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Using metagenomics and metatranscriptomics to study specific bacterial species involved in biological phosphorus removal from wastewater

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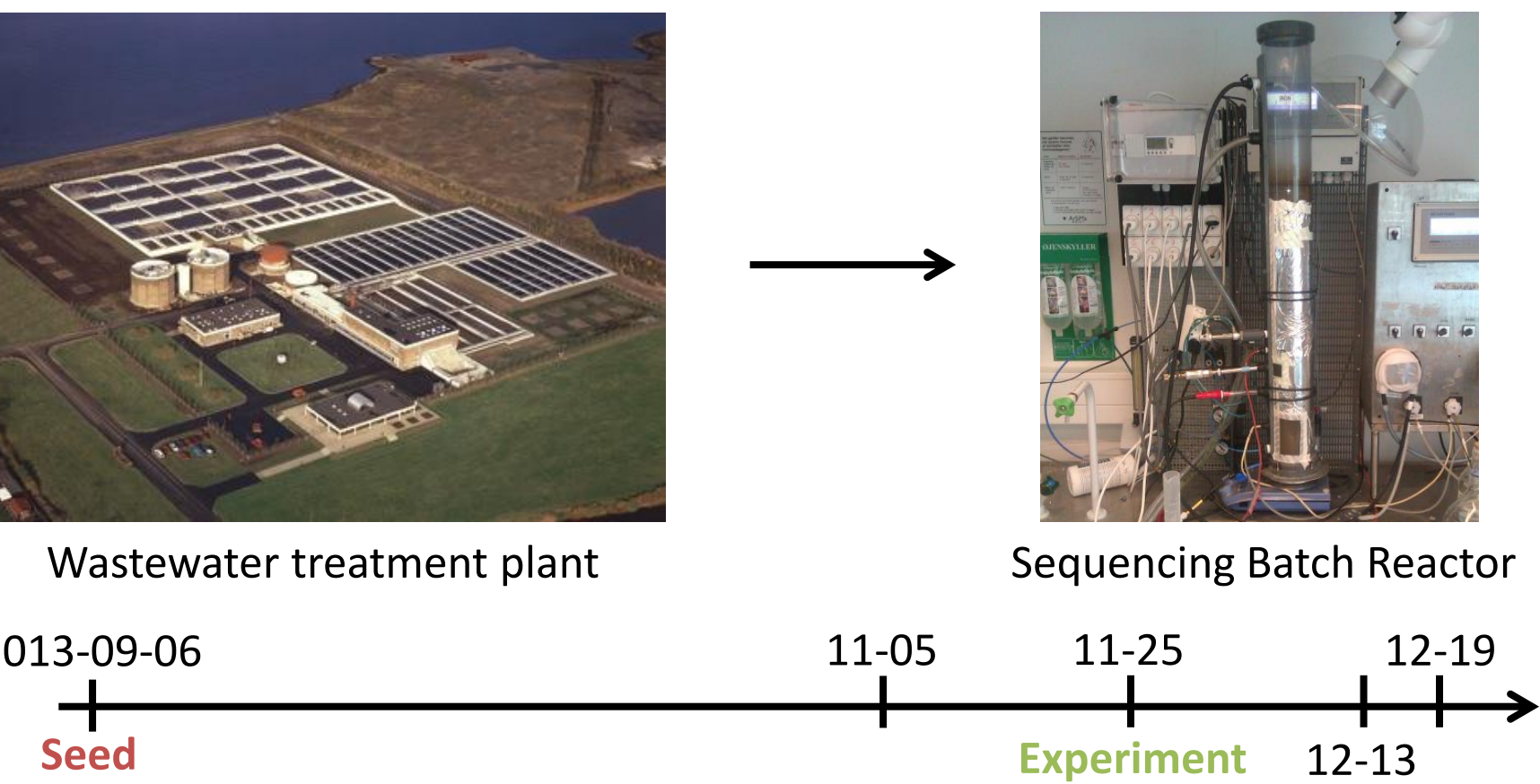
Introduction

Enhanced Biological Phosphorous Removal (EBPR) from wastewater treatment plants is managed by Polyphosphate Accumulating Organisms (PAOs). The model PAO is “*Candidatus Accumulibacter phosphatis*” (Accumulibacter) and despite a large body of knowledge, their detailed physiology remains elusive.

