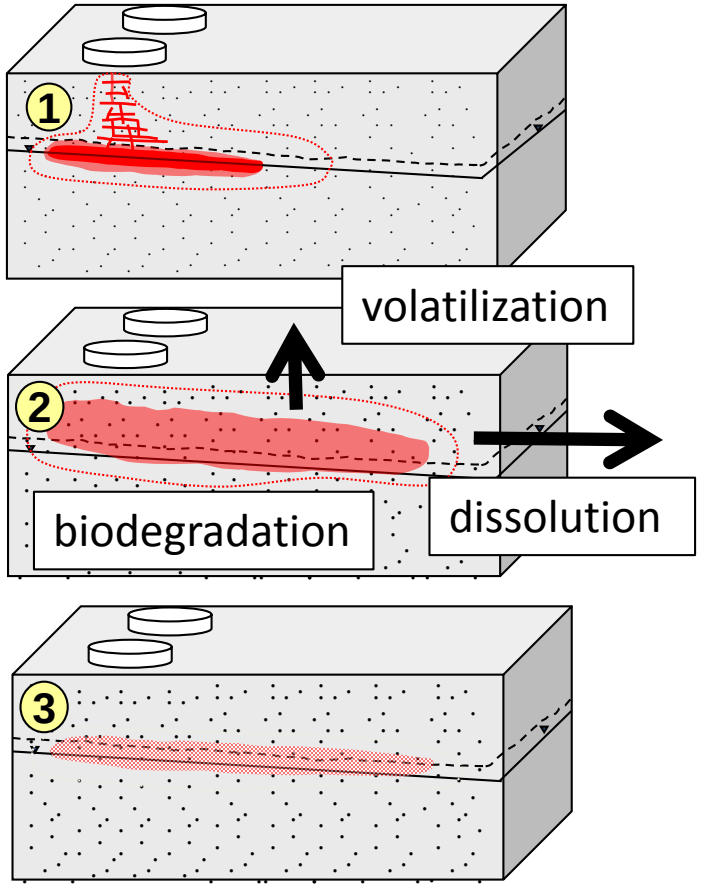
Why use meta-omics tools to identify biomarkers?

- The “menu” of available molecular assays is limited
  - For some contaminant(s)-redox conditions, none are available
  - For some, available assays can be highly inaccurate
- Available biomarker assays frequently based on pure cultures
  - Development time consuming
  - Assays more likely to be inaccurate
- Approaches needed that can be *directly applied to field-relevant mixed microbial communities*

Tracking contaminant removal with biomarkers

Molecular monitoring of bioremediation markers

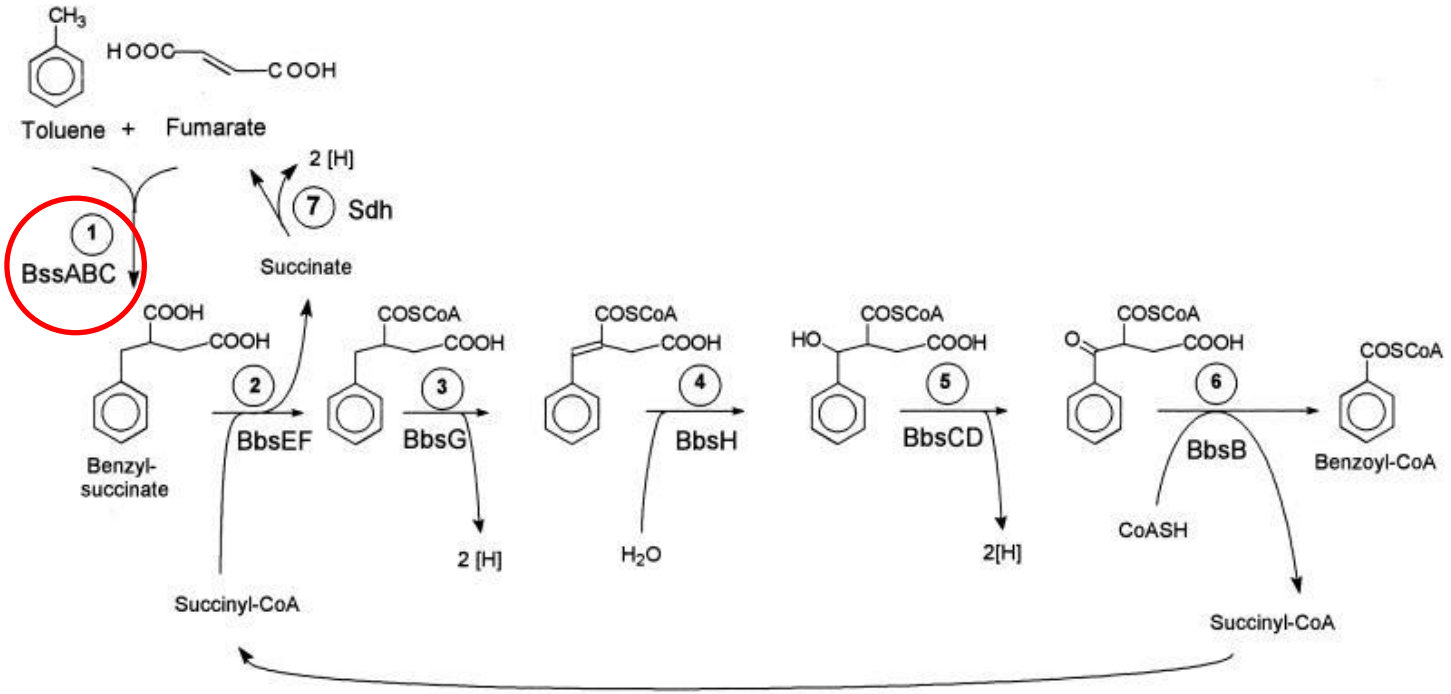
- Distinguishes between biotic and abiotic
- *In situ* tracking
  - Phylogeny (specific organisms)
  - Function or process
- Quantitate gene presence
  - qPCR
- Directly predict activity
  - RT-qPCR



Molecular tools for biomarker assays

Biomarker assay targeting functional genes

- Anaerobic biodegradation of toluene
- Initiated by fumarate addition by benzylsuccinate synthase

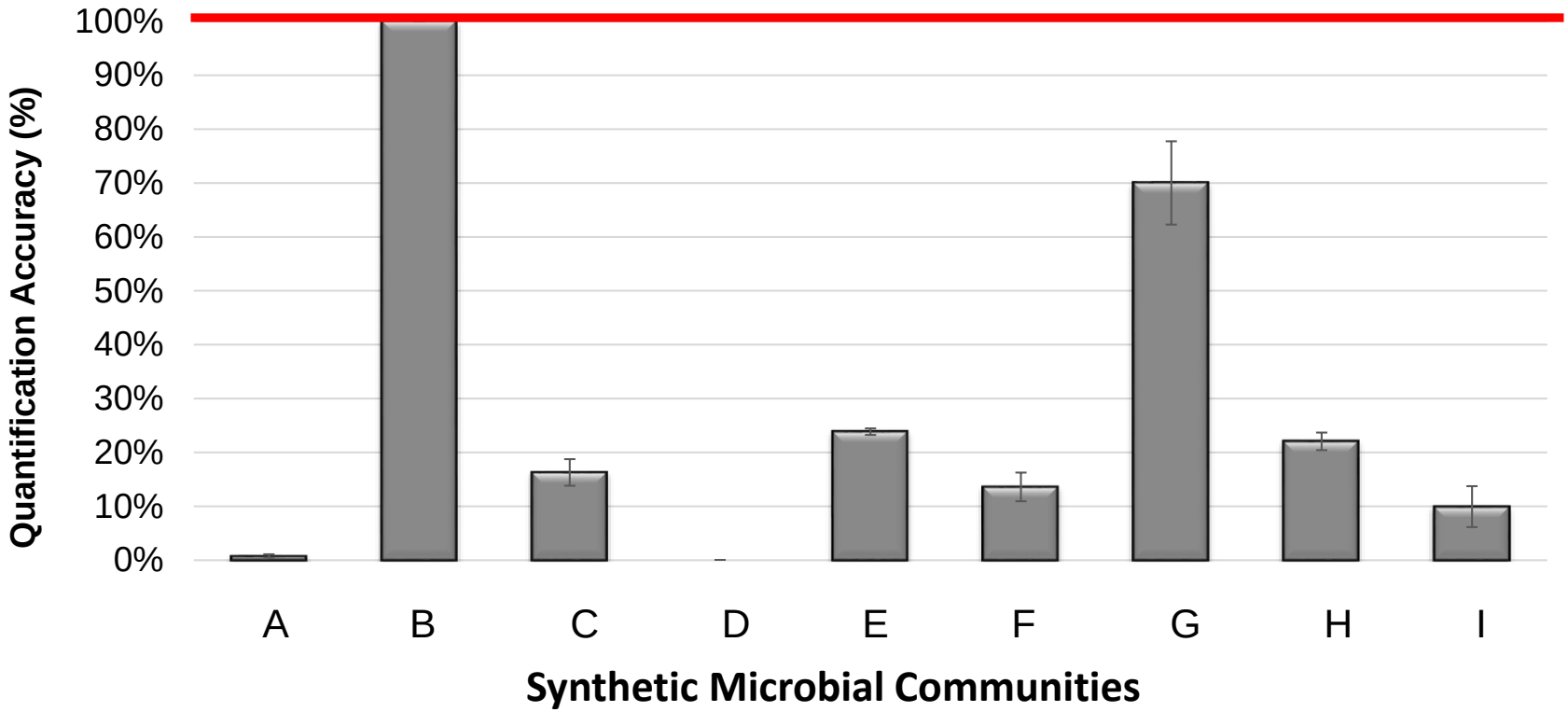


Leuthner B, Heider J. 2000. Anaerobic toluene catabolism of *Thauera aromatica*: the bbs operon codes for enzymes of beta oxidation of the intermediate benzylsuccinate. Journal of Bacteriology 182:272-277.

qPCR assays targeting *bssA*

Reference	Primer name	Amplicon size (bp)	*bssA* Target lineage
Beller *et al.*, 2002	BellerF BellerR	130	Denitrifying *Betaproteobacteria*
Beller *et al.*, 2008	SRBf SRBr	100	Sulfate-reducing *Deltaproteobacteria*
Staats *et al.*, 2011	bssA3f bssAr	500	Denitrifying and iron-reducing *Betaproteobacteria*
Fowler *et al.*, 2014	MbssA1F MbssA1R	223	*Clostridia*

(RT-q)PCR accuracy depends on primer design



Known number of copies to calculate amplification accuracy

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.

