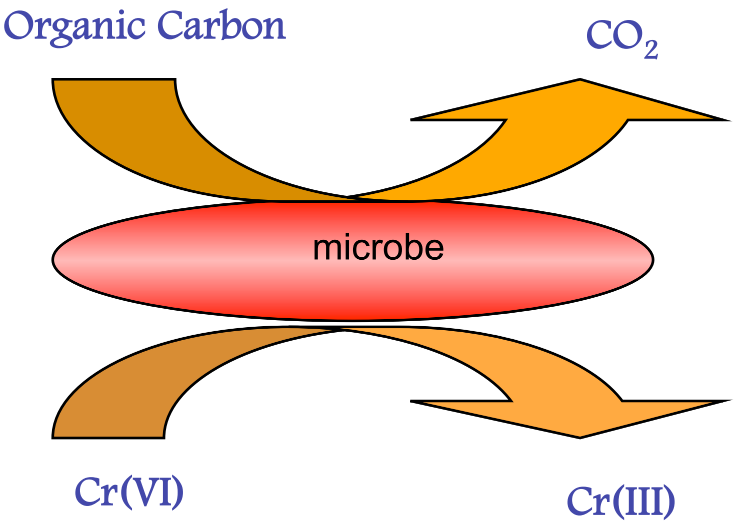
Integrated ecogenomics study for bioremediation of Cr(VI) at Hanford 100H area

Romy Chakraborty

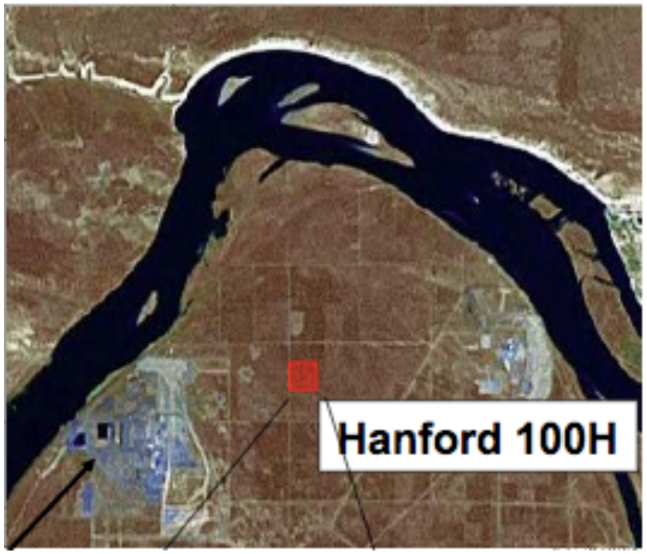
Microbial Ecology
Lawrence Berkeley National Lab

Chromium(VI)contamination at Hanford

- Cr(VI) highly soluble, toxic compound
- Chemical manufacturing, waste streams from electroplating, leather tanning, textile industries, dyes and pigments industry, reactors, coal ash.
- Causes ulcer, convulsions, lung cancer, asthma, organ damage



- 450 billion gallons of waste from nuclear reactors at Hanford released that made its way to groundwater.
- Treatability tests employed by pumping HRC an injection well



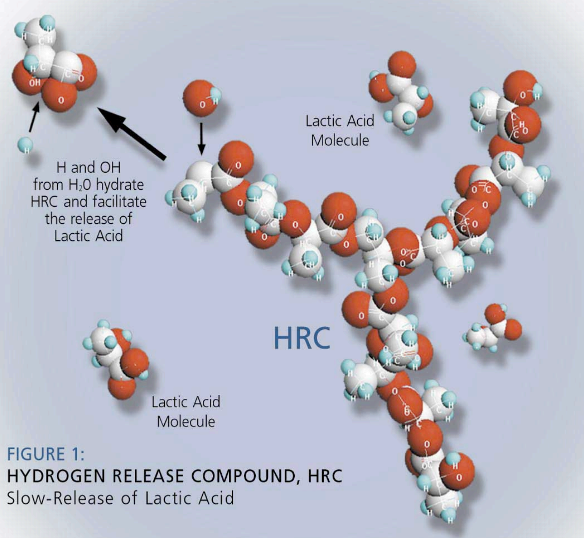
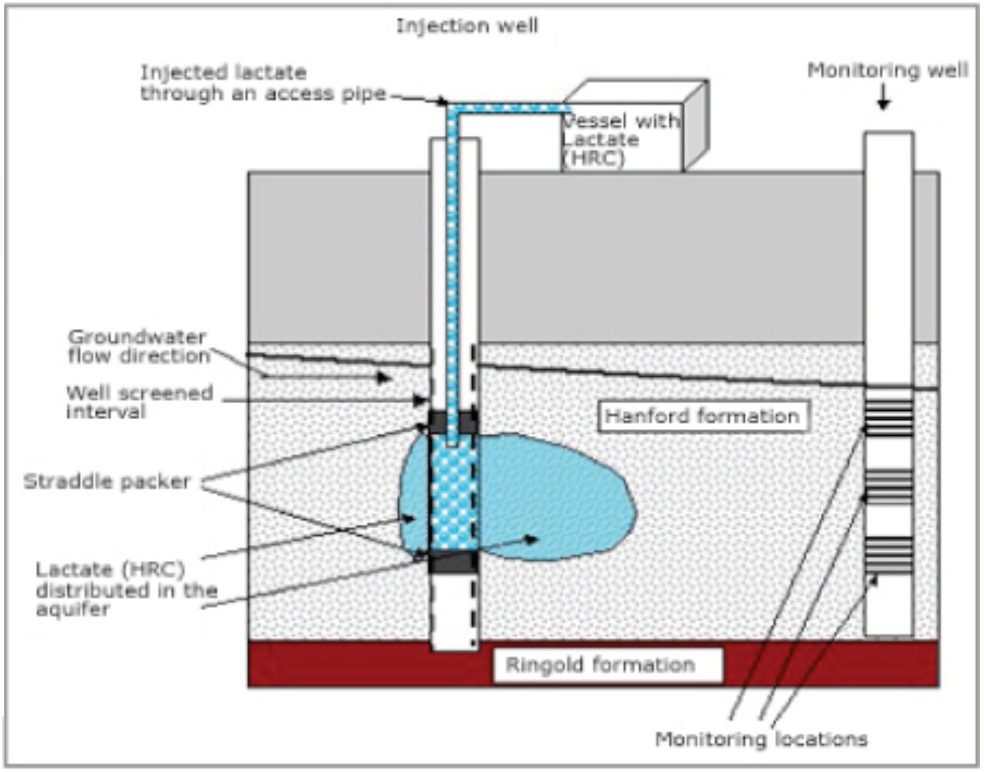