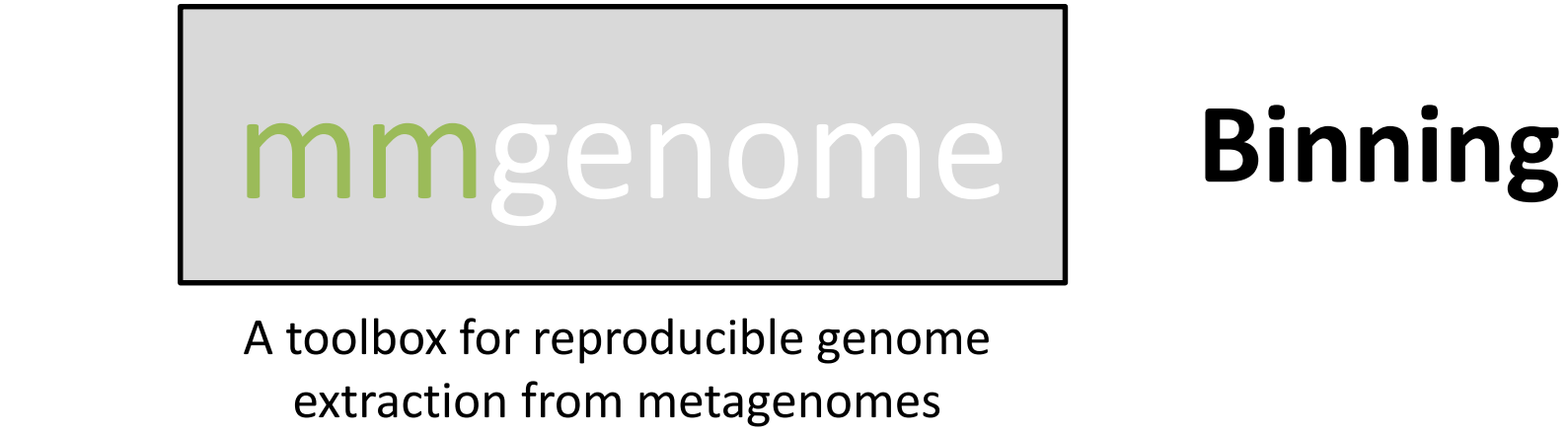
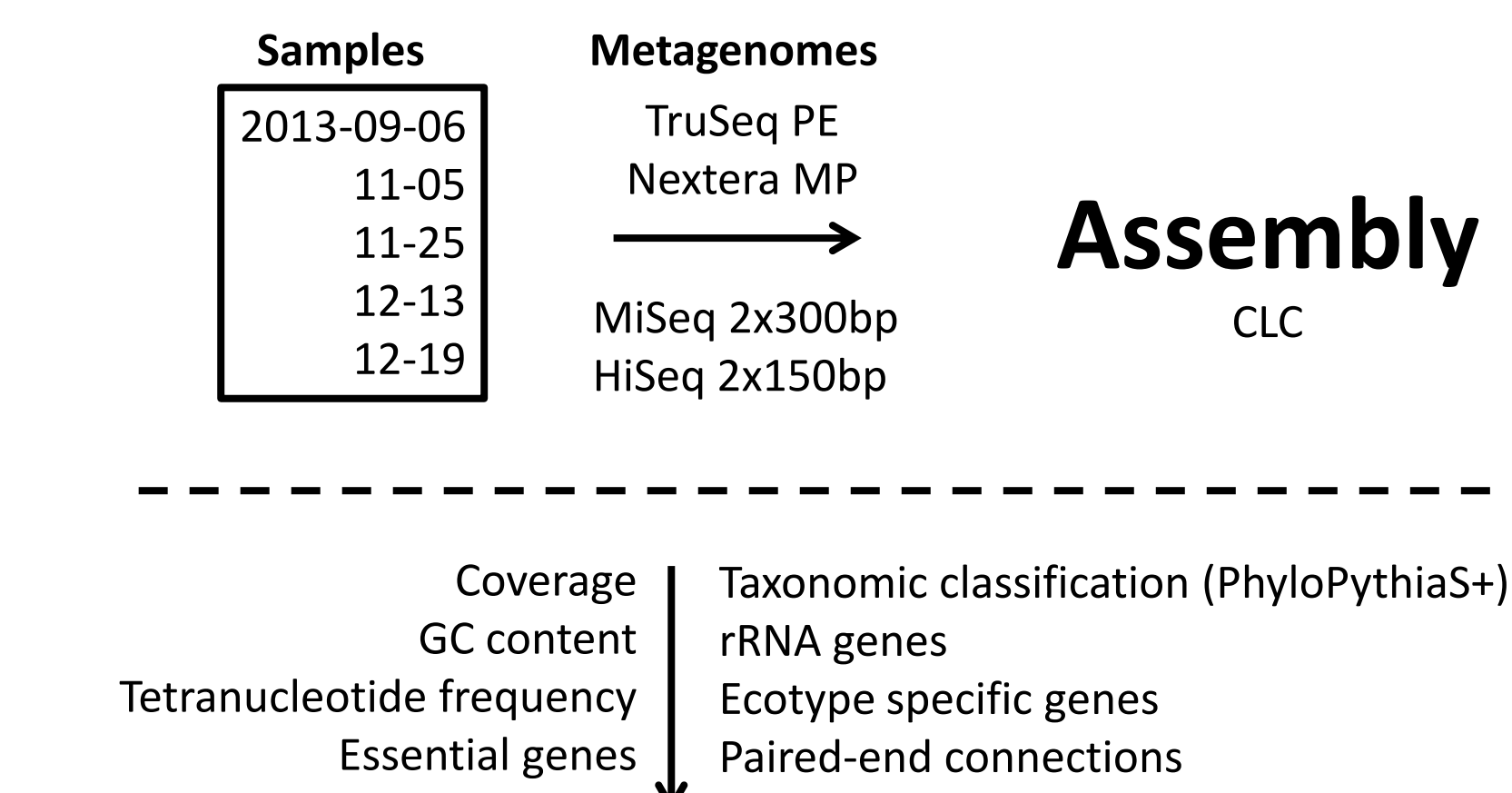
In this study we combined metagenomics, metatranscriptomics and laboratory scale enrichment in order to examine the gene expression of the uncultured Accumulibacter and the co-enriched associated community.