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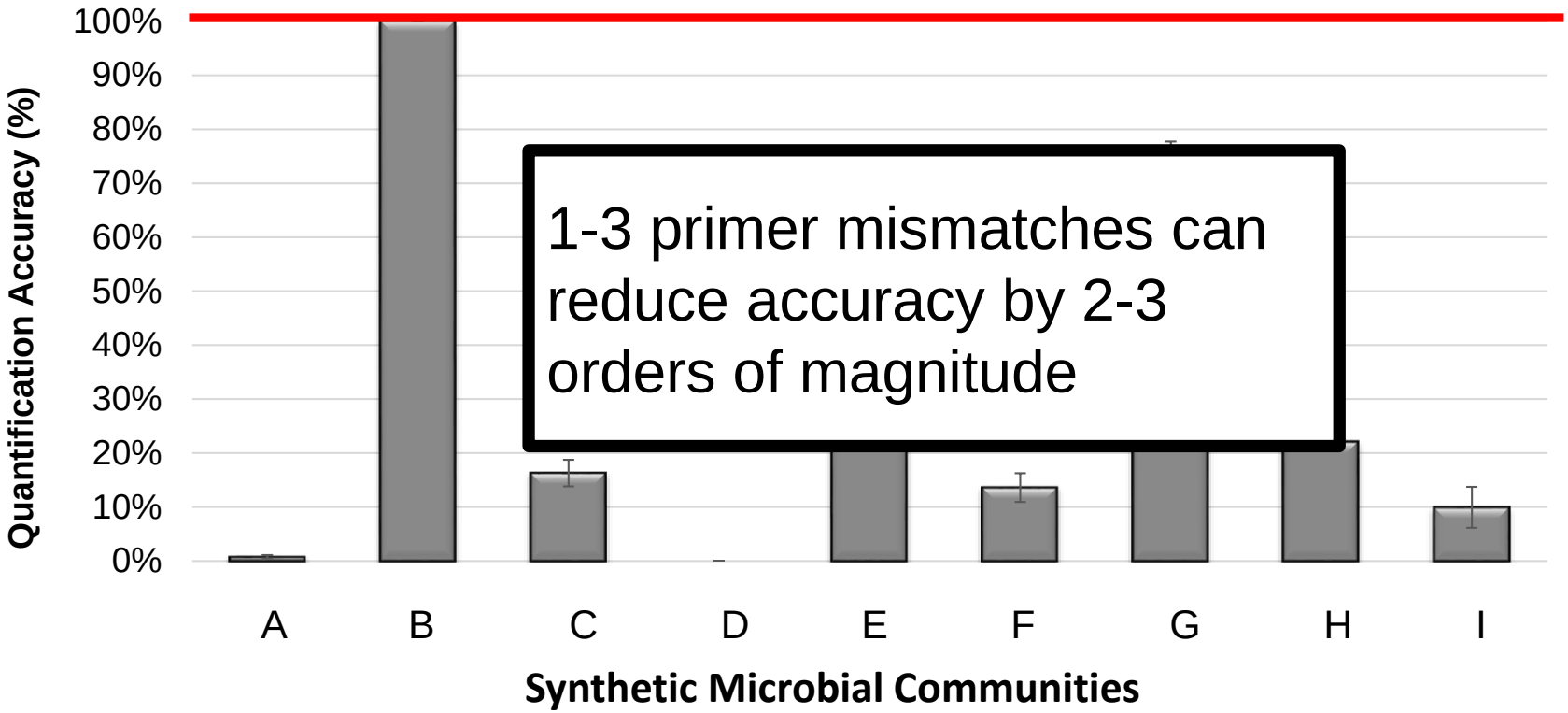

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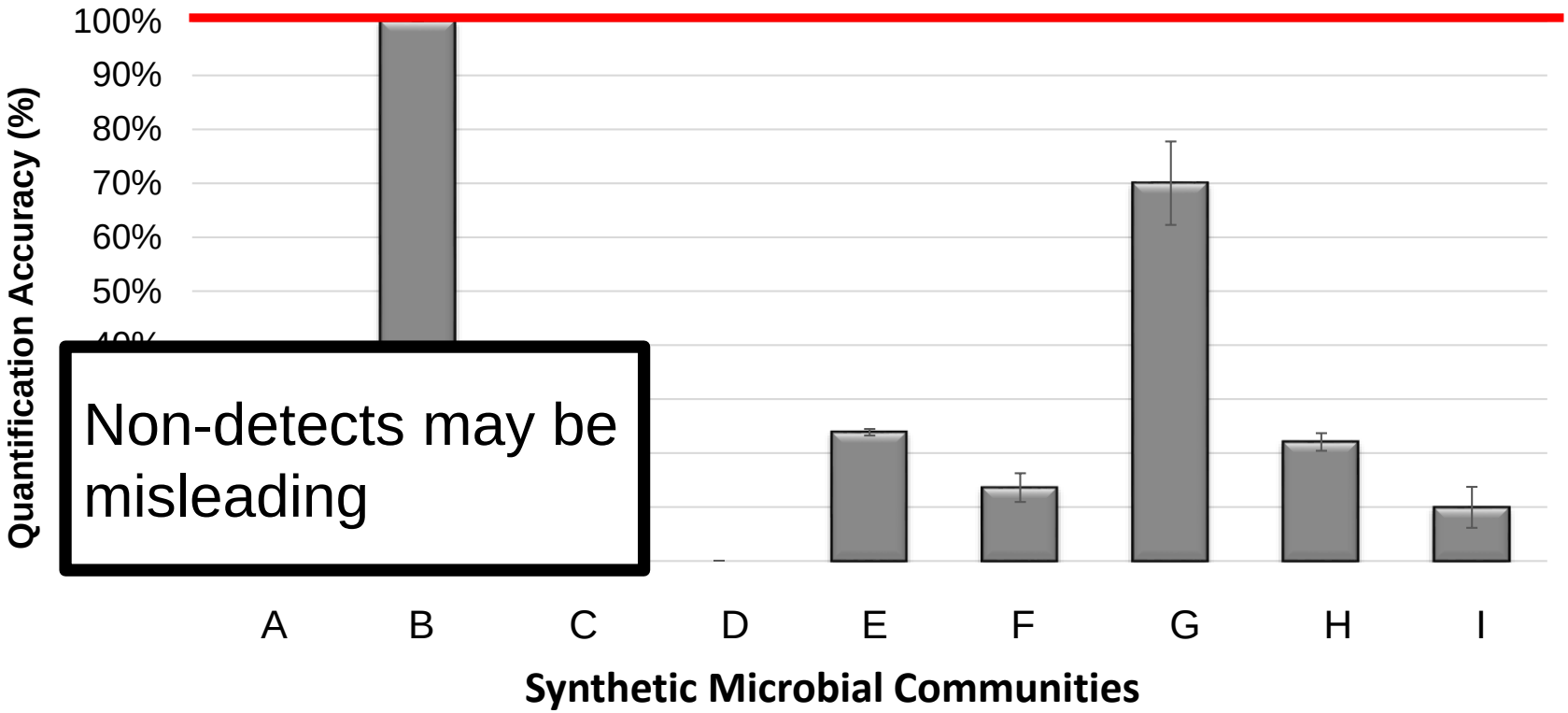

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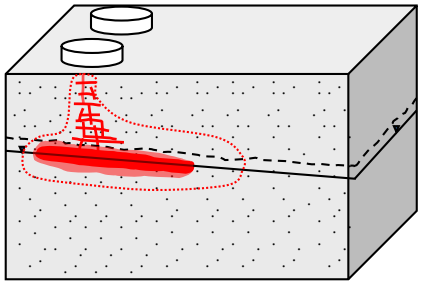
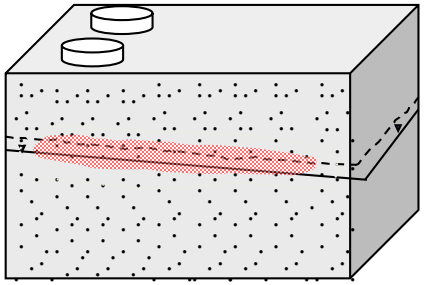
De novo strategy for designing primers

