Next Generation Molecular Biology Methods for Advancing the Activated Sludge Process

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AAEES WORKSHOP
100 YEARS OF THE ACTIVATE SLUDGE PROCESS

AMERICAN ACADEMY OF ENVIRONMENTAL ENGINEERS & SCIENTISTS®

NJWEA 99th Annual Conference
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DREXEL UNIVERSITY
Civil, Architectural, and Environmental Engineering
College of Engineering

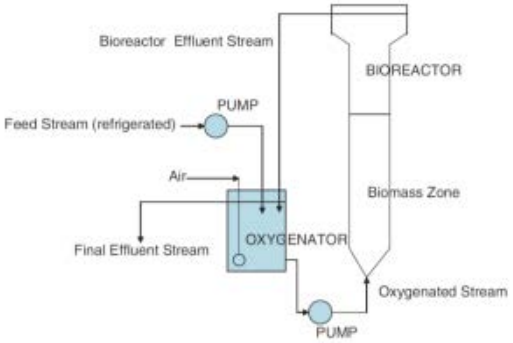
Outline

- ASP, Molecular Biology, & ME
- Dogma of Molecular Biology
- History of Molecular Biology and ASP
- Next-Generation Molecular Biology Methods
- Applications of Next-Generation Molecular Biology Methods to ASP

ACTIVATED SLUDGE PROCESS, MOLECULAR BIOLOGY, AND ME

Undergraduate Research

- Dr. Wen K. Shieh's Bioenvironmental Engineering Laboratory
  - Developed novel bioreactors seeded with activated sludge



WATER RESEARCH 40 (2006) 1442–1448

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WATER RESEARCH

Performance of an aerobic/anaerobic hybrid bioreactor under the nitrogen deficient and low F/M conditions

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Penn
University of Pennsylvania

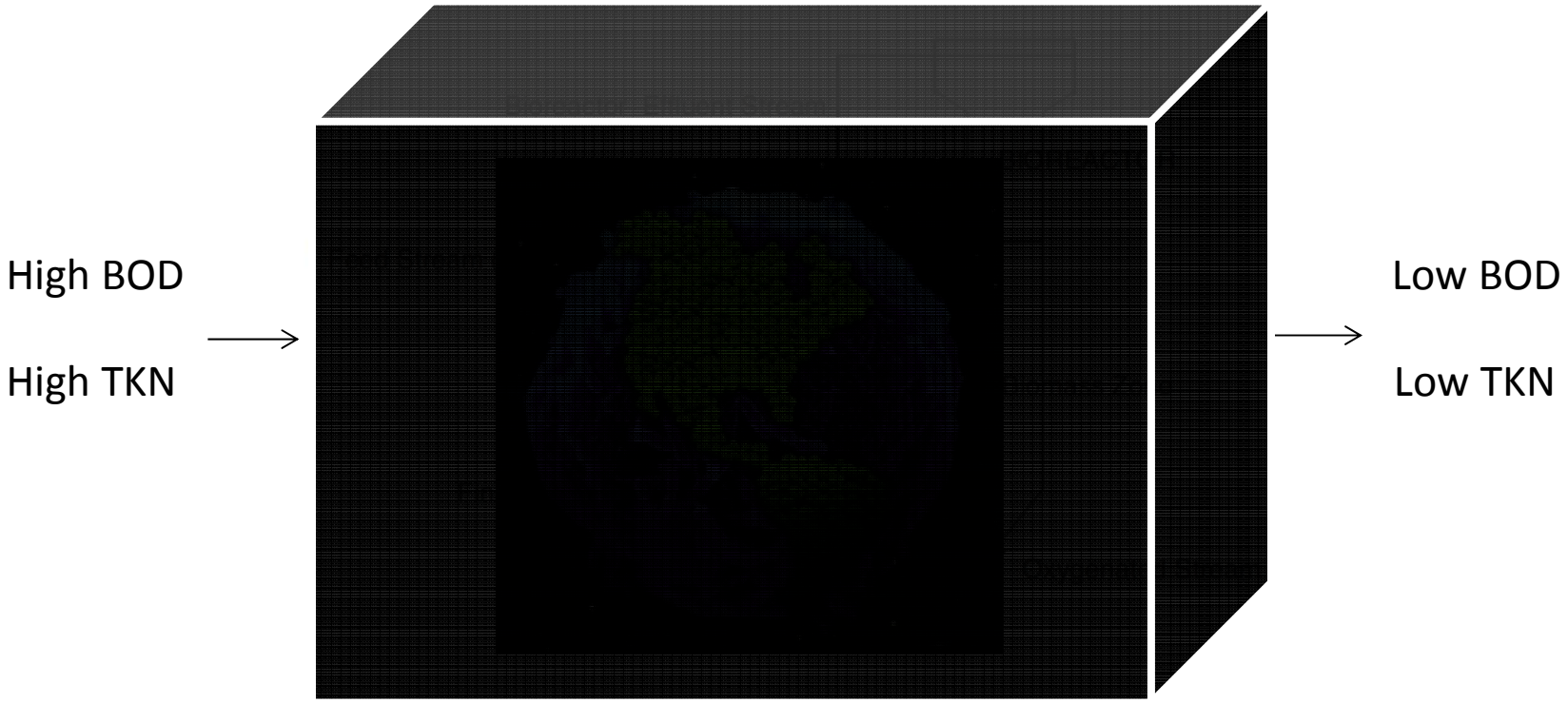
“Black Box”

Applied reactor theory and chemical kinetics to study and engineer the bioreactor but knew very little about the microbes.

$$V\frac{d(substrate, product, cells)}{dt} = r_{bioprocess}V$$

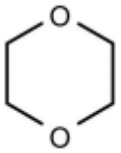
What's inside the "Black Box"?

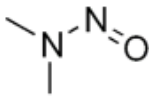
What microbes are there? What are they doing? How do they do it?



Doctoral Studies

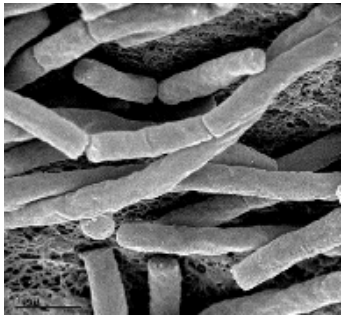
- Applied Environmental Microbiology and Molecular Biology
  - PhD Advisor: Lisa Alvarez-Cohen
  - Thesis: "Functional Genomics of Bacterial Degradation of the Emerging Contaminants: 1,4-dioxane and N-nitrosodimethylamine (NDMA)"