Methods

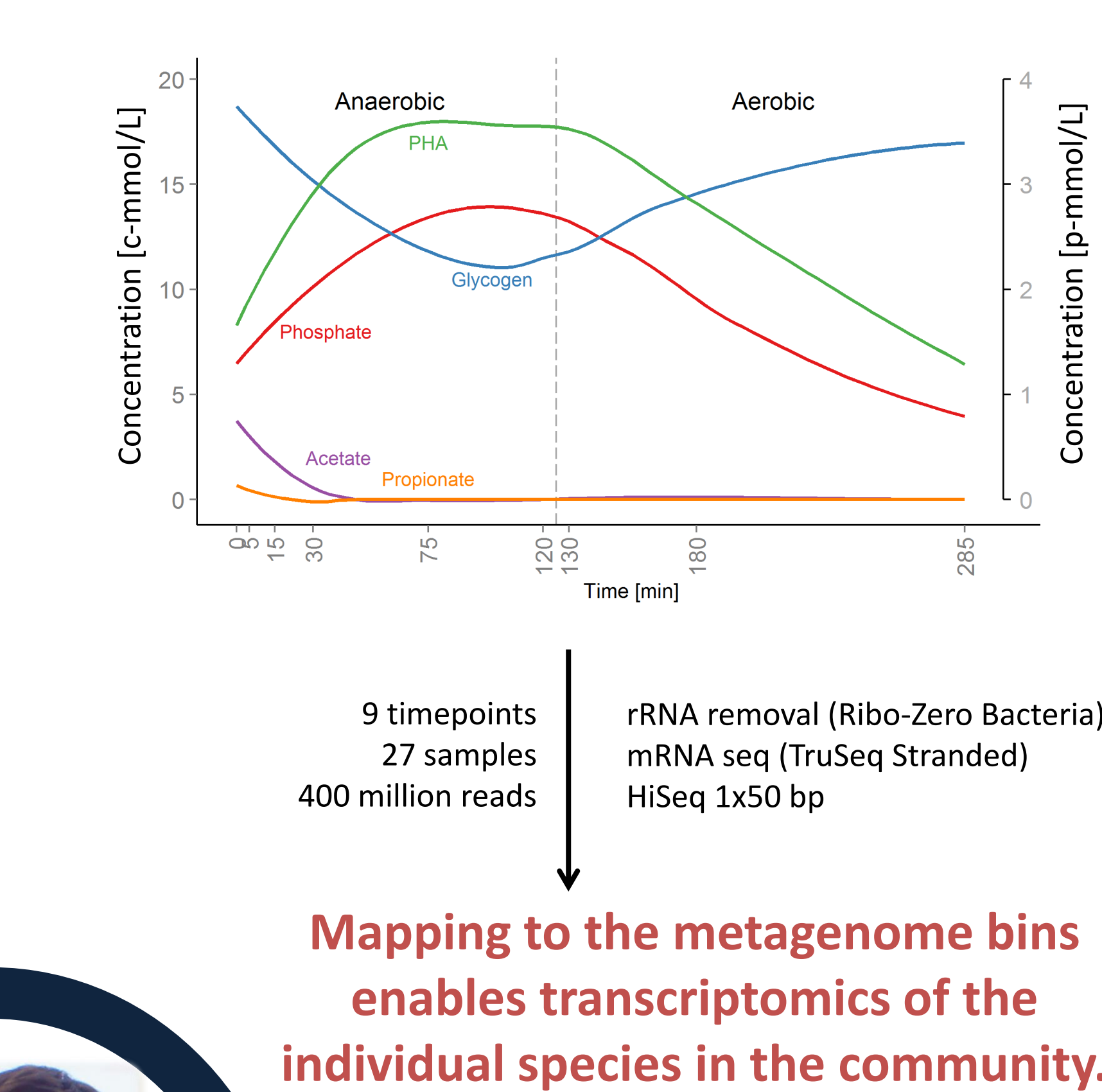
Enrichment



Genome recovery



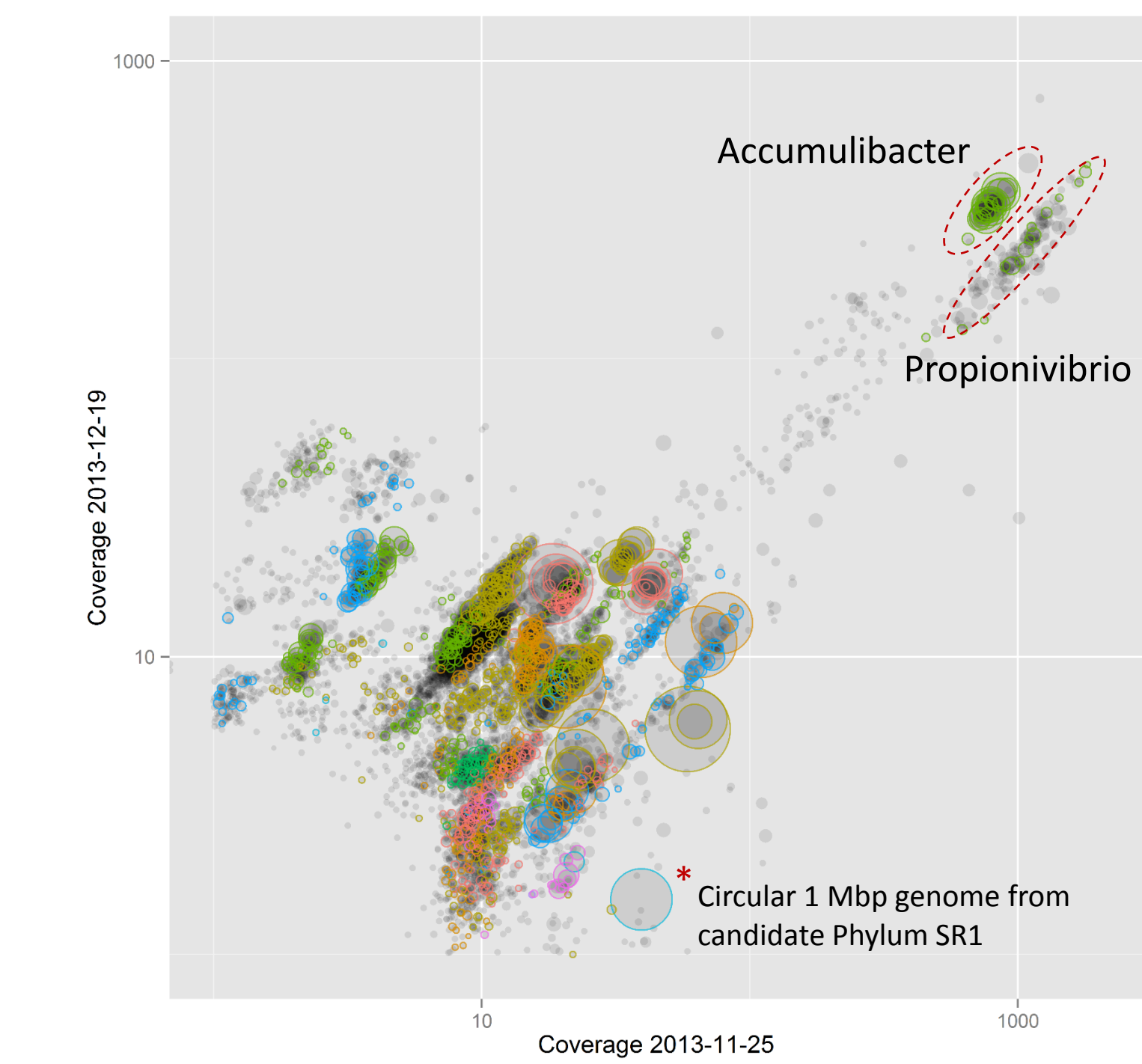
Transcriptomics



Conclusions

- Combining metagenomics, metatranscriptomics and laboratory scale enrichments enables transcriptome studies of most individual species in the community.
- For the first time we reveal the detailed transcriptome landscape of Accumulibacter during the anaerobic feast and aerobic famine conditions of the EBPR process.
- A new GAO (competitor to PAOs with a negative impact on EBPR) was discovered. Propionivibrio is closely related to Accumulibacter and hit by the current FISH probes used to define PAOs.