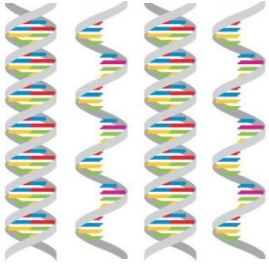


Non-degrading conditions

Extract nucleic acids

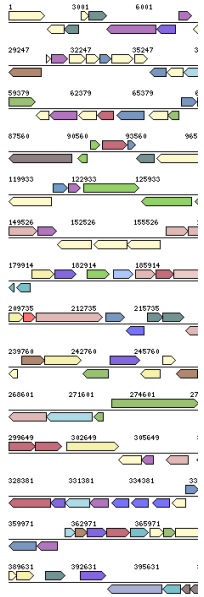


Degrading conditions



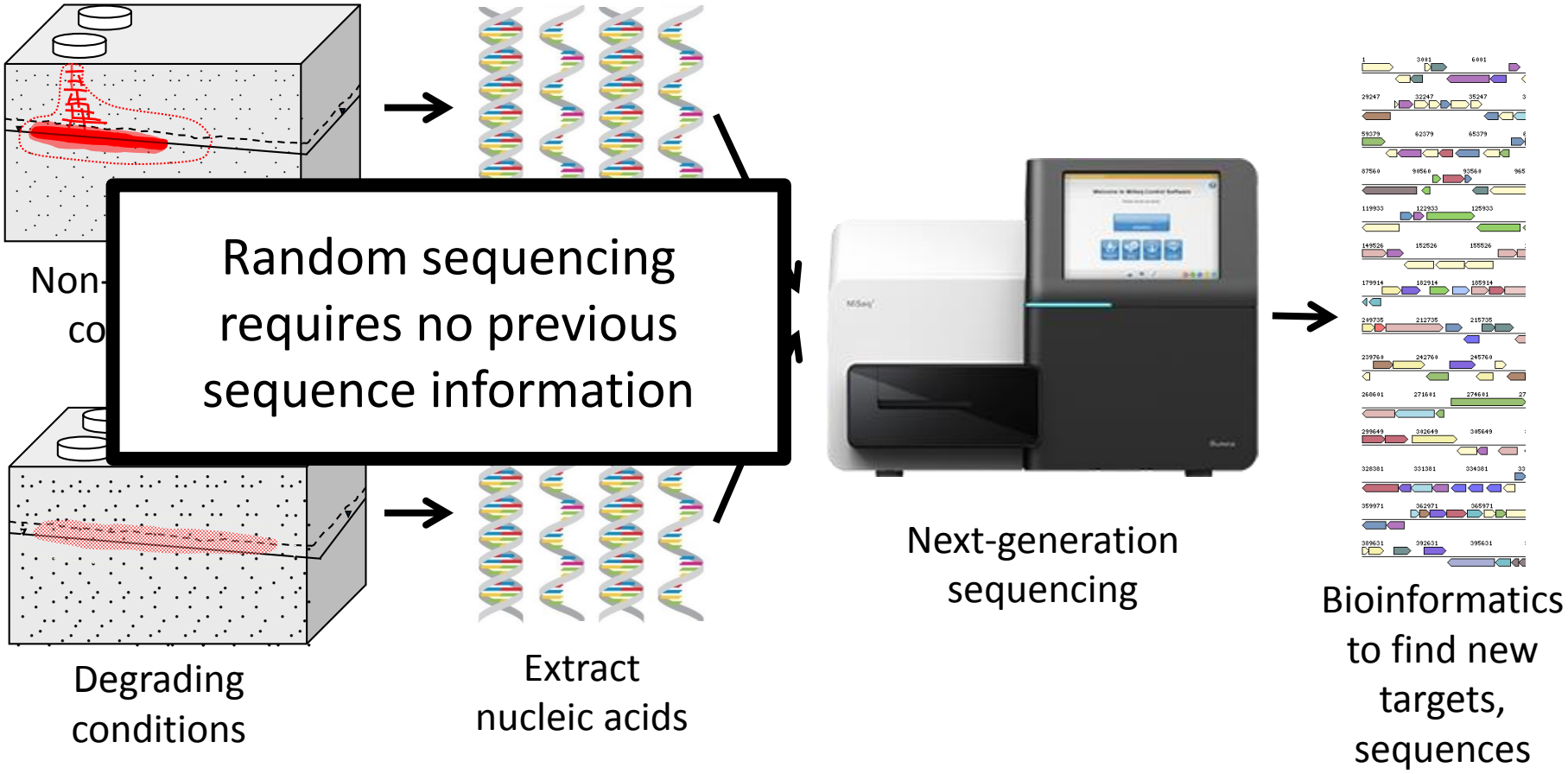
Extract nucleic acids

Next-generation sequencing

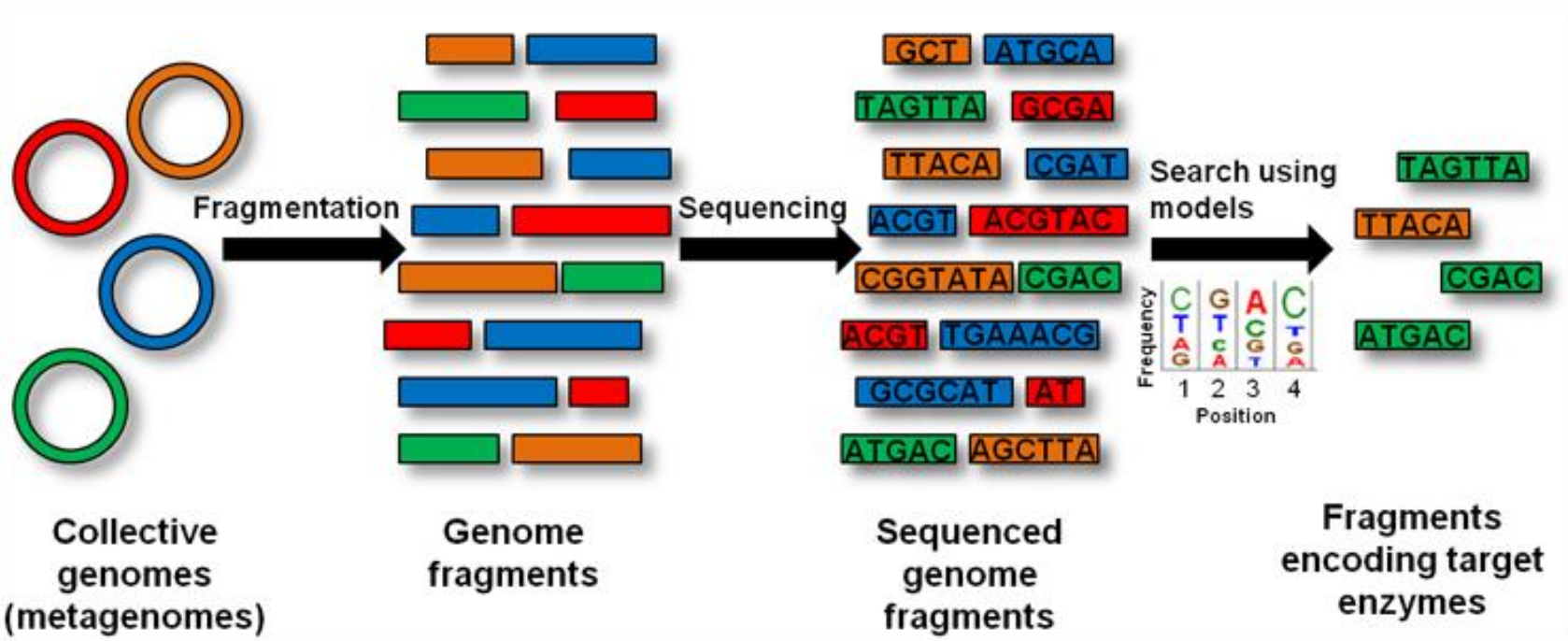


Bioinformatics to find new targets, sequences

De novo strategy for designing primers



De novo strategy for designing primers: Metagenomics

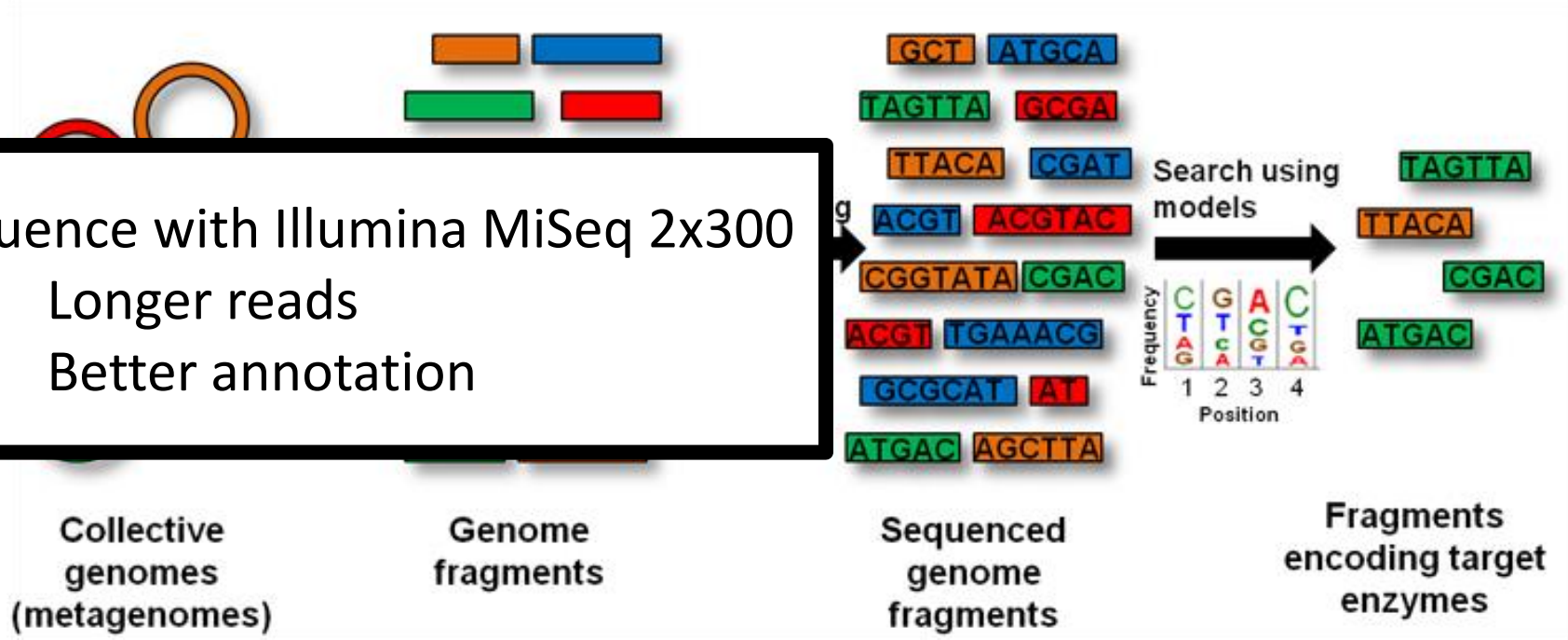


http://www.cebitec.uni-bielefeld.de/clib-gc/index.php/alumni/20-martha-zakrzewski