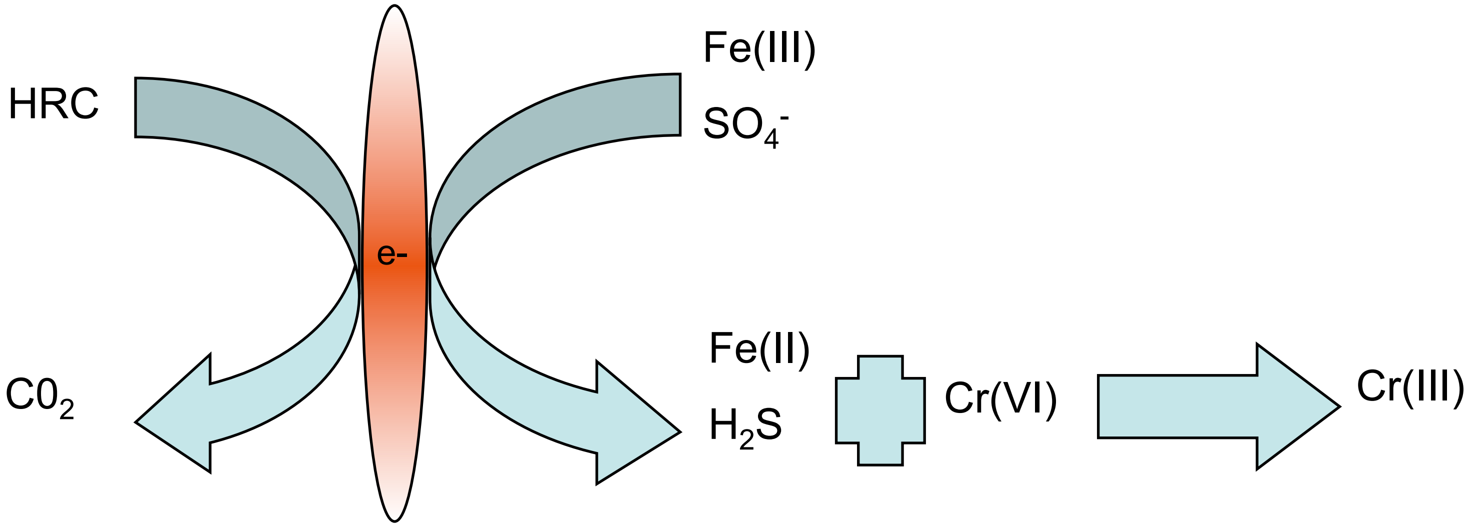
FIGURE 1:
HYDROGEN RELEASE COMPOUND, HRC
Slow-Release of Lactic Acid