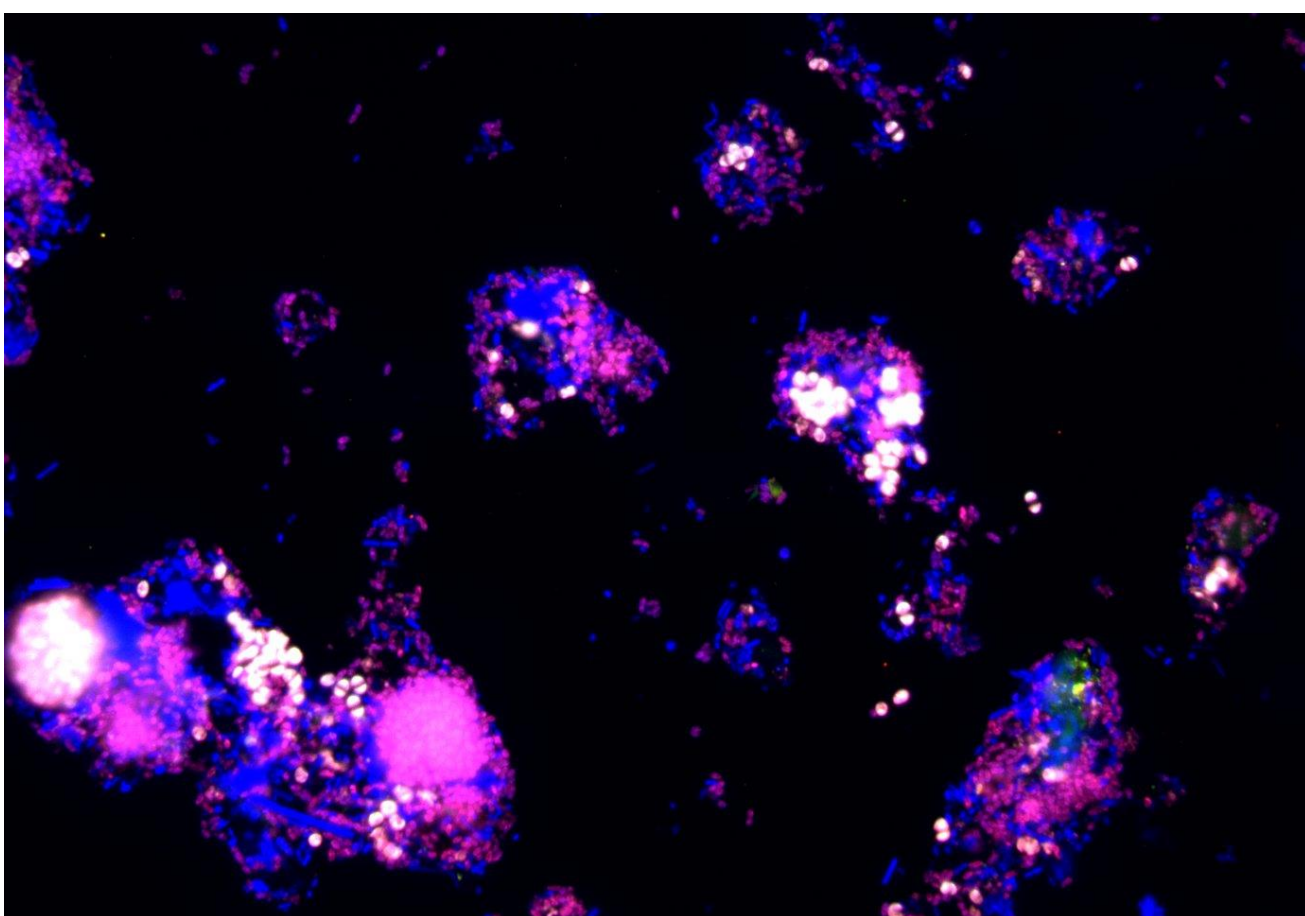
Results



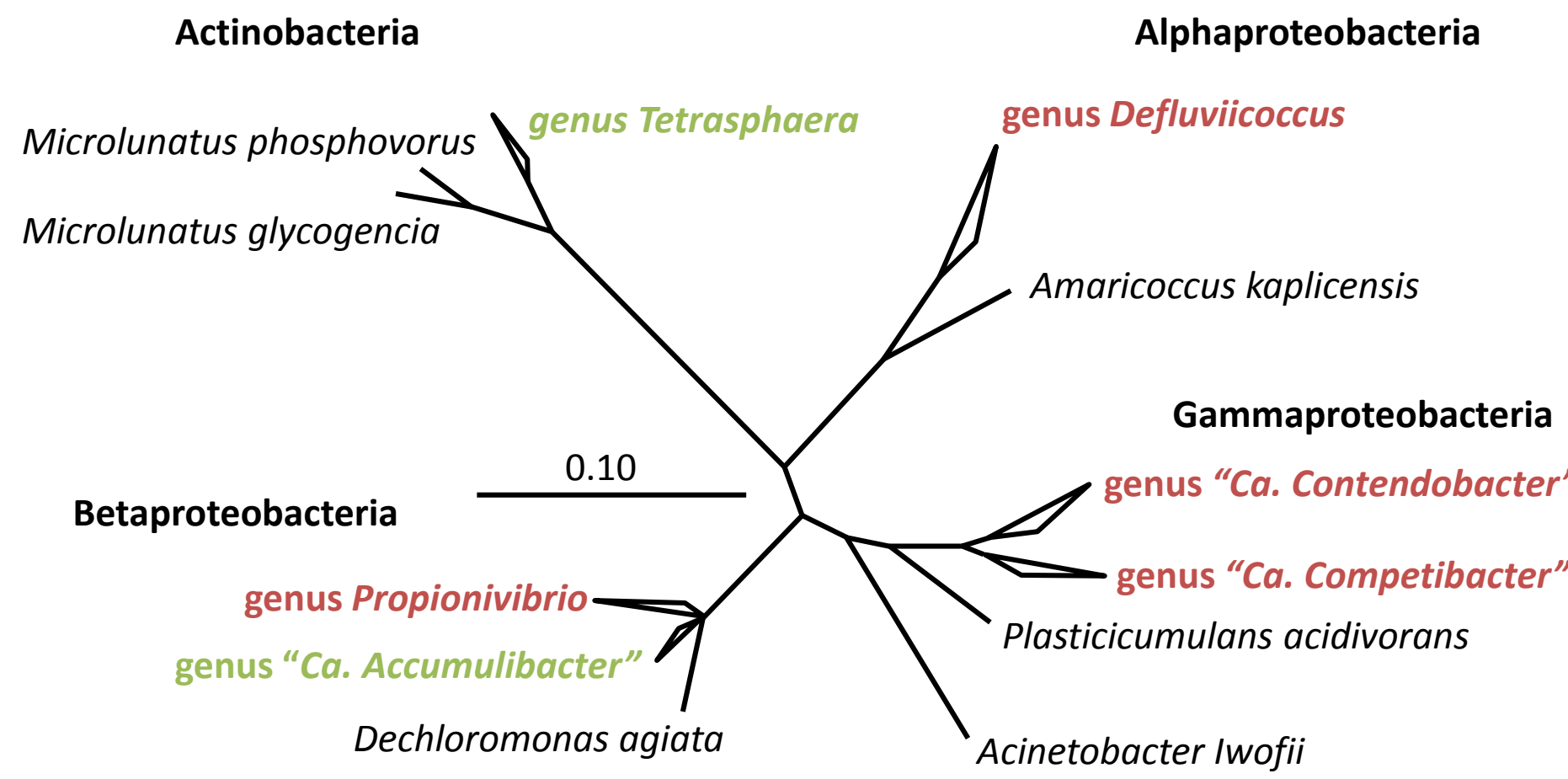
Genome extraction from metagenomes. Differential coverage binning is used as the first step in extracting individual genomes from the metagenomes. Each point represents a scaffold, colored by essential single copy genes and scaled by scaffold length. The plot was made using the mmgenome R package. Surprisingly a bacteria from the genus *Propionivibrio* was enriched along with Accumulibacter.

	Accumulibacter	Propionivibrio
Genome size	4.7 Mbp	3.8 Mbp
Scaffolds	30	88
Completeness	100 %	> 95 %
Strains	1	2-3
Plasmids	1	1-2
Non-rRNA transcriptome reads	70 million	159 million
Abundance: Experiment	15 %	32 %
Abundance: Seed WWTP	0.2 %	0.2 %
Abundance: Other WWTPs*	< 0.1 - 0.2 %	0.1 - 0.8 %