De novo strategy for designing primers: Metagenomics

Sequence with Illumina MiSeq 2x300

- Longer reads
- Better annotation



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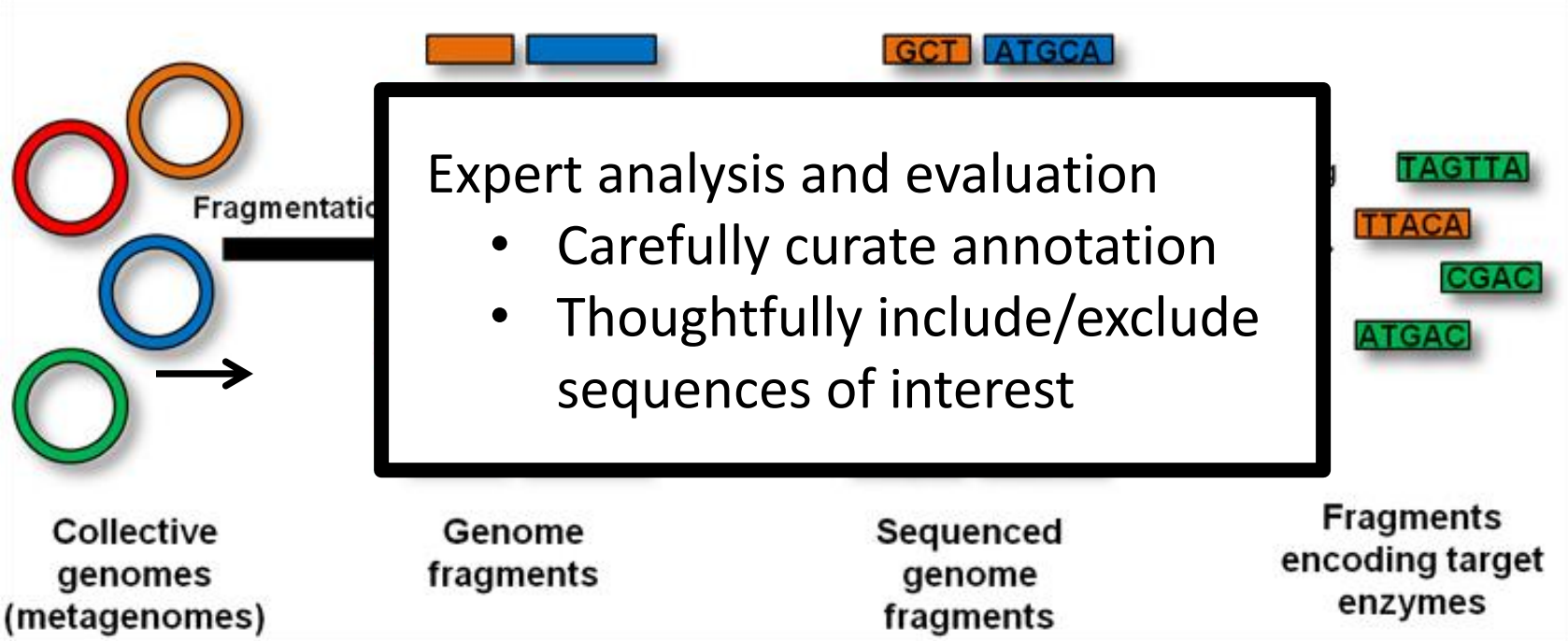
De novo strategy for designing primers: Metagenomics



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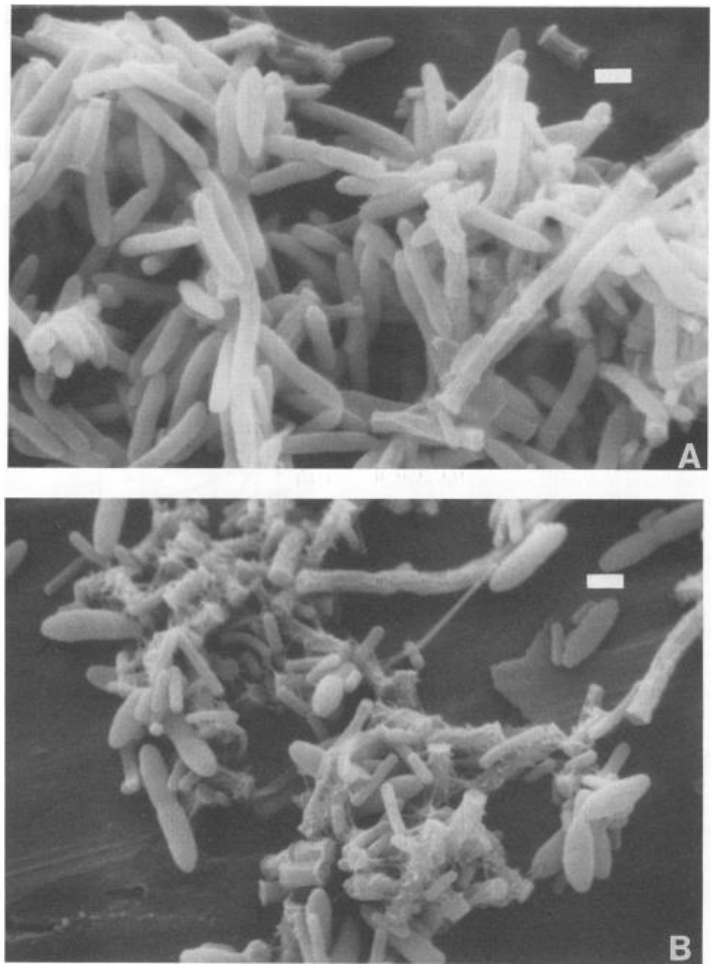
Model system to develop new strategy

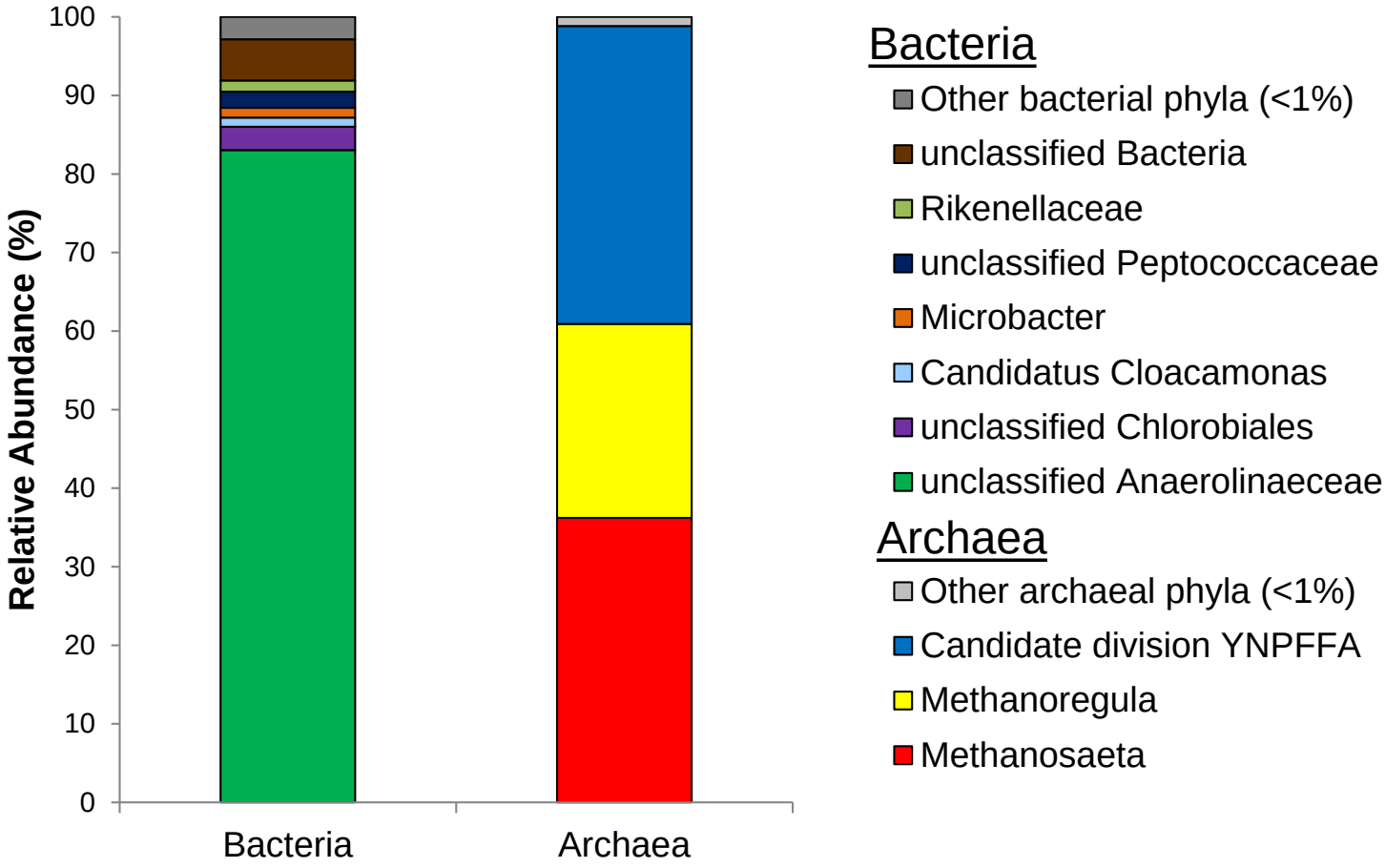
Field-derived methanogenic enrichment culture (Edwards *et al.* 1994)