Regenesis

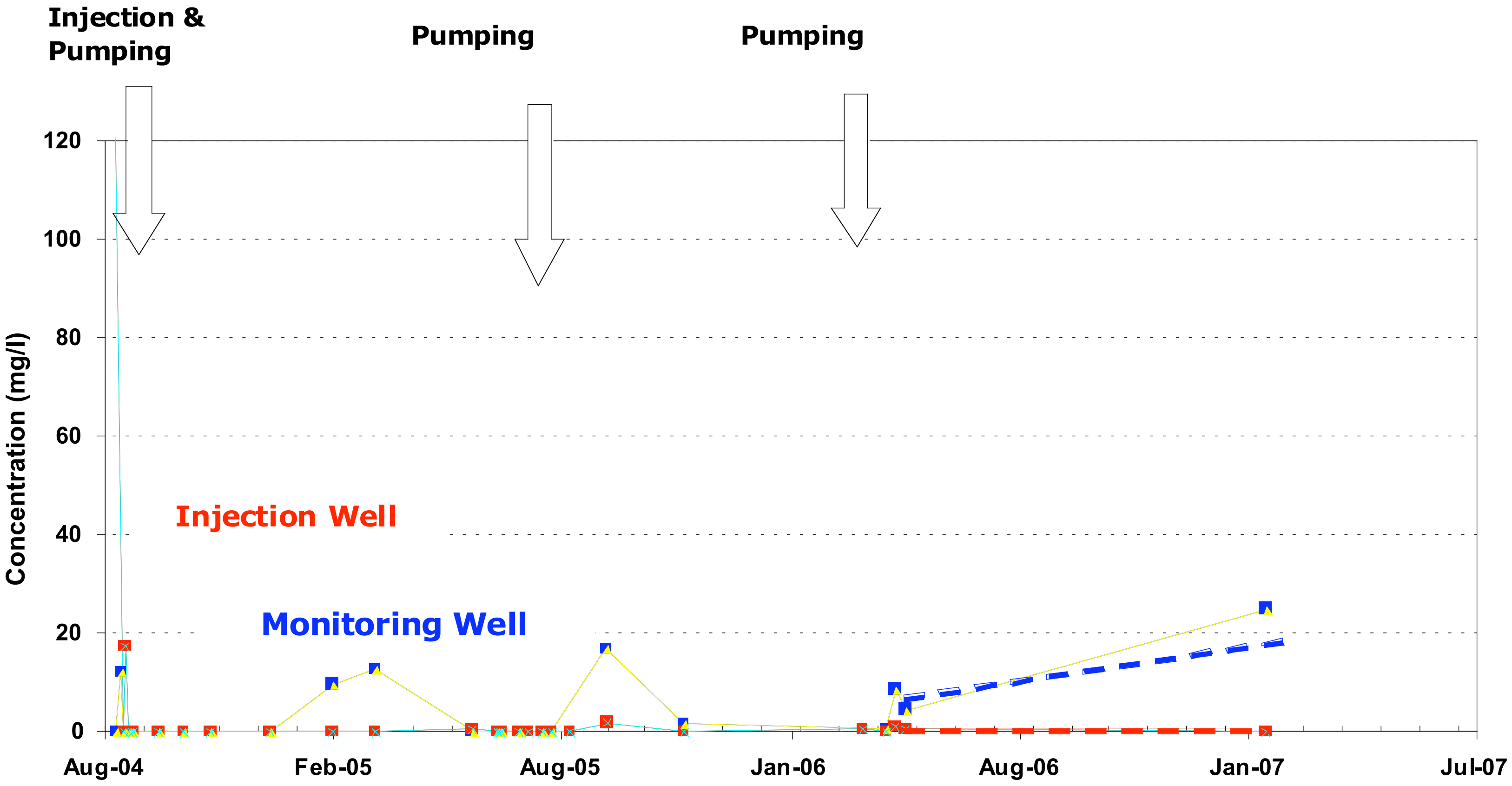


In-situ bioremediation at Hanford 100H area

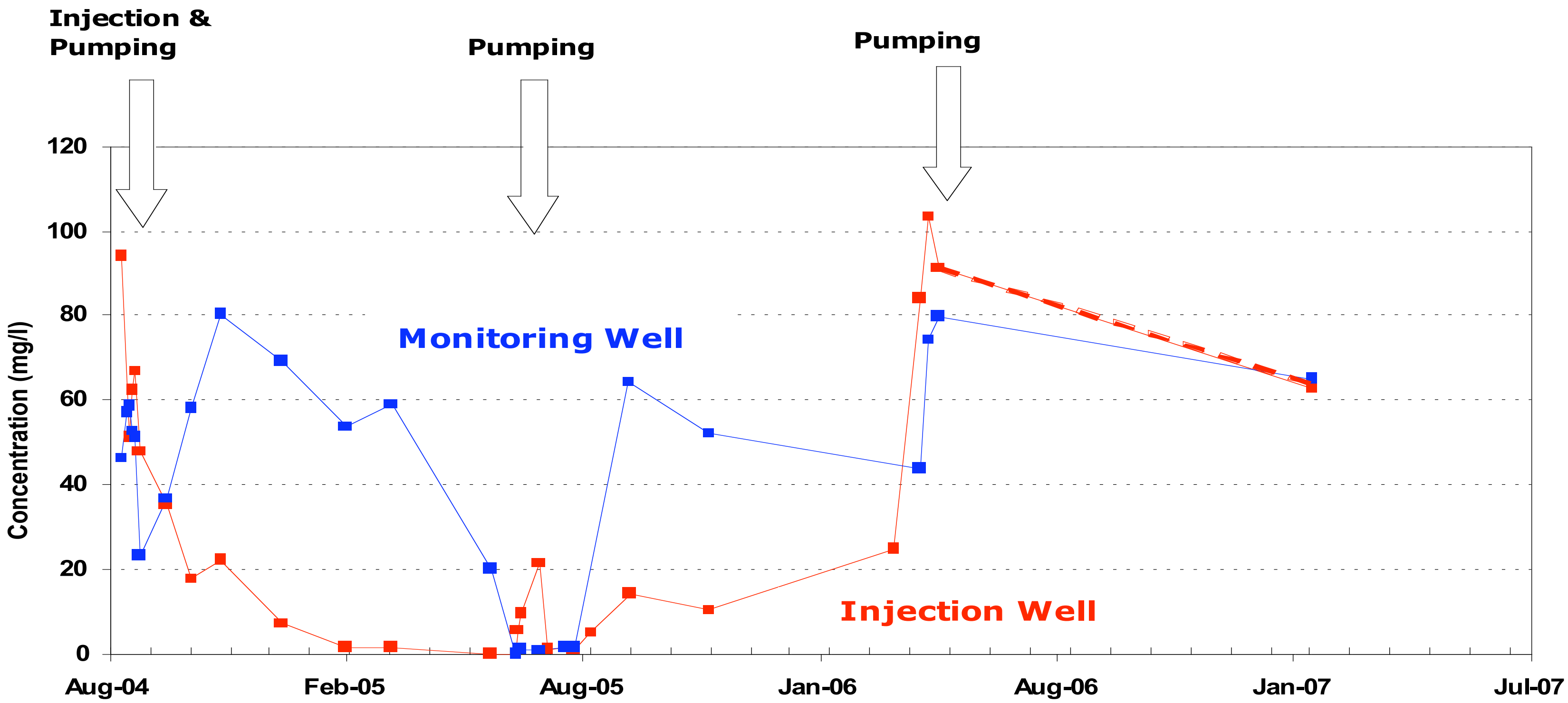
- Stimulate indigenous microbial populations
- Immobilize Cr(VI) through maintaining reduced conditions