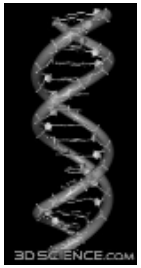
Microarrays for Transcriptomics

Quantiative PCR for gene expression

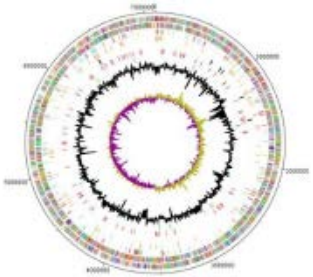


P. dioxanivorans CB1190

Isolation of genomic DNA



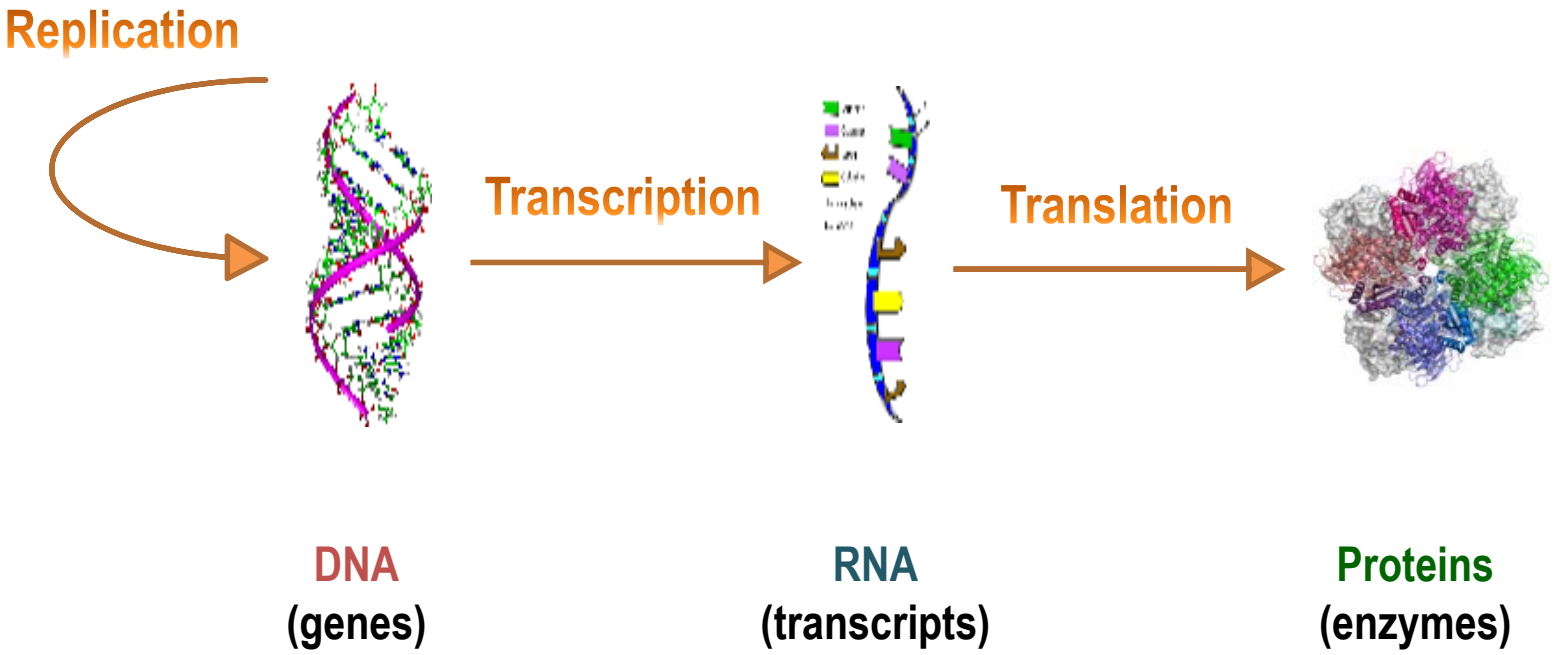
Genome sequencing



Genome Map

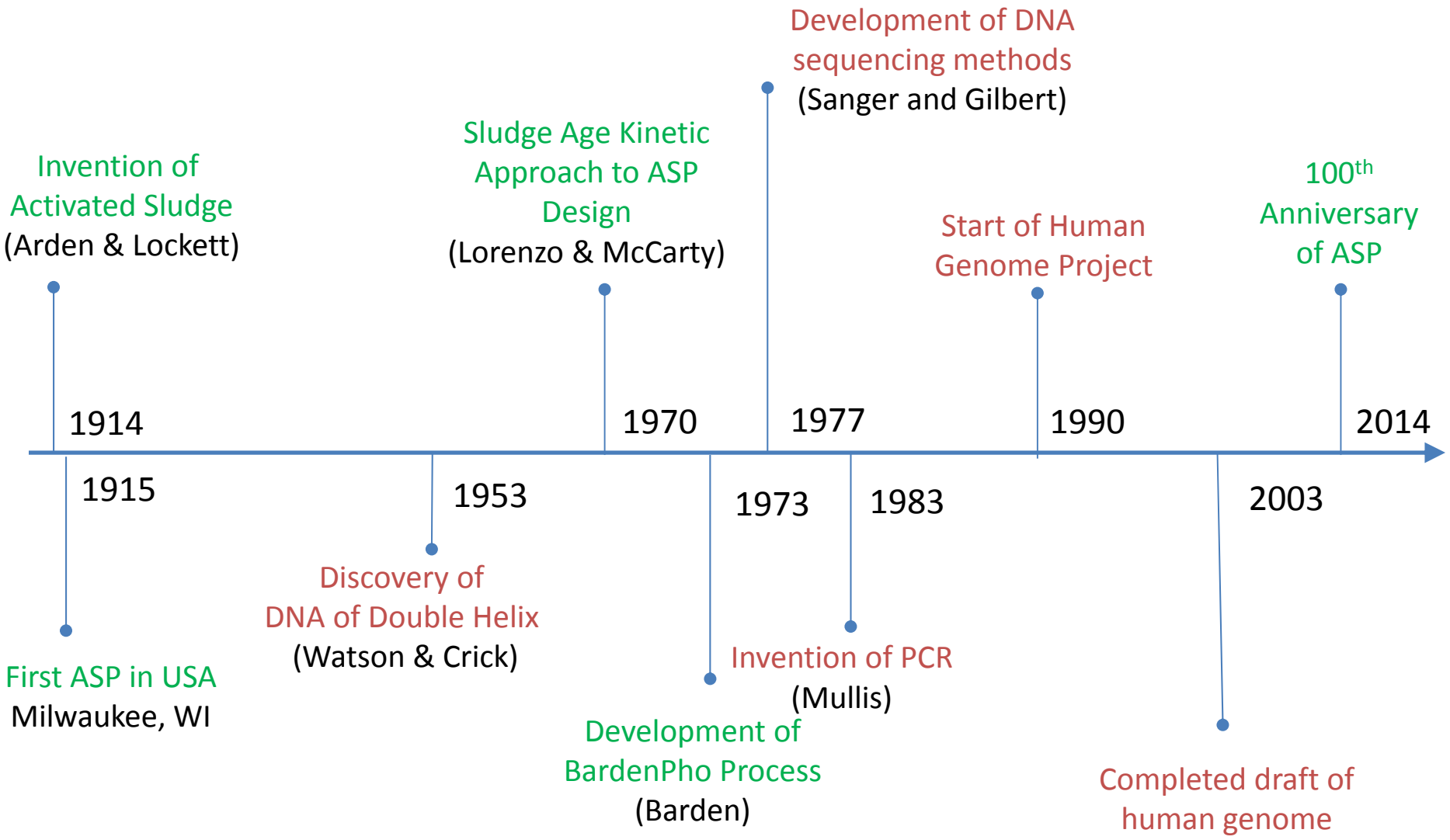
Central Dogma of Molecular Biology

Biochemical reactions are catalyzed by enzymes!