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

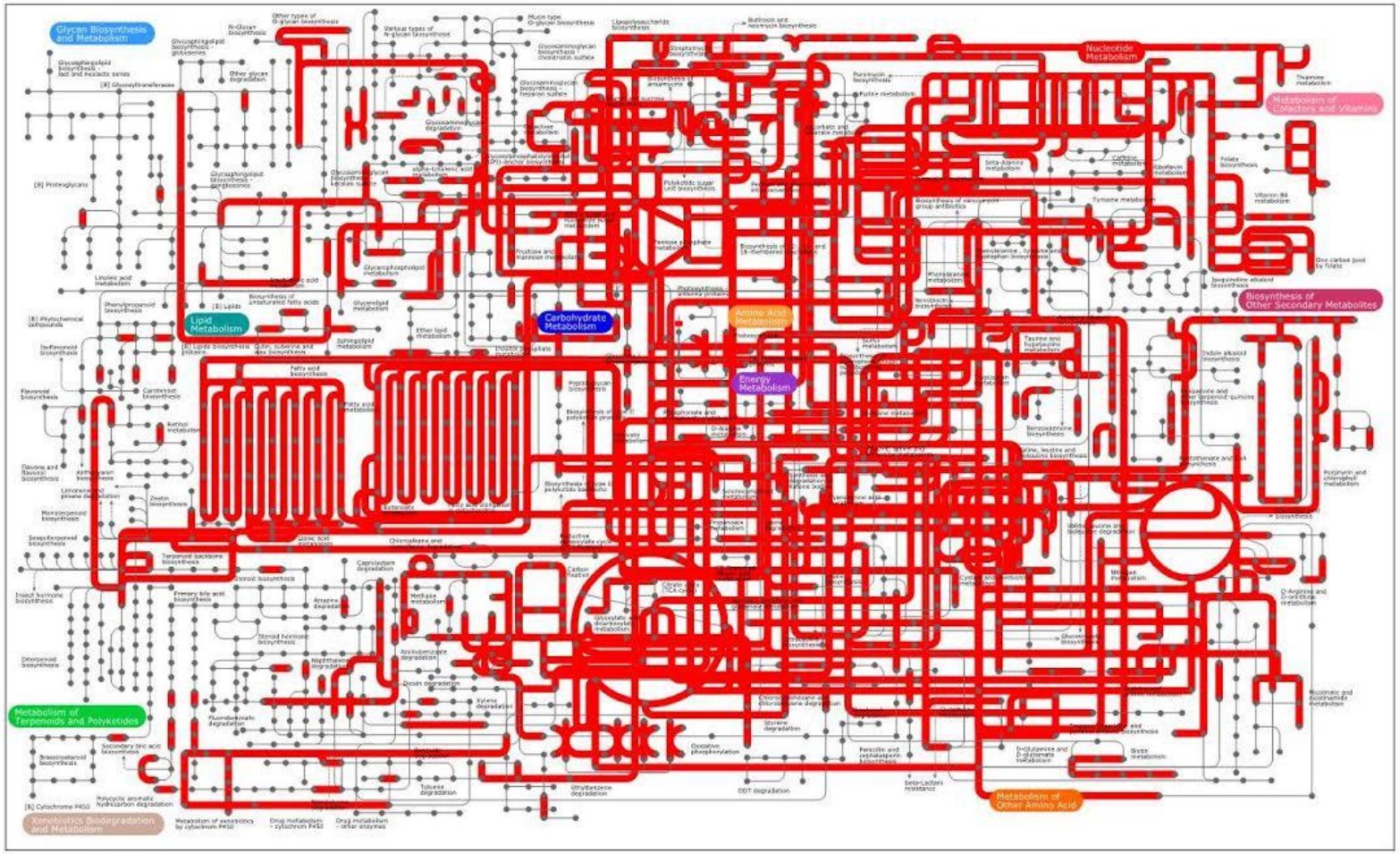


Edwards EA, Grbic-Galic D. 1994. Anaerobic degradation of toluene and *o*-xylene by a methanogenic consortium. Applied and Environmental Microbiology 60:313-322.