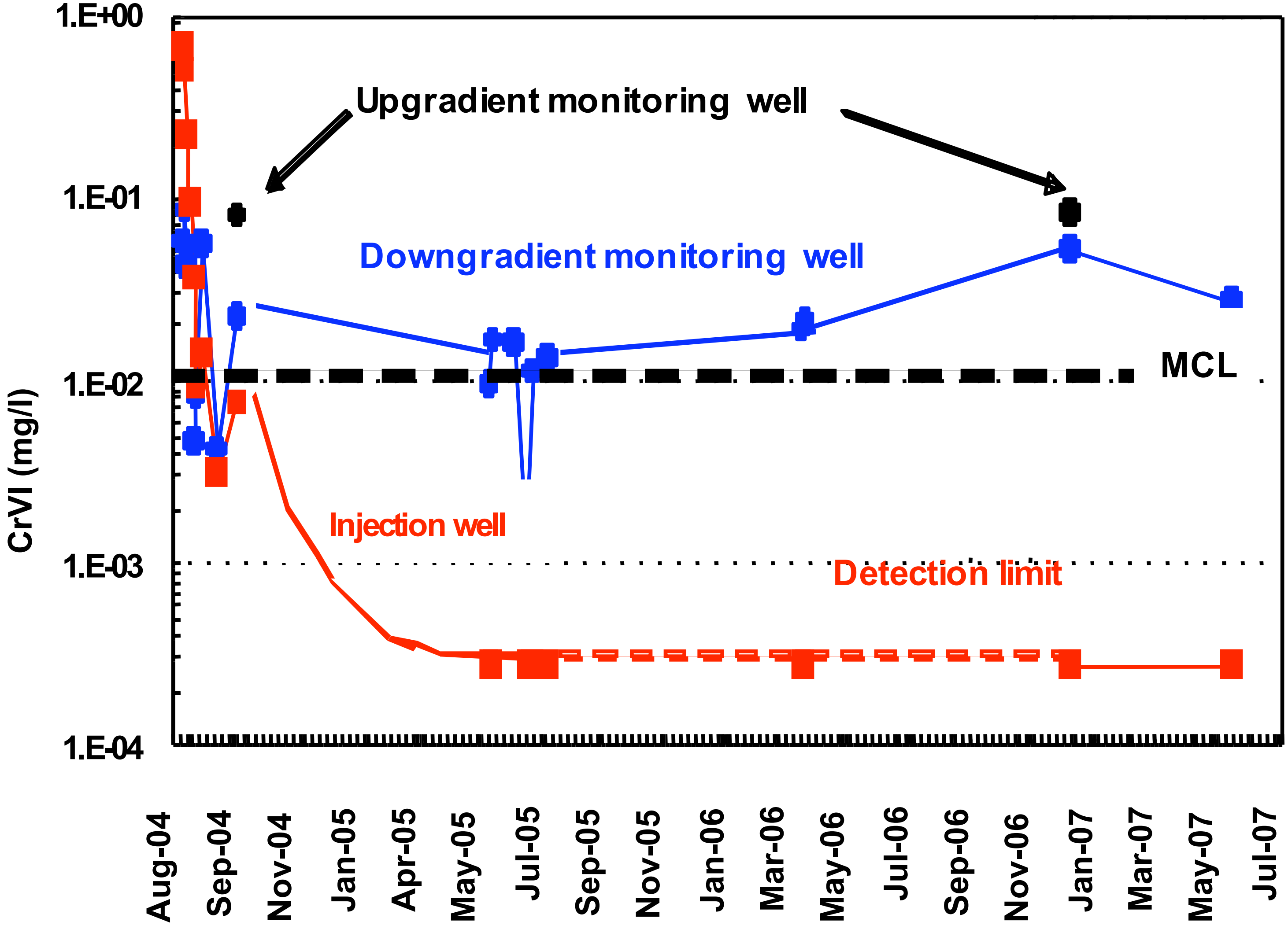
NO_3 Concentration after HRC Injection



SO_4 Concentration after HRC Injection



Average Cr(VI) Concentration after HRC Injection



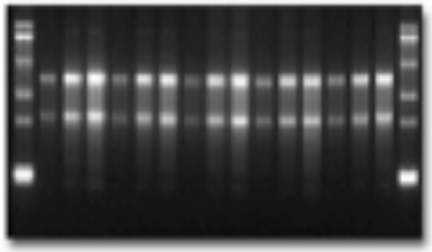
Genomic approach

- **Phylochip:** 16S rRNA based microarray
- **Clone libraries:** by MDA
- **Geochip:** Functional gene based microarray

- **Isolation, 16S~phylogenetic analysis and physiology**

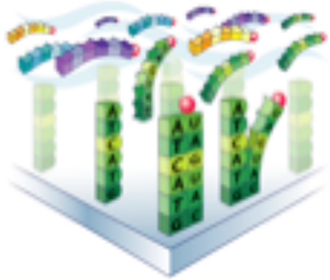
➢ PhyloChip – 500,000 probes (300k target 16S)

16S rRNA gene used as biomarker due to large database and availability of "universal" primers.

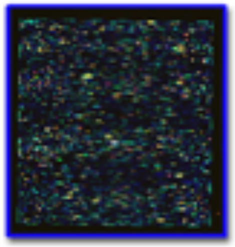


16S rRNA gene is amplified from genomic extract or 16S rRNA molecules are used directly