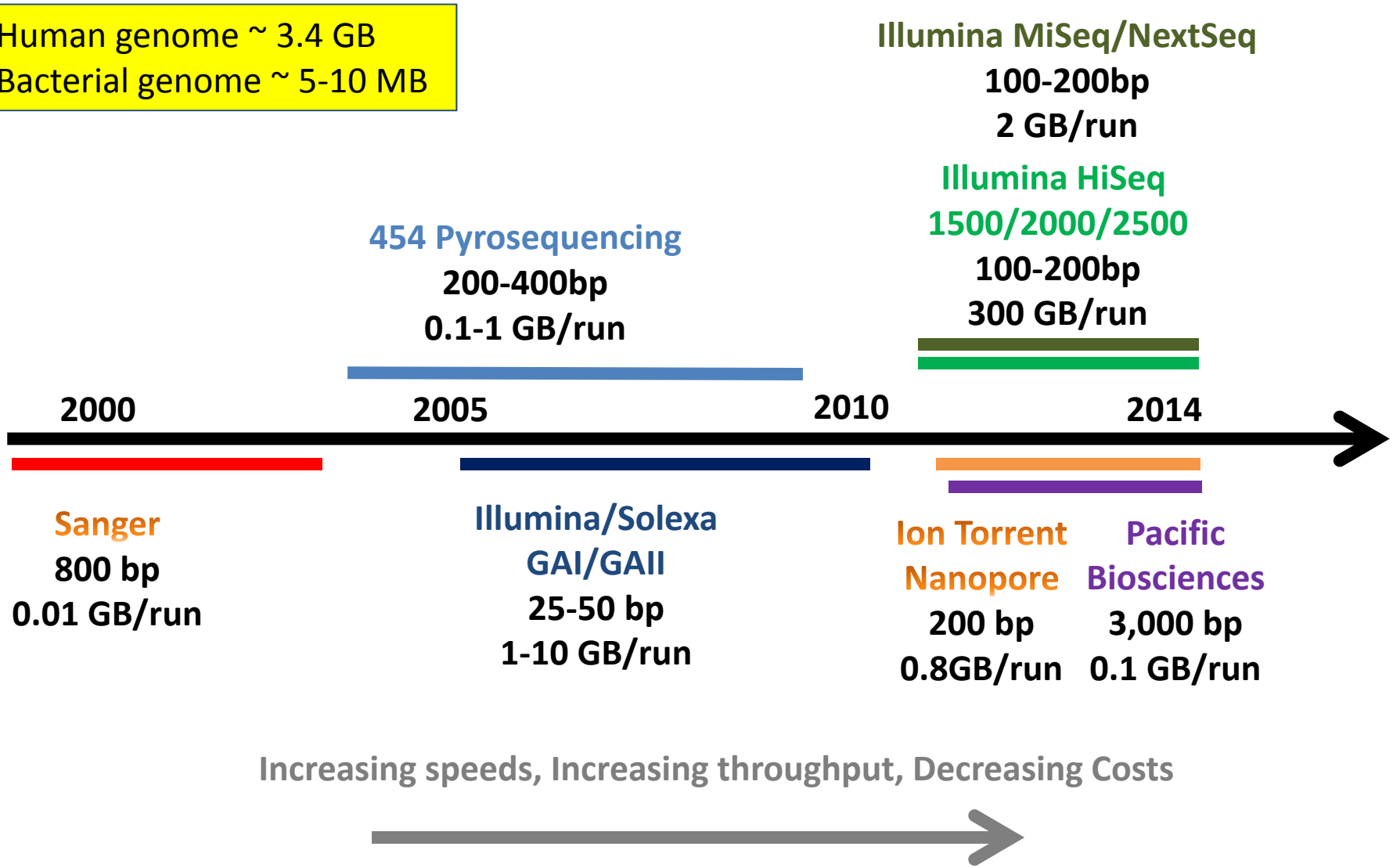
HISTORY OF
THE ACTIVATED SLUDGE PROCESS
AND MOLECULAR BIOLOGY

History of ASP and Molecular Biology

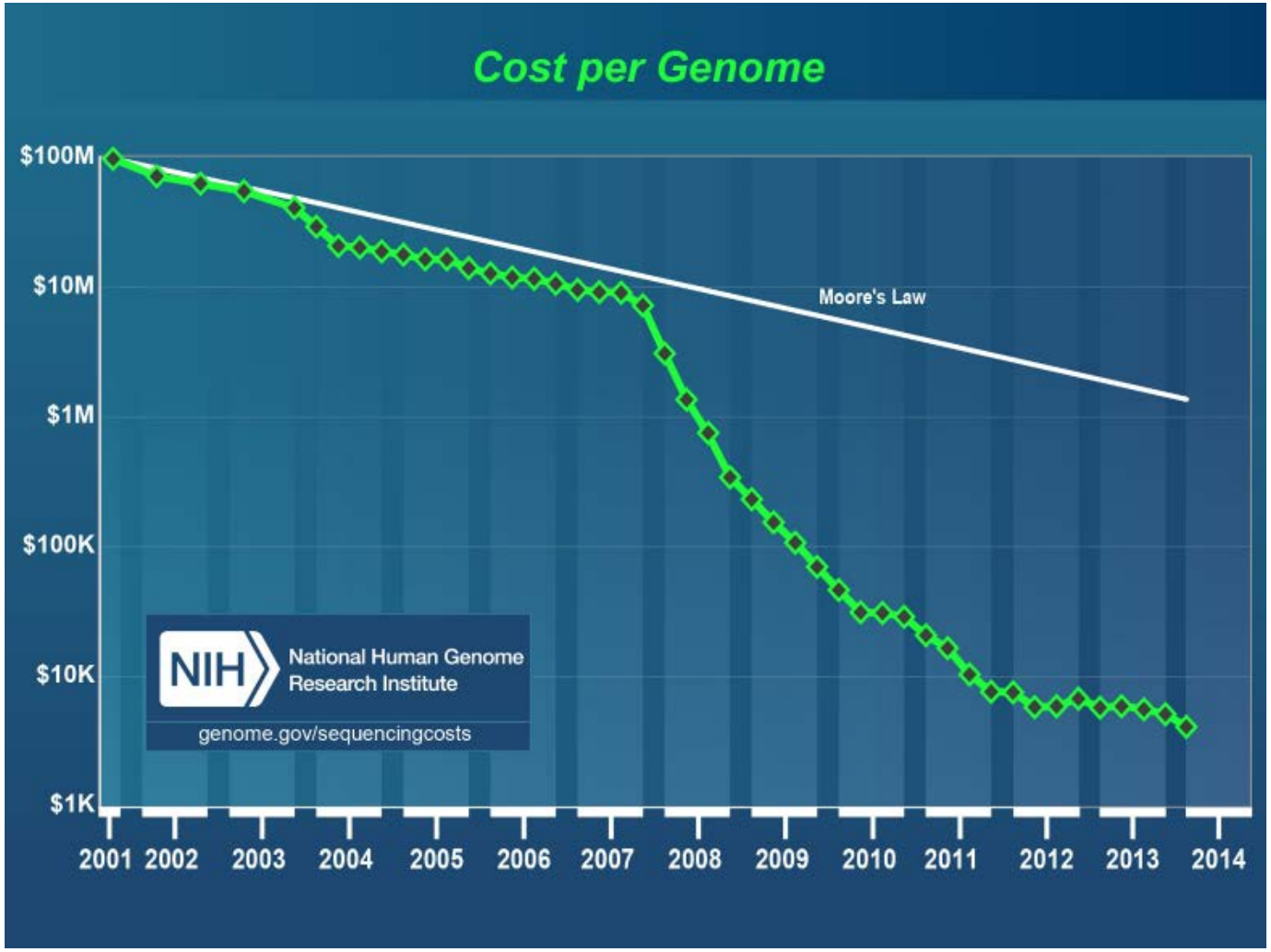


Rapid Advances in Next-Generation Sequencing

Human genome ~ 3.4 GB
Bacterial genome ~ 5-10 MB

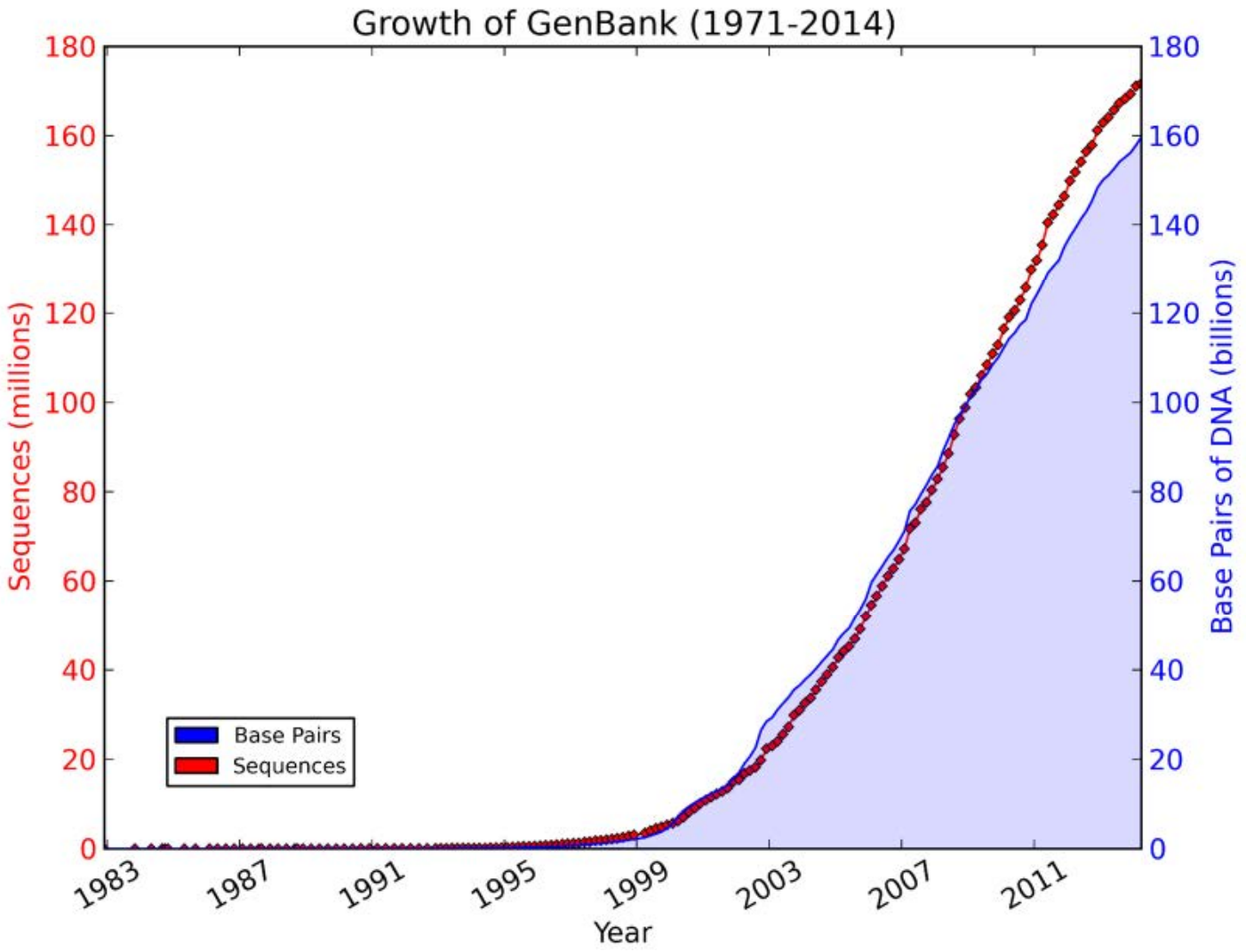


Rapid Advances in Next-Generation Sequencing



Moore's law: long-term trend in the computer hardware industry that involves the doubling of 'compute power' every two years for the same cost

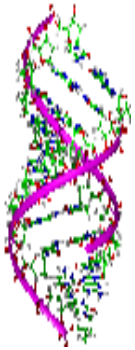
Rapid Advances in Next-Generation Sequencing



http://genome.ist.unomaha.edu/cgi-bin/genbank.cgi

Era of “omes” and “omics”

Advances in high throughput techniques, such as next generation sequencing technologies, enable the study of “everything” in microbiology.