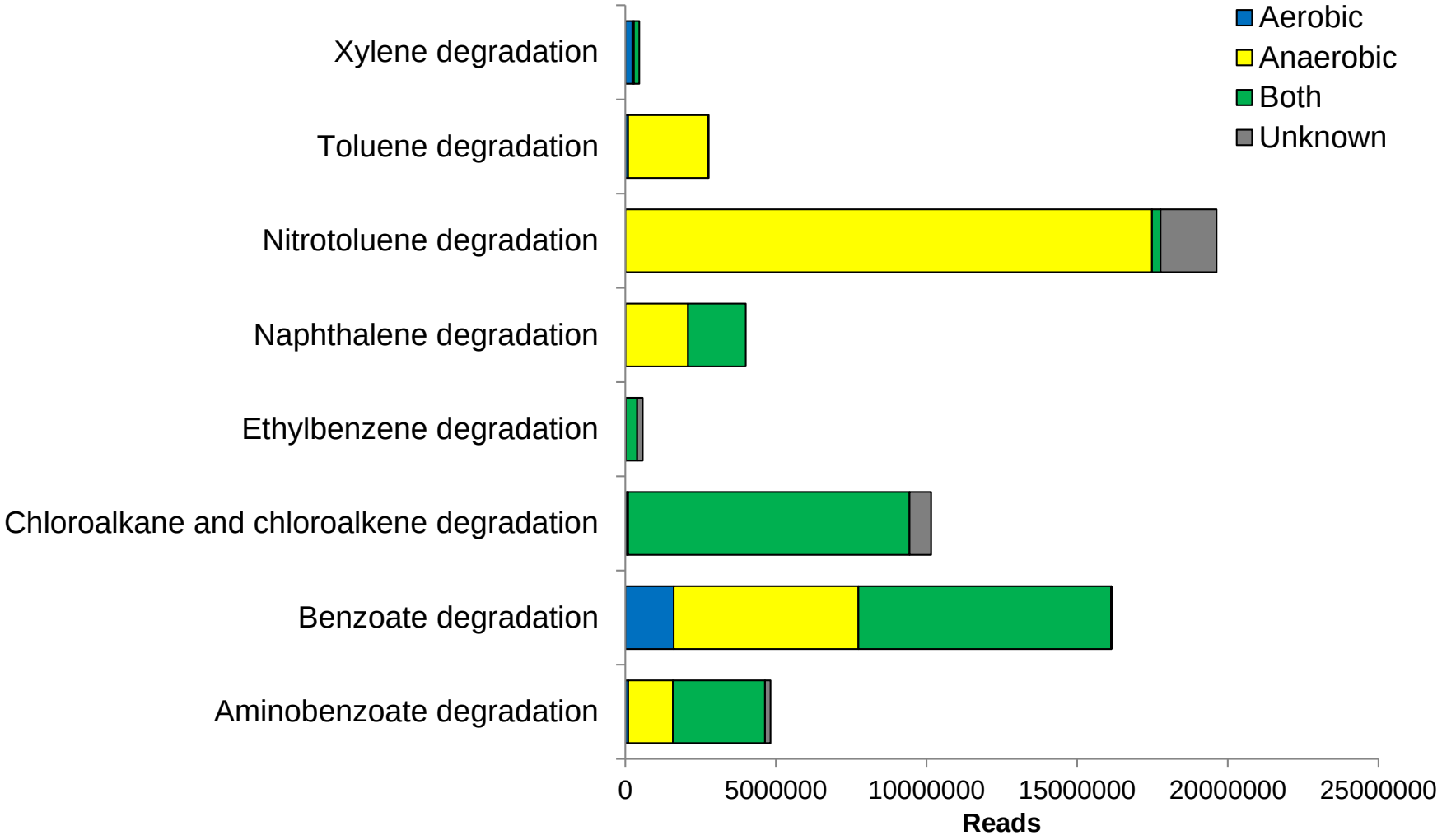
Community structure of model system



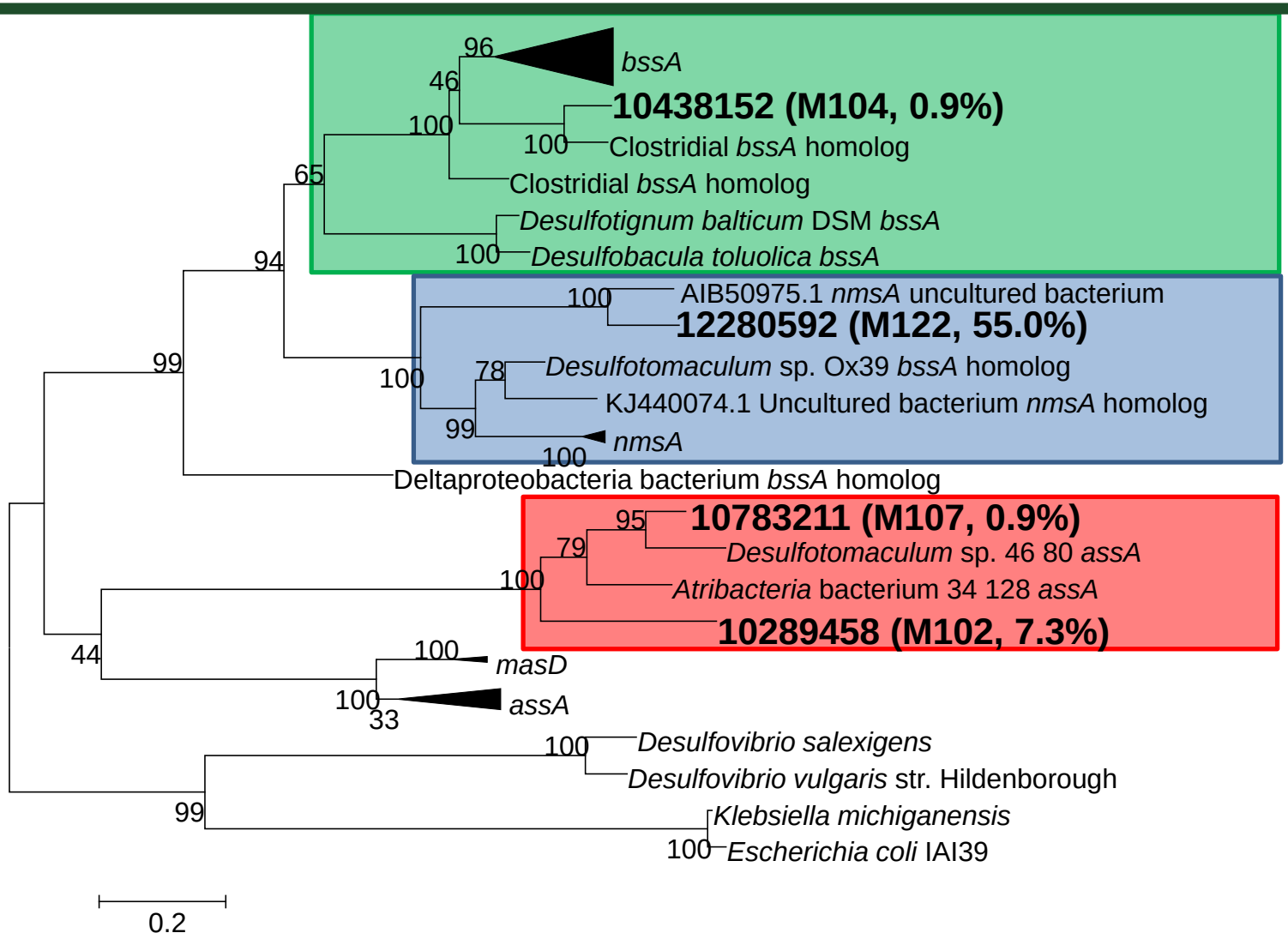
Functional potential of metagenome



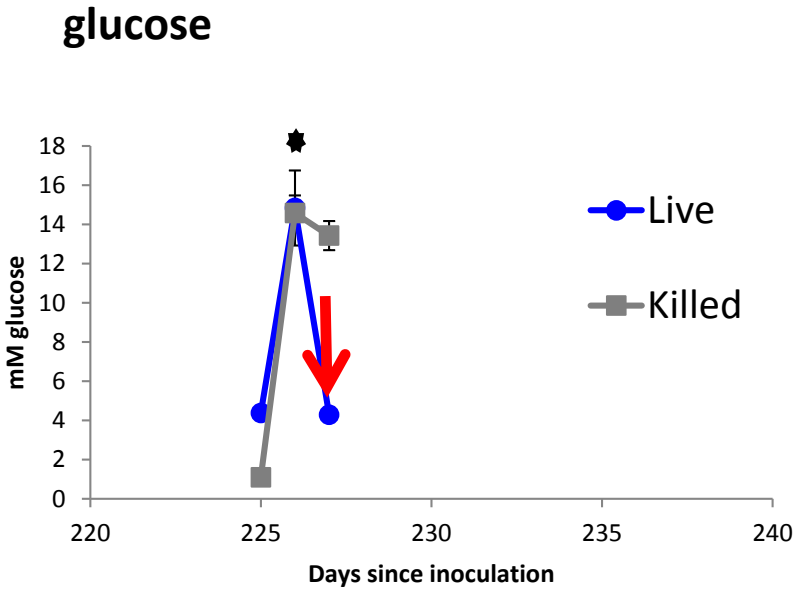
Xenobiotics degradation in metagenome

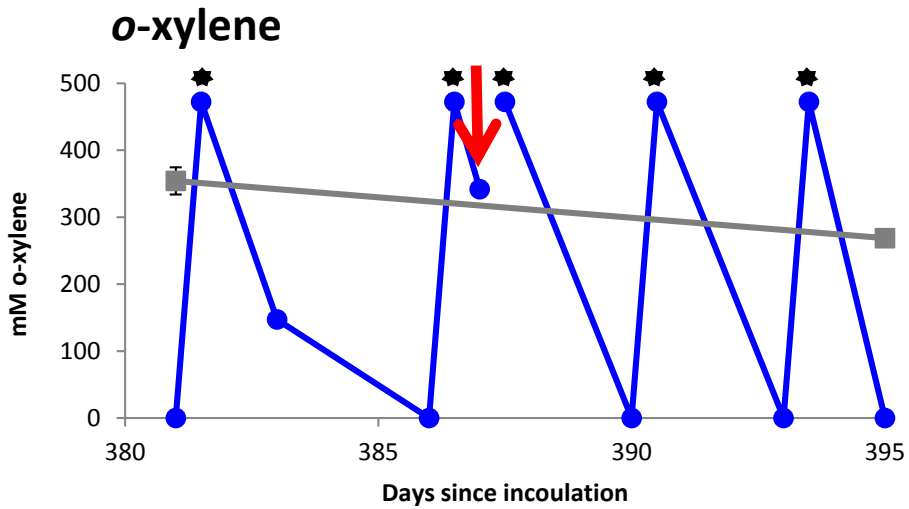


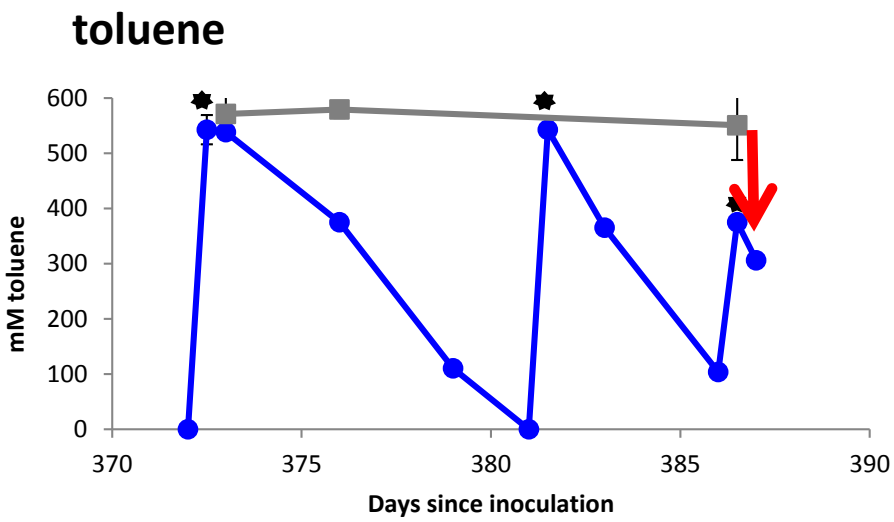
bssA-like genes in metagenome



Cultures for studying differential expression







Sequences compared to previously published *bssA* primers

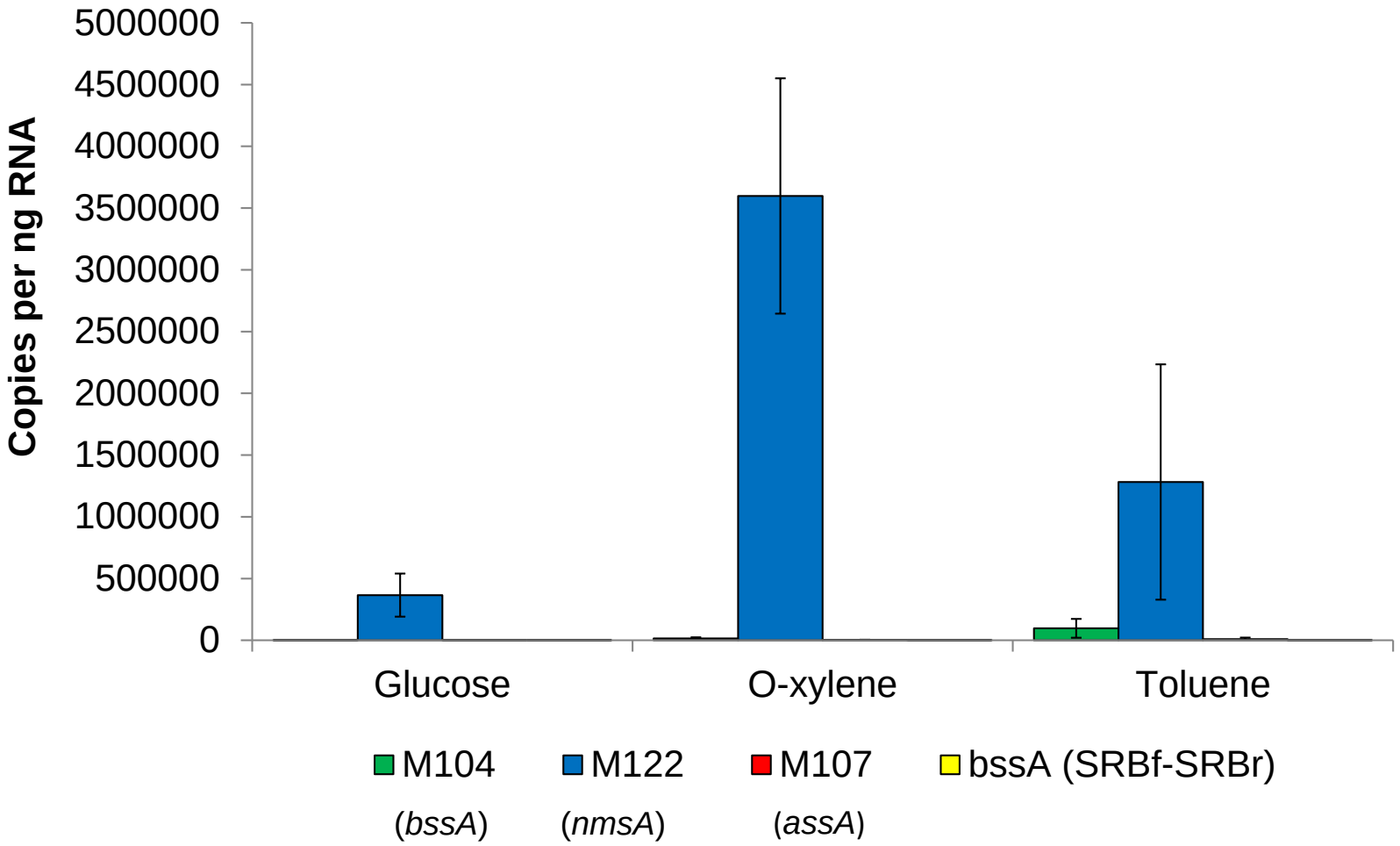
Reference	Primer name	Mismatches against M102 (F, R)	Mismatches against M104 (F, R)	Mismatches against M107 (F, R)	Mismatches against M122 (F, R)
Beller *et al.*, 2002	BellerF BellerR	**7, 5**	**2, 2**	**7, 4**	**8, 8**
Beller *et al.*, 2008	SRBf SRBr	**4, 5**	**3, 3**	**8, 7**	**7, 5**
Staats *et al.*, 2011	bssA3f bssAr	**7, 5**	**0, 3**	**7, 6**	**7, 5**
Fowler *et al.*, 2014	MbssA1F MbssA1R	**6, 6**	**4, 6**	**7, 6**	**6, 5**