Amplicon pool fragmented, biotin labeled

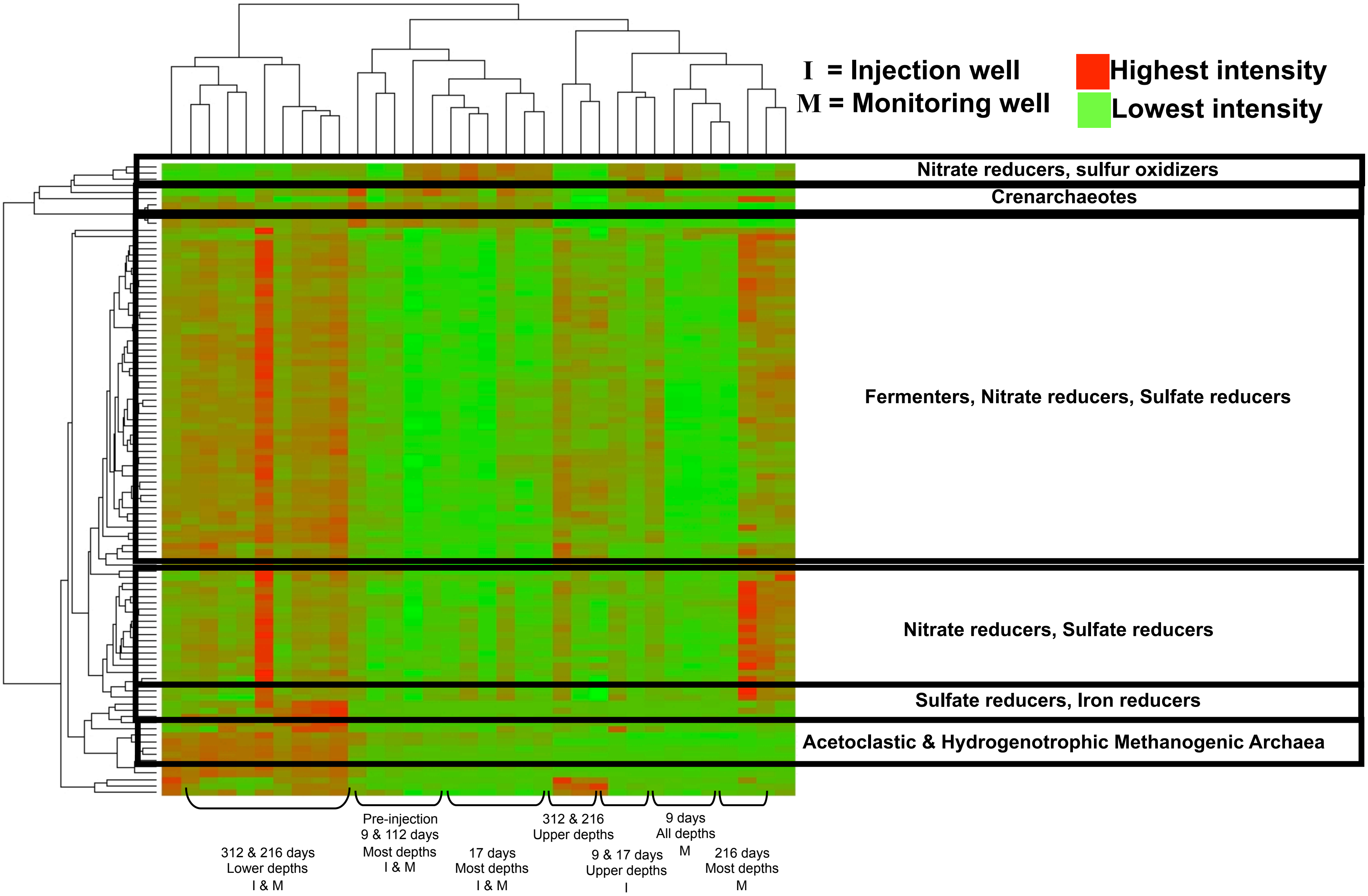


PhyloChip stained and washed using automatic fluidics station