DNA
(genes)

single genes

↓

genomes

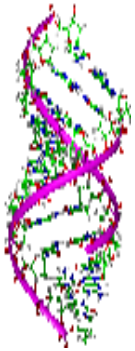
all genes of an organism

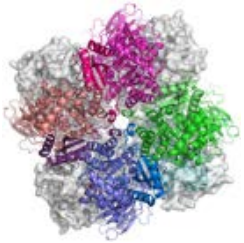
↓

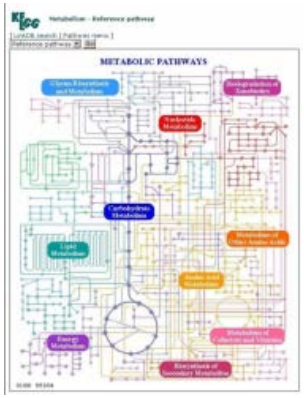
METAGENOMES

all genes of a microbial community

Era of “omes” and “omics”







DNA
(genes)

RNA
(transcripts)

Proteins
(enzymes)

Metabolites

genomes

transcriptomes

proteomes

metabolome

METAGENOMES

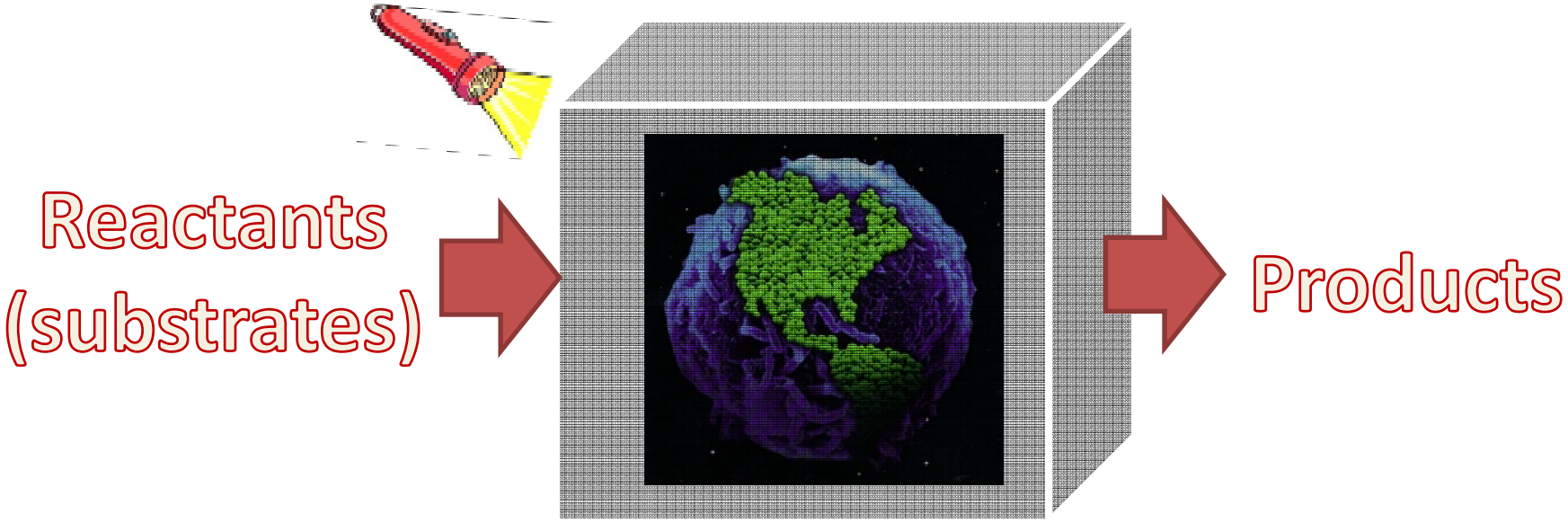
METATRANSCRIPTOMES

METAPROTEOMES

METAMETABOLOME

Application of “omics” to ASP

“Omics” technologies provide tools for a systems biology approach to study the complex interactions that are central to the physiology and function of environmental biological processes, such as the ASP



Application of “omics” to ASP

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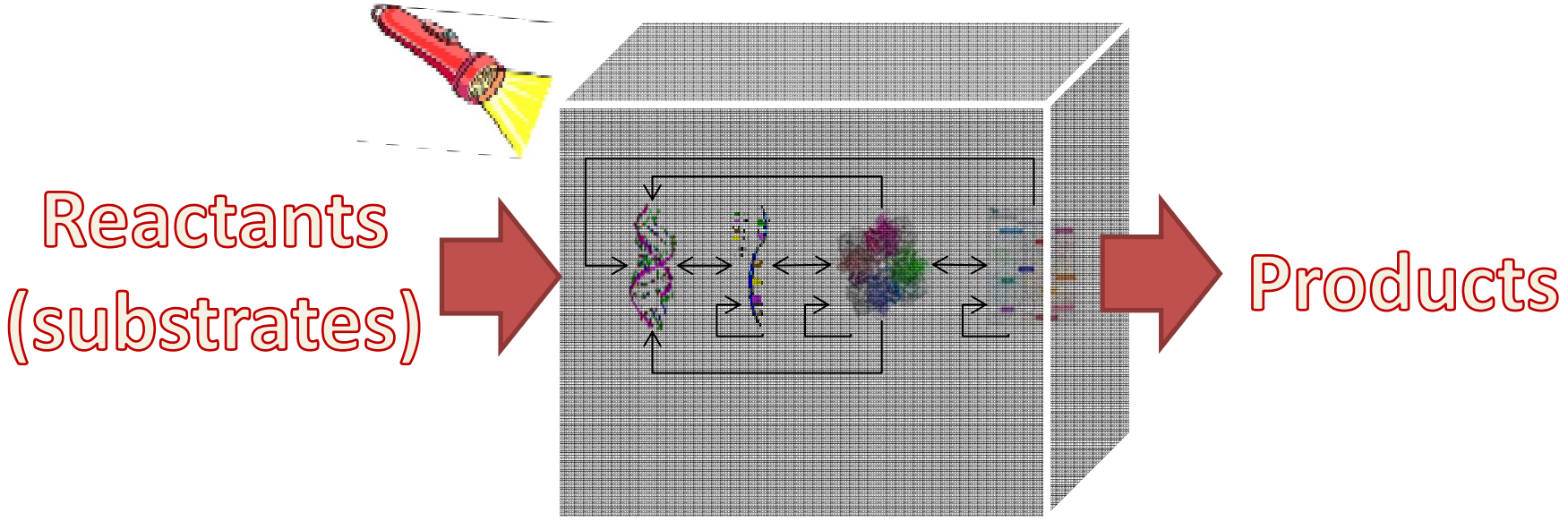


APPLICATION OF OMICS TO ASP

Example:

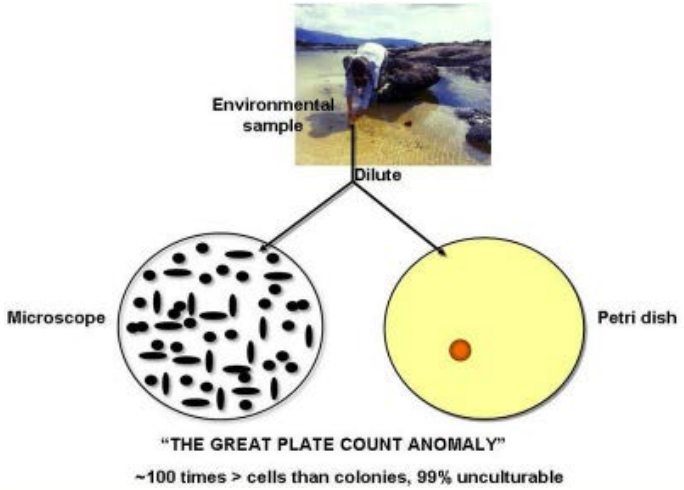
Enhanced Biological Phosphorous Removal (EBPR)