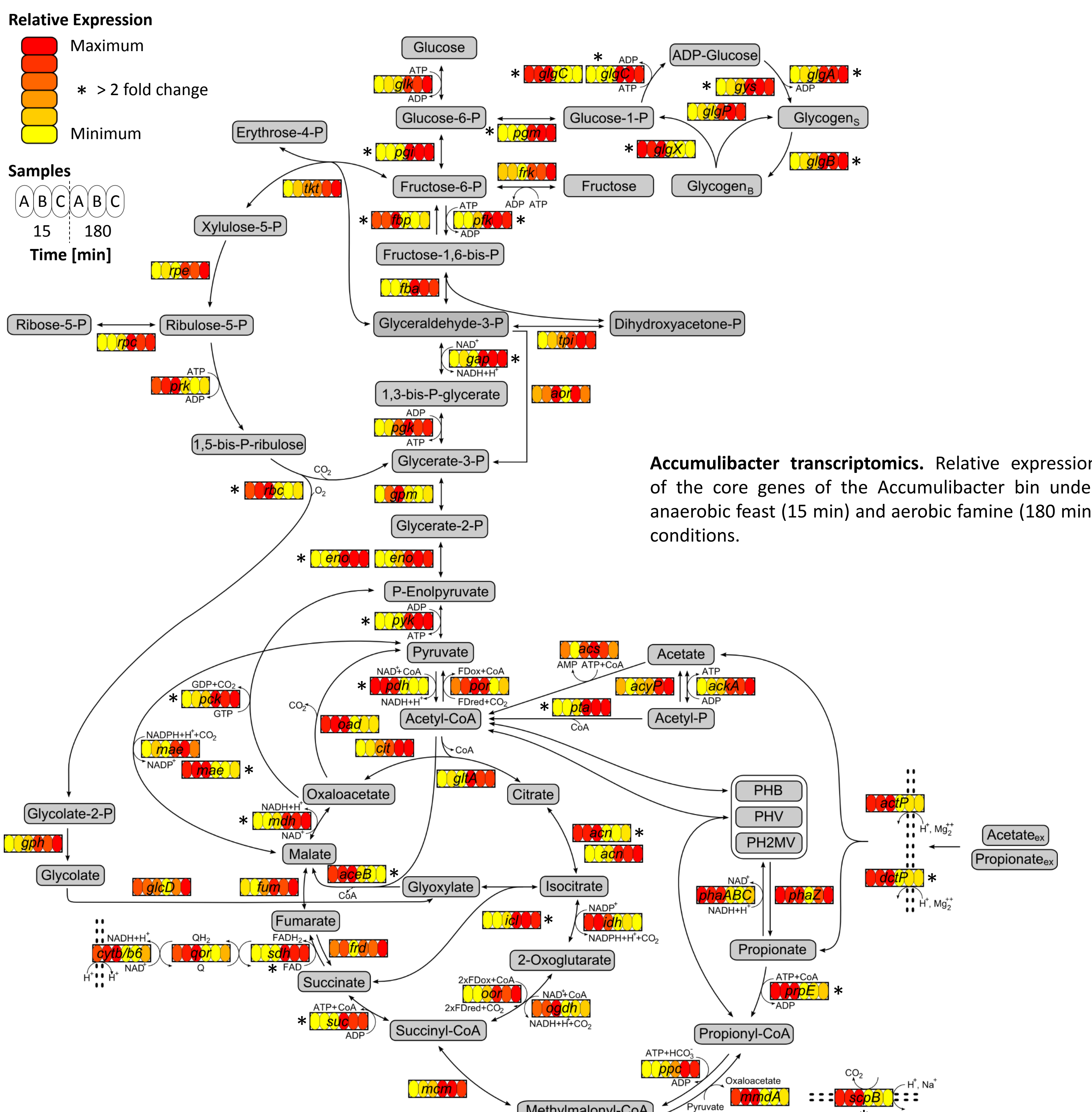
*Estimated from 15 full scale WWTP metagenomes (2.3 bn reads).



A GAO disguised as a PAO. The putative GAO *Propionivibrio* (magenta) is hit by the probes used to quantify the PAO *Accumulibacter* (white). However, it does not accumulate excessive amounts of polyphosphate. Probes: PAO651 (FLUOS, Accumulibacter); PAO462 (Cy3, both); PAO846 (Cy3, both); EUBmix (Cy5, all bacteria).



Phylogenetic tree (16S rRNA) of known PAOs and GAOs. Until now the PAOs and GAOs have been separated into distant phylogenetic lineages. The discovery of a GAO from the genus *Propionivibrio* makes interpretations more difficult. The binned Accumulibacter genome was closely related to Accumulibacter clade IIC based on phylogenetic analysis of the *ppk1* gene.



Accumulibacter transcriptomics. Relative expression of the core genes of the Accumulibacter bin under anaerobic feast (15 min) and aerobic famine (180 min) conditions.



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