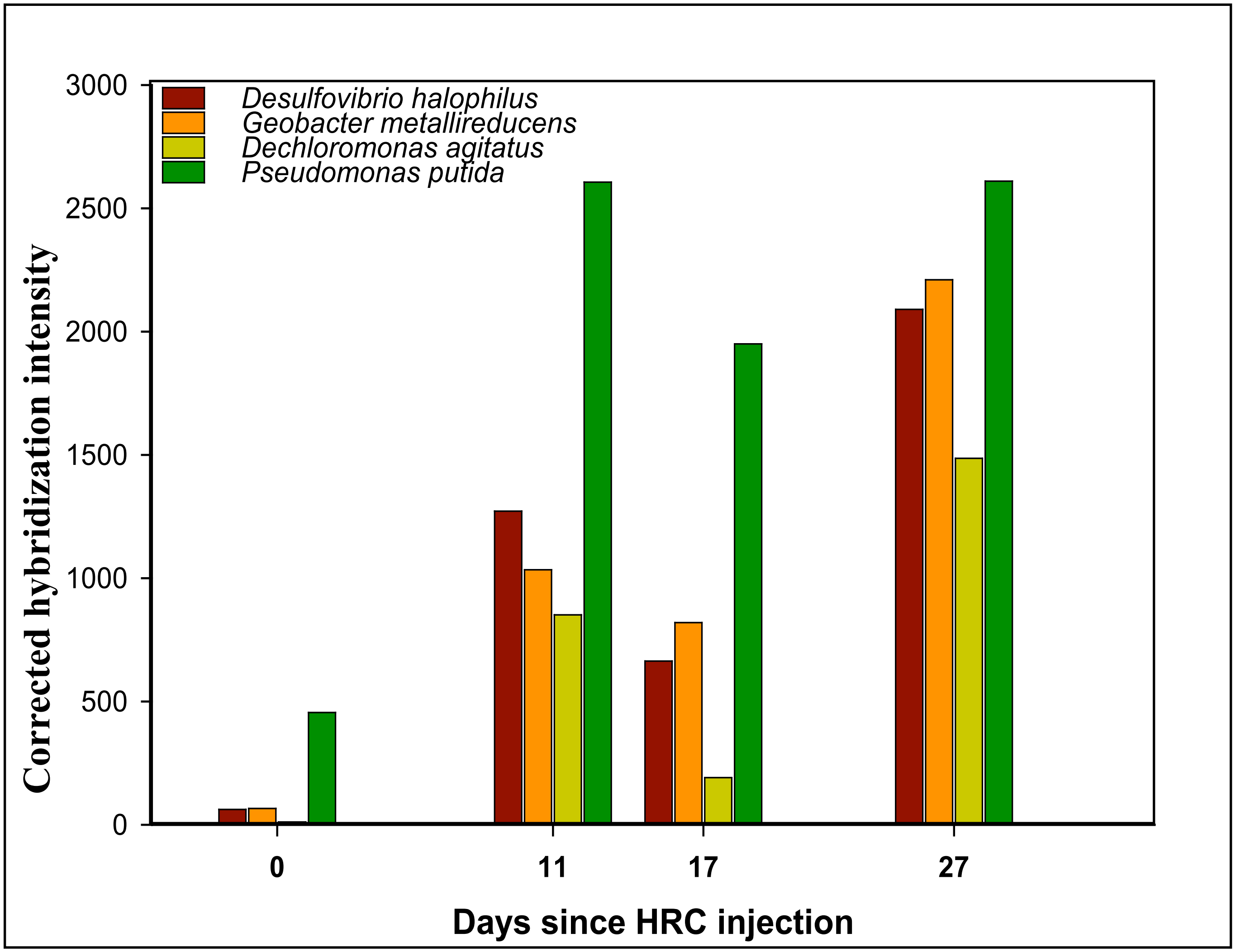


PhyloChip is scanned, fluorescence data analyzed and probe sets with >90% probes positive are considered present