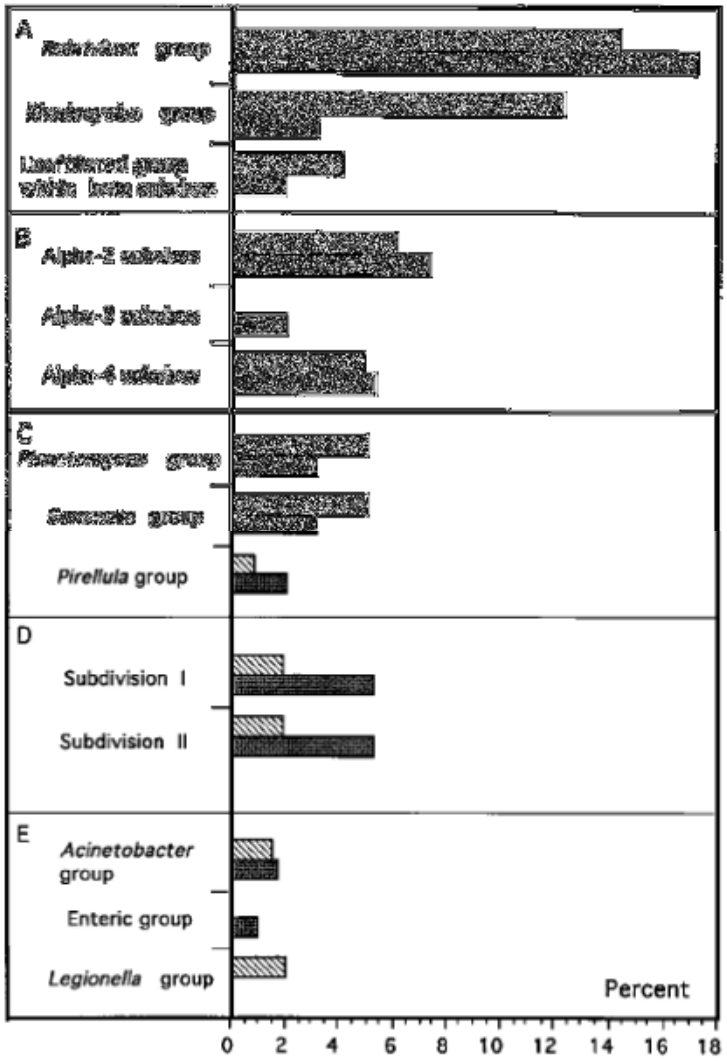
Microbial Misconceptions of the EBPR

- Fuhs and Chen (1978) implicated *Acinetobacter* spp. as being the key organism in EBPR via culturing methods

- However, 16s clone libraries (culture-independent) studies, by Bond et al (1995), identified *Rhodocyclus* group of bacteria as being more dominant in phosphate-removing sludge (gray) than non-phophsate-removing sludge (black).

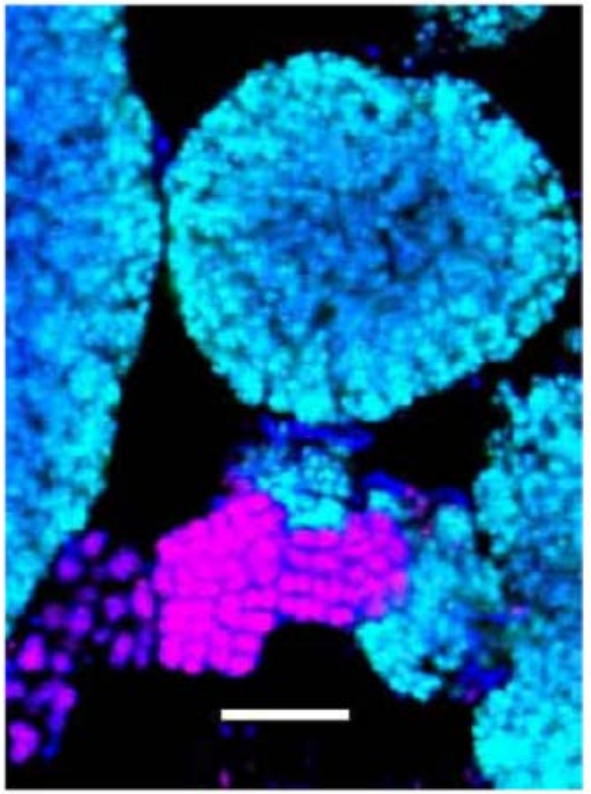
- <u>Great Plate Count Anomaly</u>





First Metagenomics Study of Activated Sludge

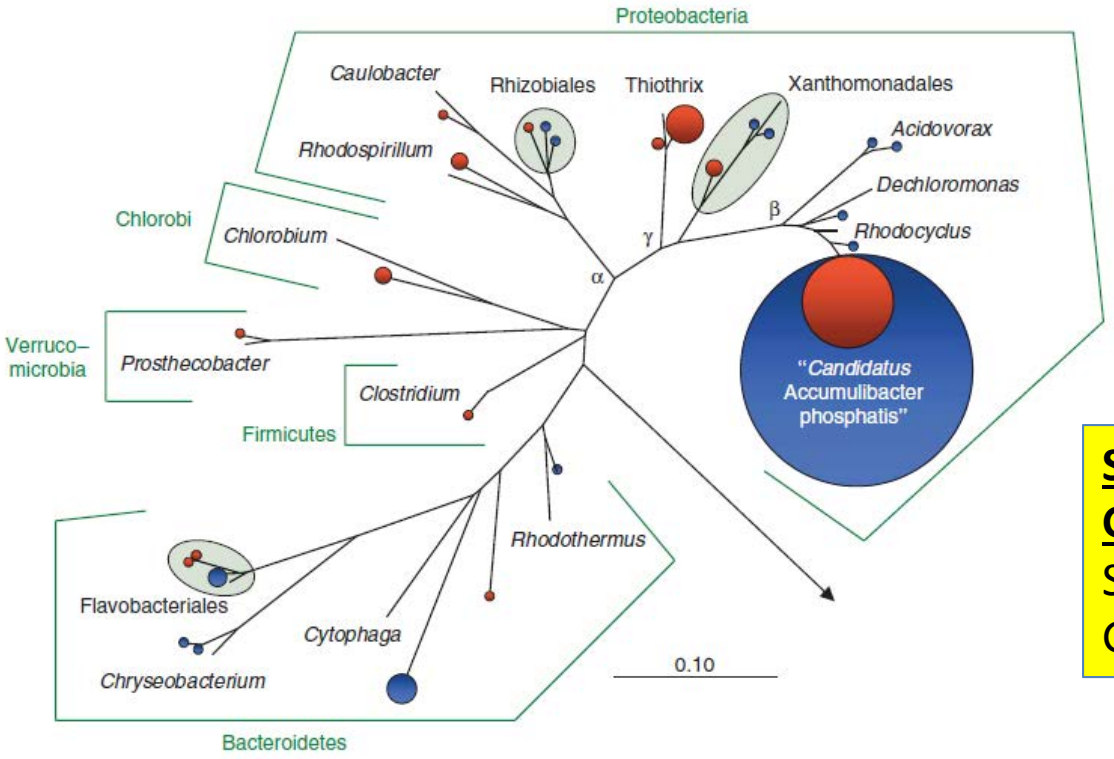
- Garcia Martin et al (2006) *Nat Biotechnol*
  - Sludge from 2 lab-scale SBRs performing EBPR:
    - Madison, Wisconsin (US)
    - Brisbane, Australia (OZ)
  - Shotgun sequenced libraries of DNA extracted from each sludge using the Sanger method: 98 Mbp (US) and 78 Mbp (OZ)
  - Previously, *Candidatus* Accumulibacter phosphatis [cyan; all other bacteria, pink] comprised 80% (US) and 60% (OZ) of community by fluorescence in situ hybridization (FISH)



Oehmen et al (2006) *J Biotechnol*

First Metagenomics Study of Activated Sludge

- Garcia Martin et al (2006)
  - From shotgun metagenomic data, 16s rRNA genes also showed that Accumulibacter phosphastis was dominant in the US (blue) and OZ (red) sludges