Sequences compared to previously published *bssA* primers

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

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

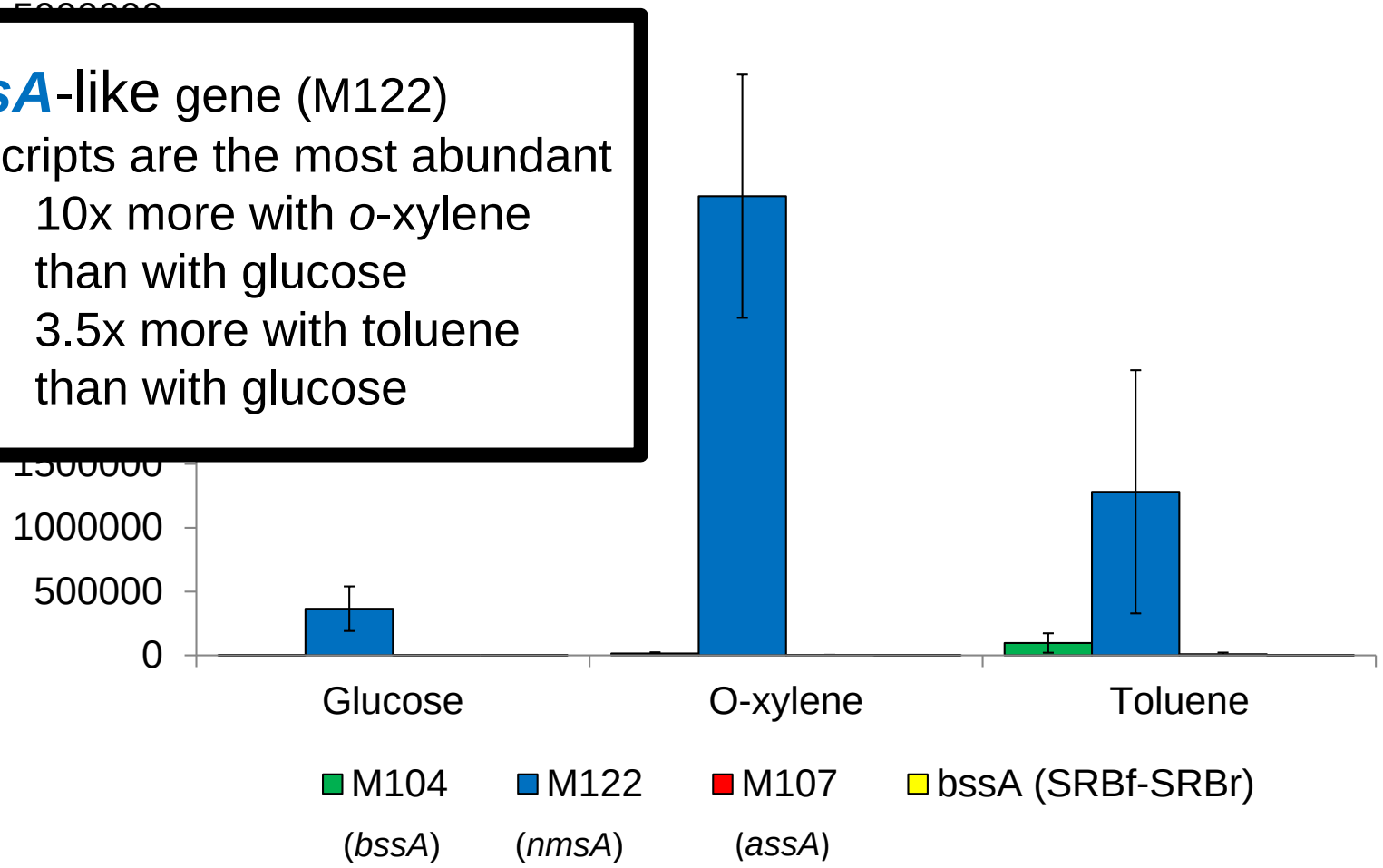
Expression of *bssA*-like genes



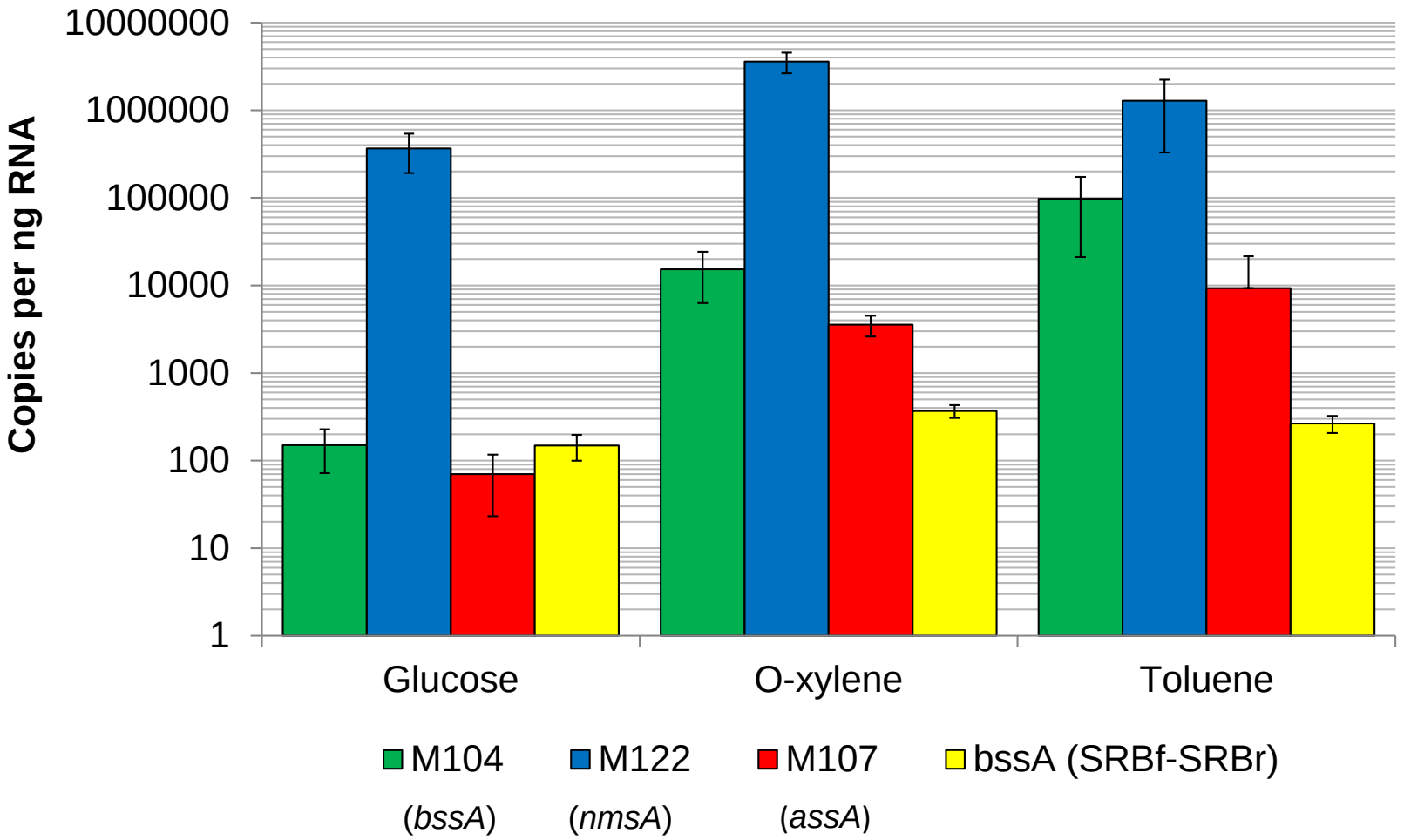
Expression of *bssA*-like genes

***nmsA*-like gene (M122)** transcripts are the most abundant

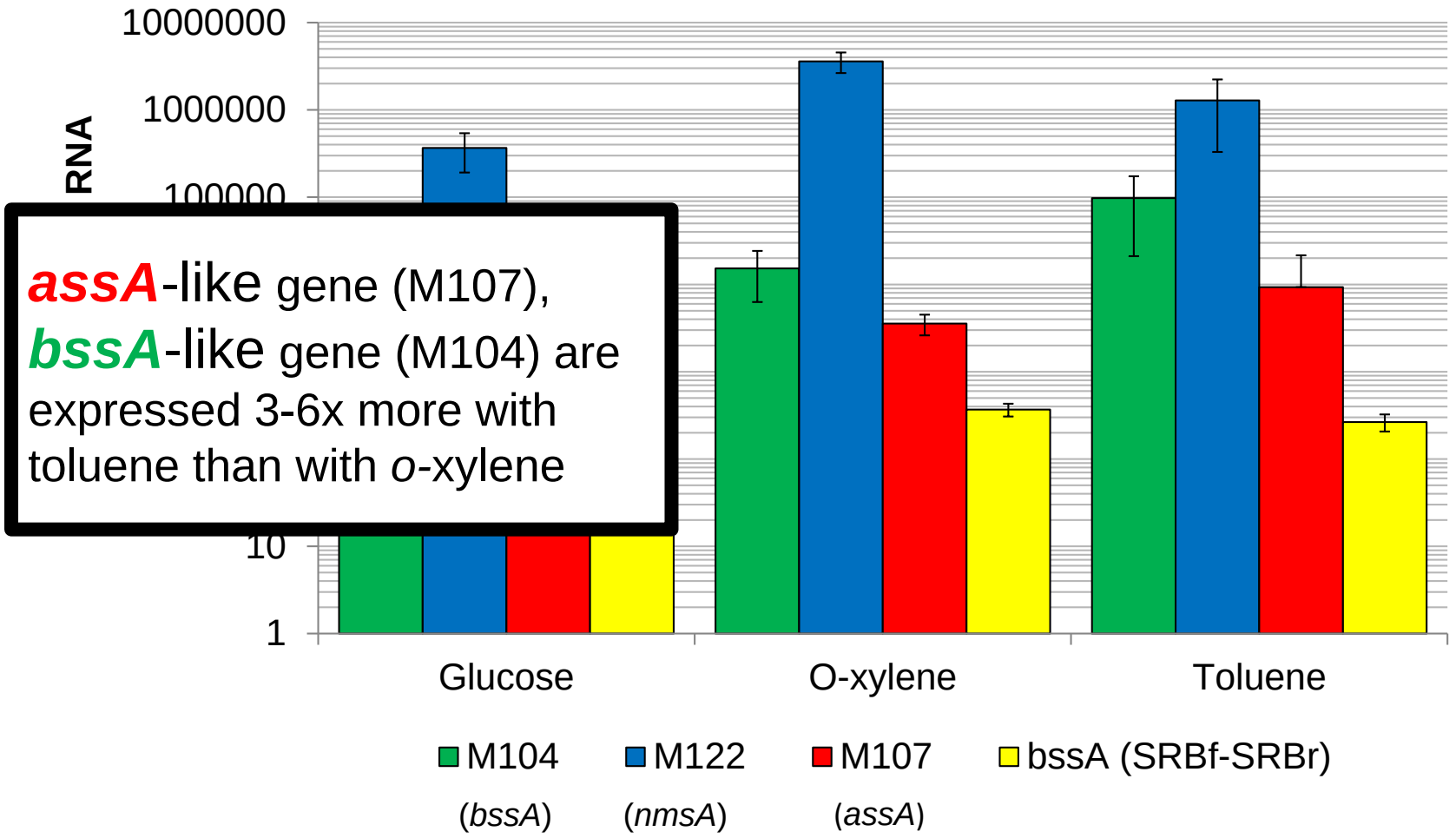
- 10x more with *o*-xylene than with glucose
- 3.5x more with toluene than with glucose