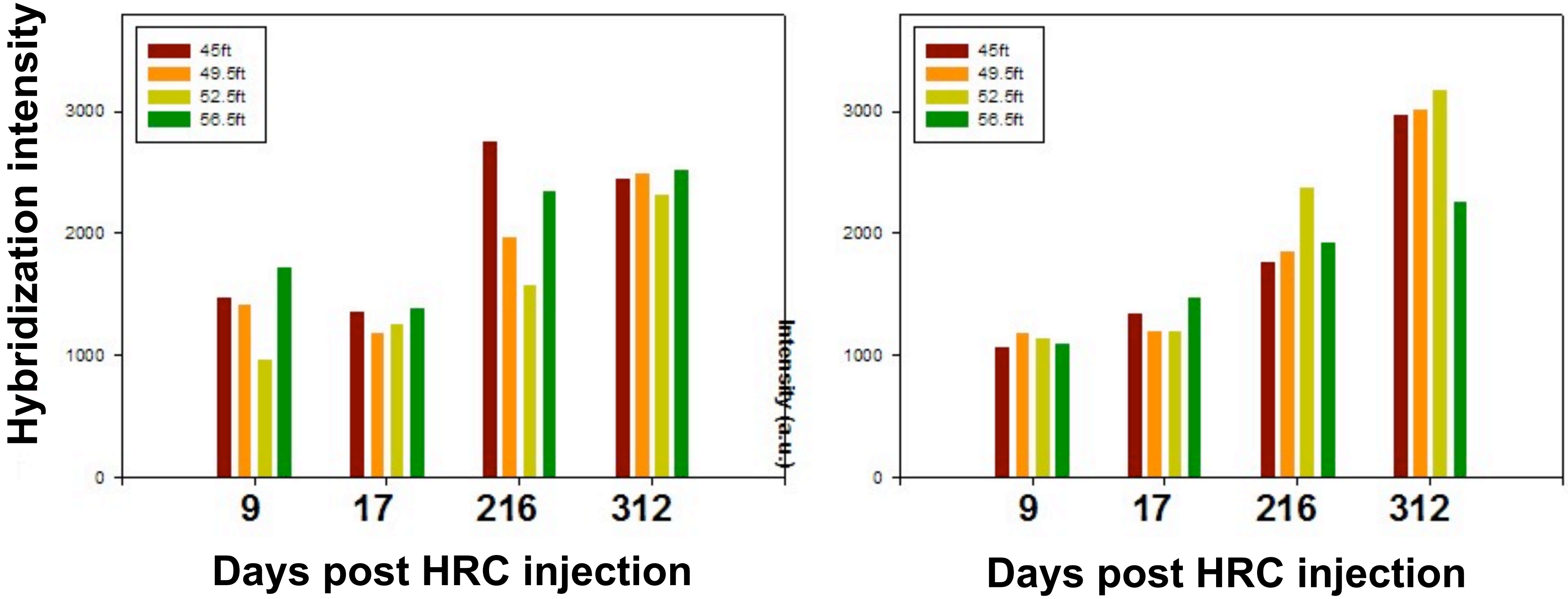
Data mining – Bidirectional clustering



Phylochip results of significant bacterial groups:



Functional groups – Iron reduction



Deltaproteobacteria (*Geobacteraceae*)