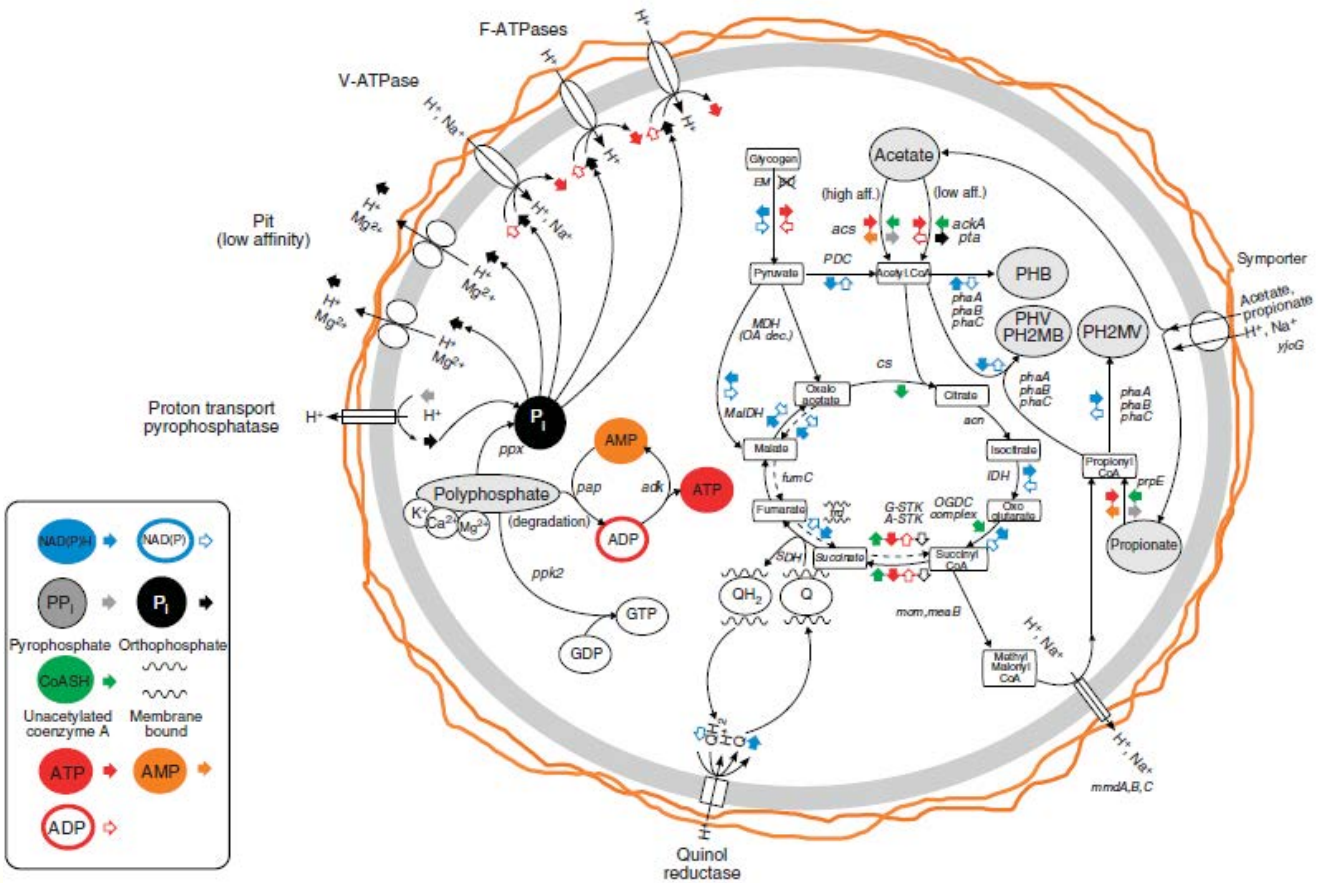
Stats for Near-Complete Genome of *A. phosphatis*

Size: 5.6±0.2 Mbp

GC-content: 63%

First Metagenomics Study of Activated Sludge

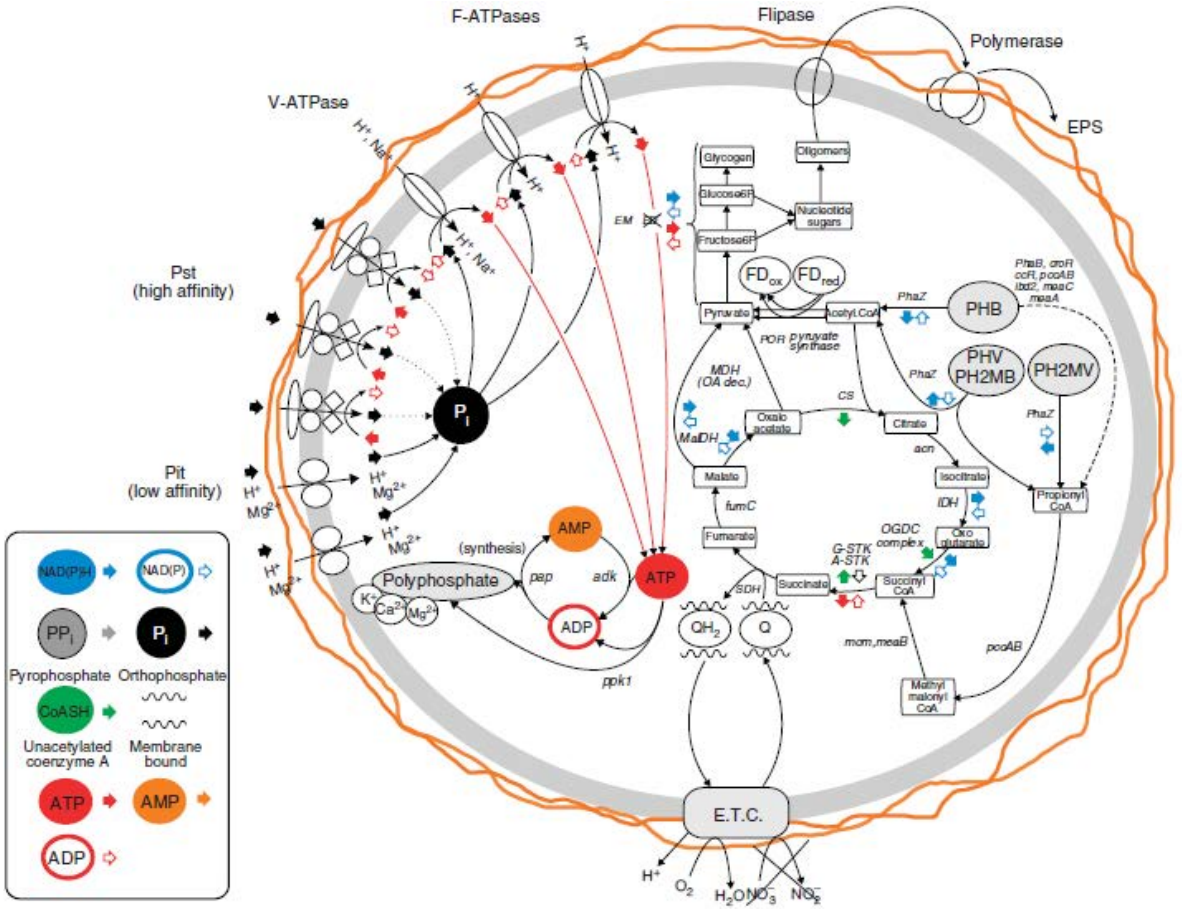
- Garcia Martin et al (2006)
  - Metabolic reconstruction of A. phosphatis
    - <u>Anaerobic phase:</u>



Garcia Martin et al (2006) Nature Biotechnol

First Metagenomics Study of Activated Sludge

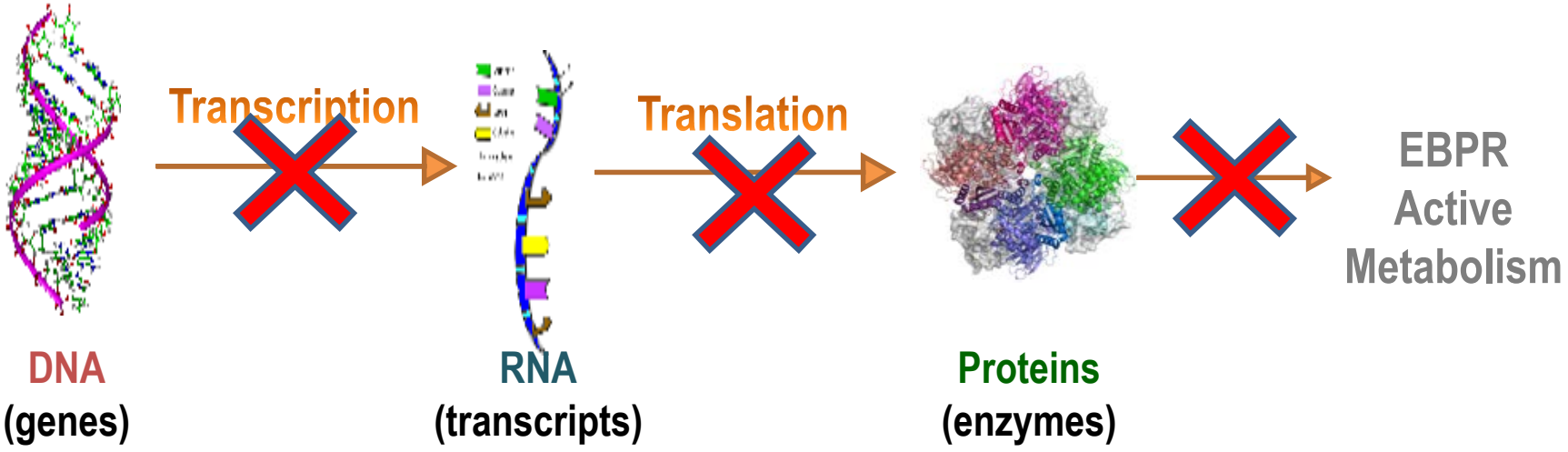
- Garcia Martin et al (2006)
  - Metabolic reconstruction of A. phosphatis
    - Aerobic phase:



Garcia Martin et al (2006) Nature Biotechnol

First Metagenomics Study of Activated Sludge

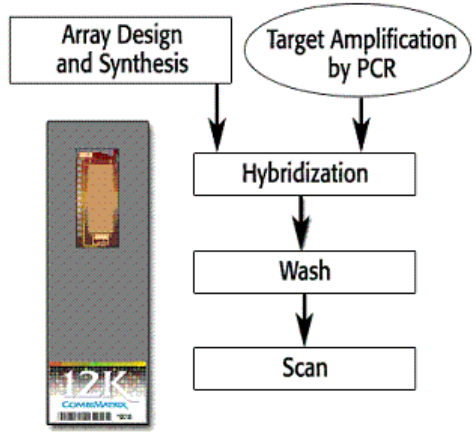
- Thoughts on Garcia Martin et al (2006)
  - Strengths:
    - Novel insights into genetic basis of EBPR w/o culturing
    - Info on metabolism can suggest better cultivation methods
  - Shortcomings:
    - Just a snapshot (sludge collected at end of anaerobic phase)
    - DNA doesn't tell us what genes are expressed and active

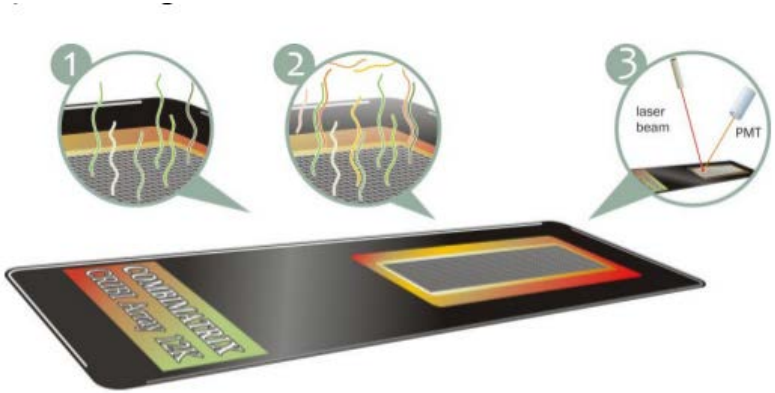


metagenomes

Functional Omics of EBPR

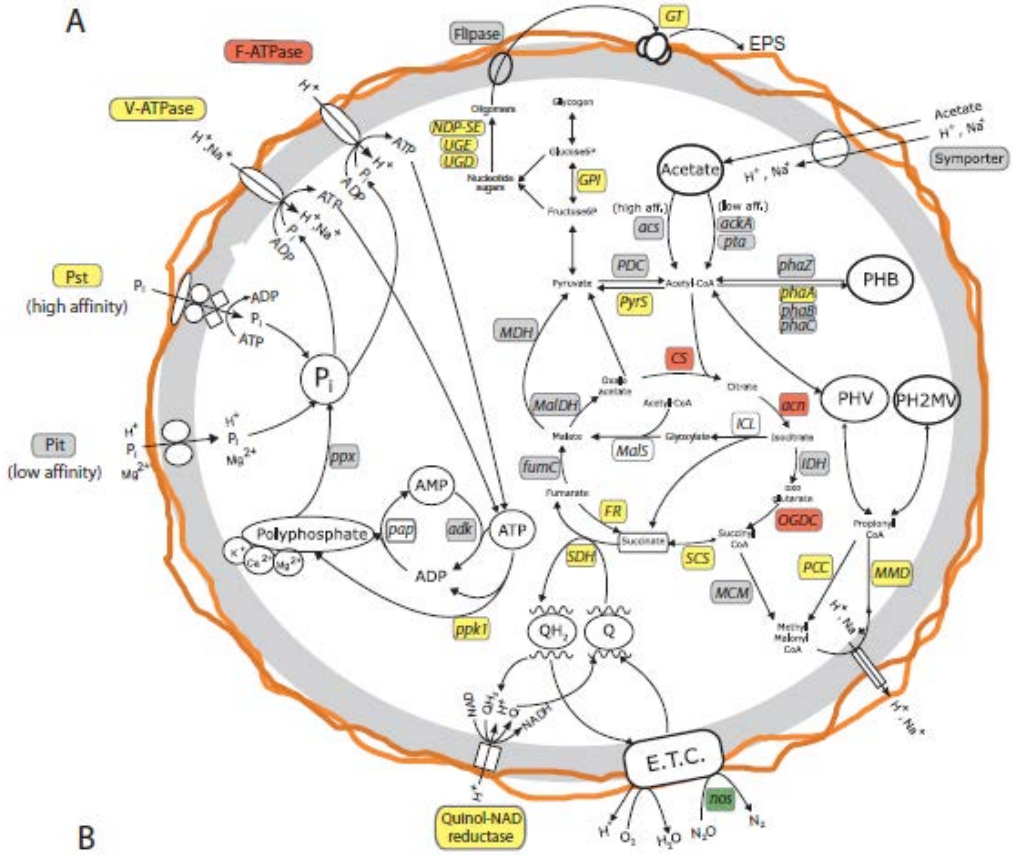
- Metatranscriptomics of lab-scale EBPR
  - He et al (2010) *Environ Microbiol*
    - Lab-scale SBR performing EBPR
    - Two RNA samples collected:
      - anaerobic phase (15 min after acetate addition)
      - early stage of aerobic phase (15 min after switching to aeration)
    - mRNA transcripts measured on Combimatix oligonucleotide microarray





Functional Omics of EBPR

- Metatranscriptomics of EBPR
  - He et al (2010)



Expressed, but not significantly

Expressed more in aerobic sample

Expressed more in anaerobic sample

Not expressed

Functional Omics of EBPR

- Metatranscriptomics of EBPR
  - He et al (2010)
    - Many genes are expressed but not significantly different between samples (i.e., constitutively), possibly because they encode genes that catalyze reversible reactions
    - Genes involved in PHA synthesis, split TCA cycle, and polyp formation were expressed

Quantitative PCR Results

Gene	*PDC*	*phaC*	*mcm*	*CS*	*nos*	*FR*	*phaA*	*PyrS*	*SDH*	*ppk1*
Ct difference in cDNA and NRTC [1]	17.5	19.4	14	16.4	17.4	20.2	20.3	21.8	14	11.8
Fold Change (AE/AN)[2]	0.81	1.26	4.09	**3.56**	**0.49**	**1.7**	**1.82**	**1.35**	**0.51**	**0.95**

Expressed, but not significantly

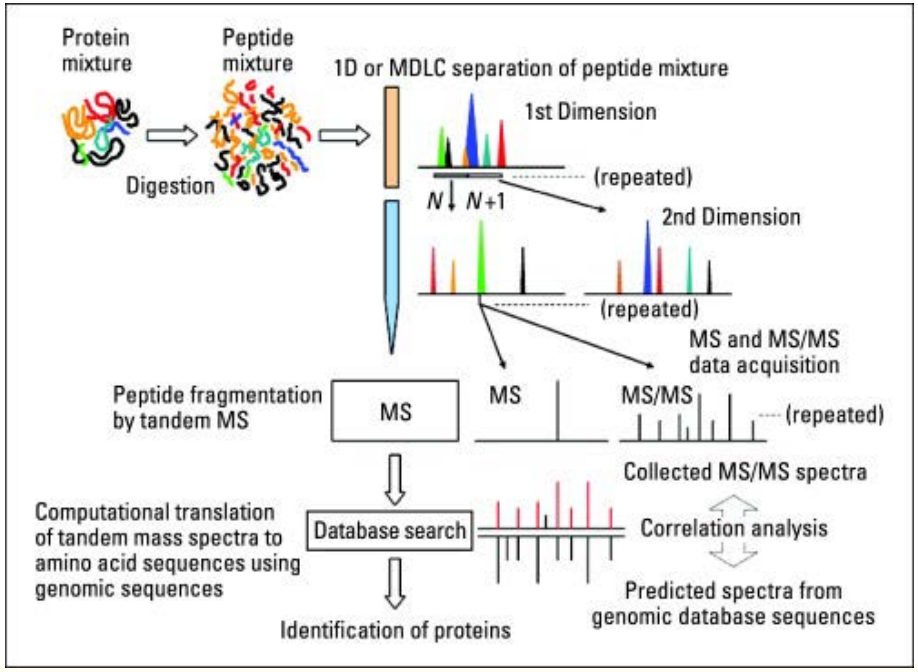
Expressed more in aerobic sample

Expressed more in anaerobic sample

Not expressed

Functional Omics of EBPR

- Metaproteomics of Lab-scale (acetate-fed) EBPR
  - Wilmes et al (2008) *ISME J*
    - Samples were collected at the end of anaerobic and aerobic phases
    - High-resolution community proteomics was accomplished via two-dimensional nano-LC by MS/MS analysis using LTQ or LTQ-Orbitap mass spectrometer