Expression of *bssA*-like genes

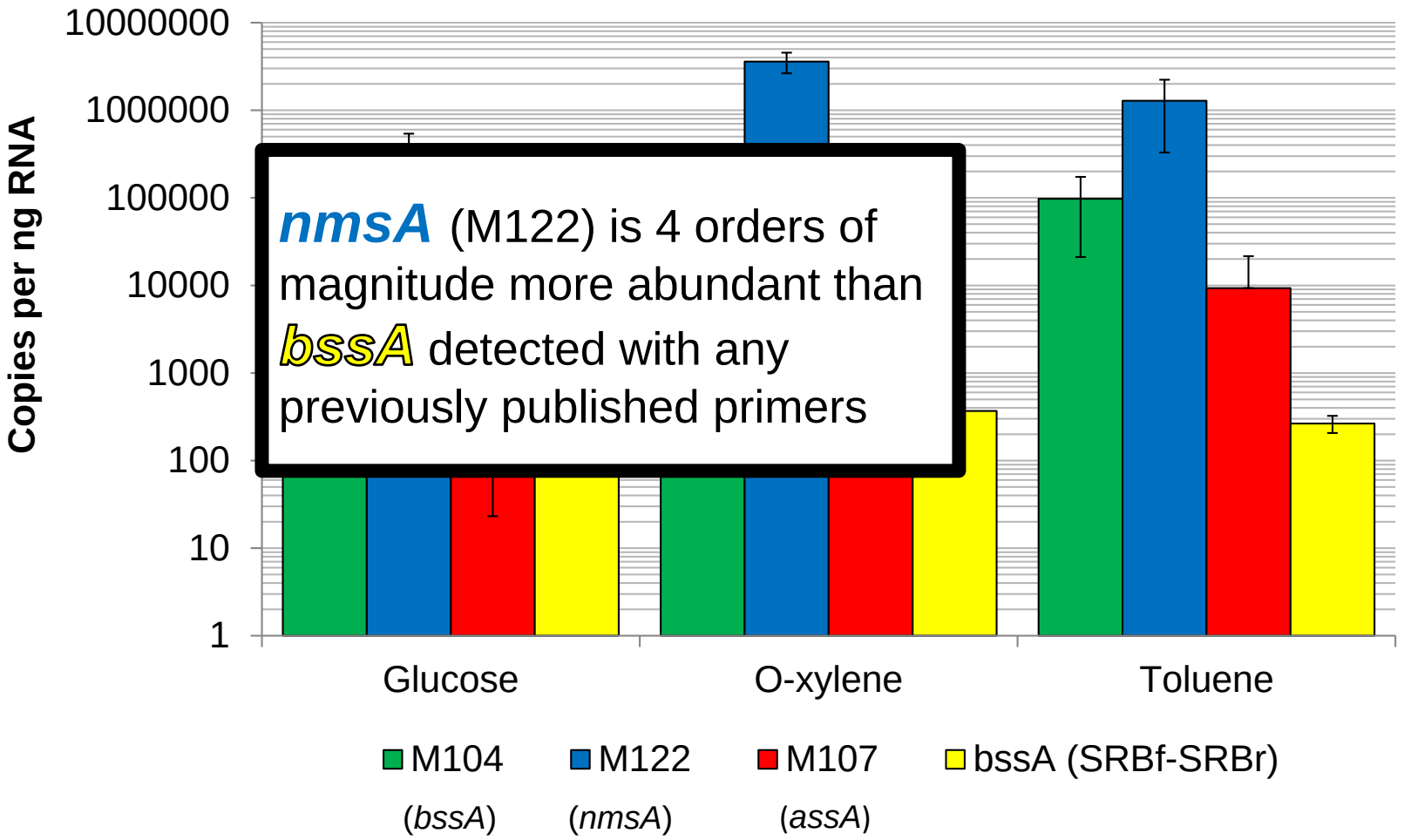


Expression of *bssA*-like genes



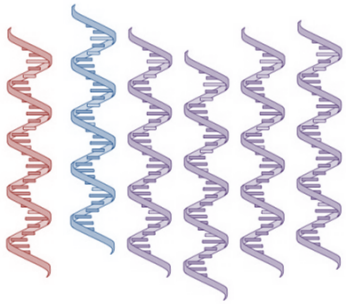
***assA*-like** gene (M107), ***bssA*-like** gene (M104) are expressed 3-6x more with toluene than with *o*-xylene

Expression of *bssA*-like genes

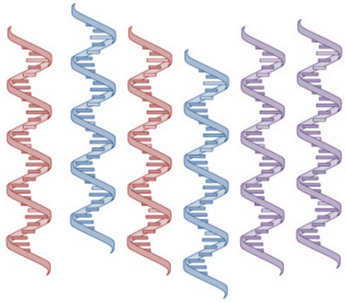


Metatranscriptomics – differential expression

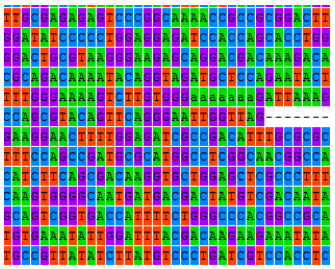
Glucose



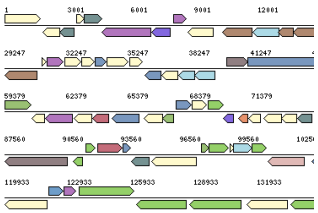
o-xylene



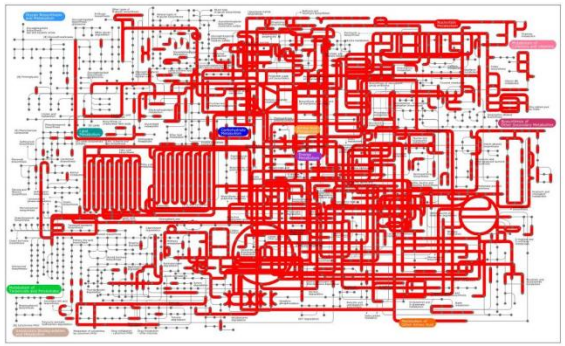
Extract RNA

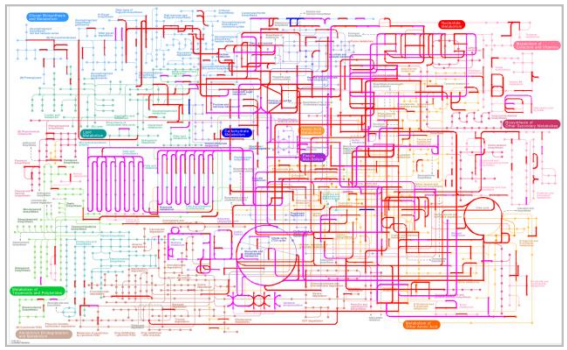


Sequence



Assemble, annotate genes

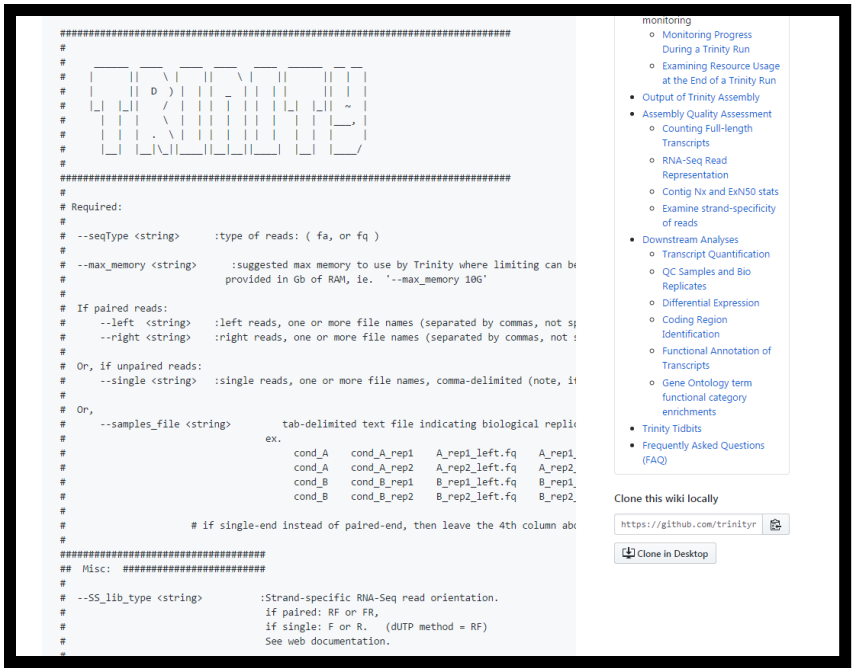




Differential expression

Identify differentially expressed genes - biomarkers

Analysis is currently under way to determine differentially expressed genes in *o*-xylene and glucose cultures



Grabherr MG, *et al*. Full-length transcriptome assembly from RNA-seq data without a reference genome. Nat Biotechnol. 2011 May 15;29(7):644-52. doi: 10.1038/nbt.1883. PubMed PMID: 21572440.

Use of meta-omics for biomarker assay development

- Metagenomics allows **improved**, **hypothesis-driven** biomarker assay development
- Metatranscriptomics allows **hypothesis-independent** biomarker discovery
- Meta-omics can be applied to mixed microbial communities, including field sites
  - New assays, biomarkers are **field-relevant**

- *In preparation*: Rossmassler, K, Snow, C, De Long, S.K, 2017 "Metagenomic-enabled Biomarker Identification: Biodegradation of *o*-xylene by a Methanogenic Consortium"

Acknowledgements

- Dr. Elizabeth Edwards
- Dr. Fei Luo
- Diana Marcela Nuñez Hernandez
- De Long lab members

- **Funding - NSF**

Questions ?