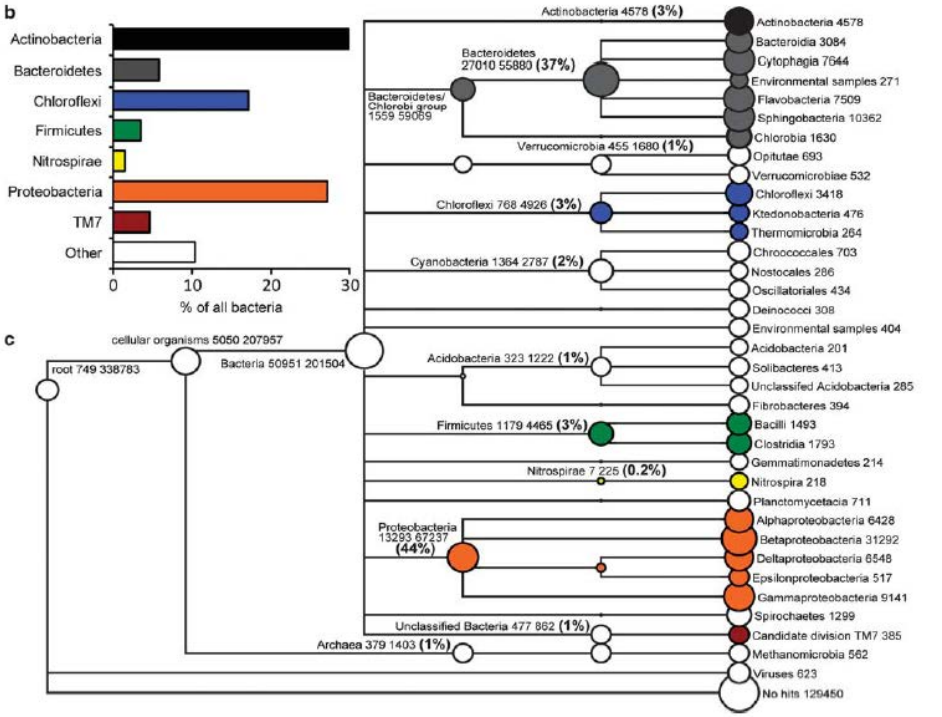
Motoyama and Yates (2008) *Anal Chem*

Functional Omics of EBPR

- Metaproteomics of Lab-scale (acetate-fed) EBPR
  - Wilmes et al (2008) ISMEJ
    - Despite collecting samples at different times in each phase, the metatranscriptomic and metaproteomic data were in good agreement
    - Metaproteomics found
      - fatty acid degradation and synthesis proteins were highly abundant
        - » suggest possible enrichment strategy based on pre-fermentation of fatty acids to produce VFAs with longer chains than acetate
      - all protein subunits for denitrification (nitrate reductase) were identified, which allow for TCA cycle and glyoxylate shunt pathway to function anaerobically
    - Metaproteomics may be better than metratranscriptomics because it detects the final product of gene expression

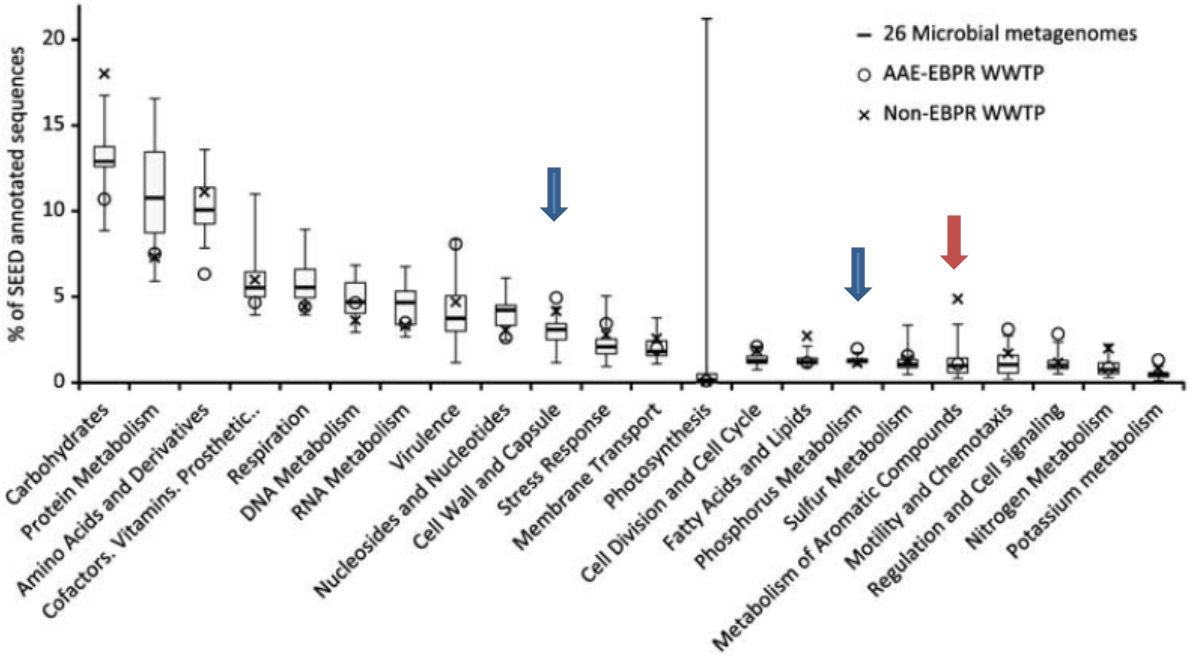
Omics and Molecular Biology of Full-Scale EBPR

- Metagenomics of full-scale EBPR (Denmark)
  - Albertsen (2011) ISME J
    - Comparison of community structure by metagenomics and quantitative FISH shows good qualitative agreement but indicates slight bias



Omics and Molecular Biology of Full-Scale EBPR

- Metagenomics of full-scale EBPR (Denmark)
  - Albertsen (2011) ISME J
    - Comparison of EBPR community to a full-scale, non-phosphate accumulating activated sludge WWTP [Sanapareddy et al (2009) AEM]



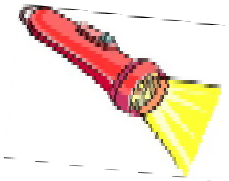
Considerations for Applying Omics to ASP

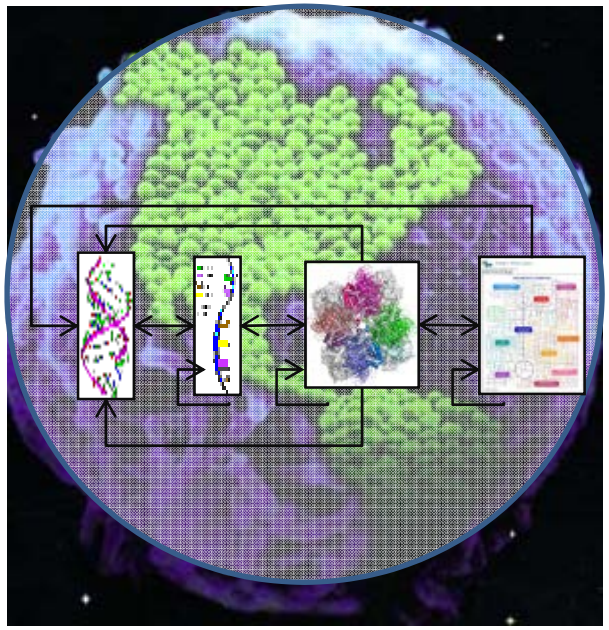
- Potential Challenges
  - Requires technicians with skills in molecular biology and bioinformatics
  - Requires high-throughput sequencers
  - Requires significant computing power and data storage
  - Time for processing samples, production and analysis of molecular data for omics is not ideal for real-time monitoring

Considerations for Applying Omics to ASP

- Potential Benefits
  - Omics can provide, often novel, insight into the microbial and molecular systems that control the function of environmental biological processes
  - Omics has the potential to revolutionize our approach to studying and engineering biological processes for environmental sustainability
  - Omics could guide developments in the design and operation of ASP to tackle future challenges (e.g., climate change, new regulations, micropollutants)

Final Remarks





- “Omics” can be applied in combination with other methods to study environmental biological processes
- “Omics” can provide insight into the microbial and molecular systems that control the function of environmental biological processes
- “Omics” has the potential to revolutionize our approach to studying and engineering biological processes for environmental sustainability

QUESTIONS?