Injection well

Monitoring well

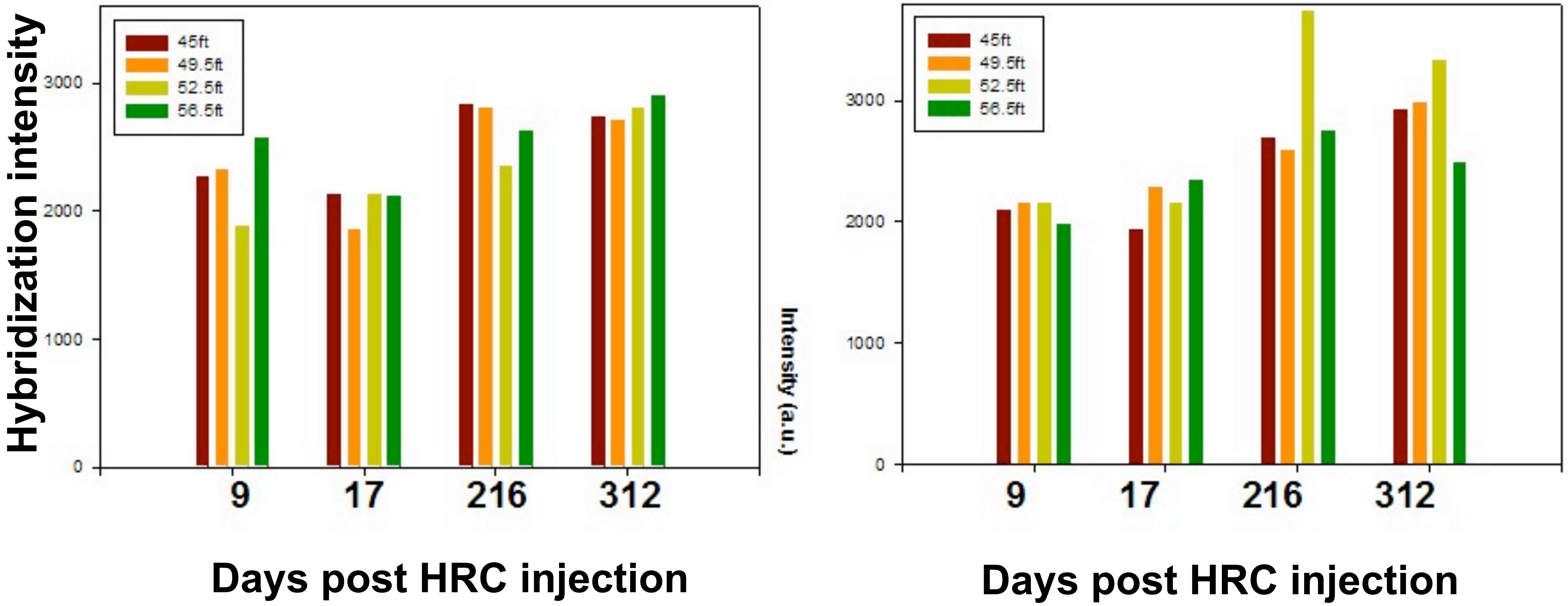
Fe(II) can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Sulfate reduction

Injection well

Deltaproteobacteria
(*Desulfovibrionaceae*)

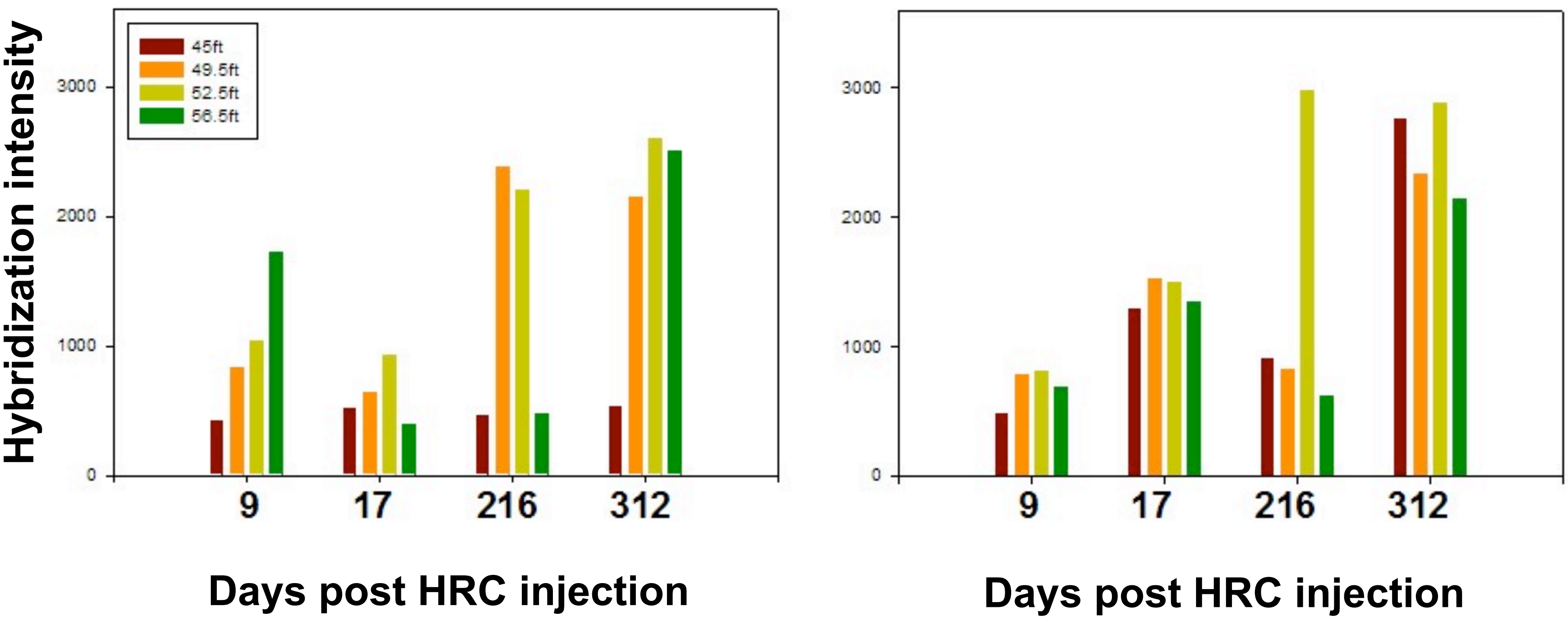
Monitoring well



H_2S can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Methanogenesis

Euryarchaeota (Methanogens)



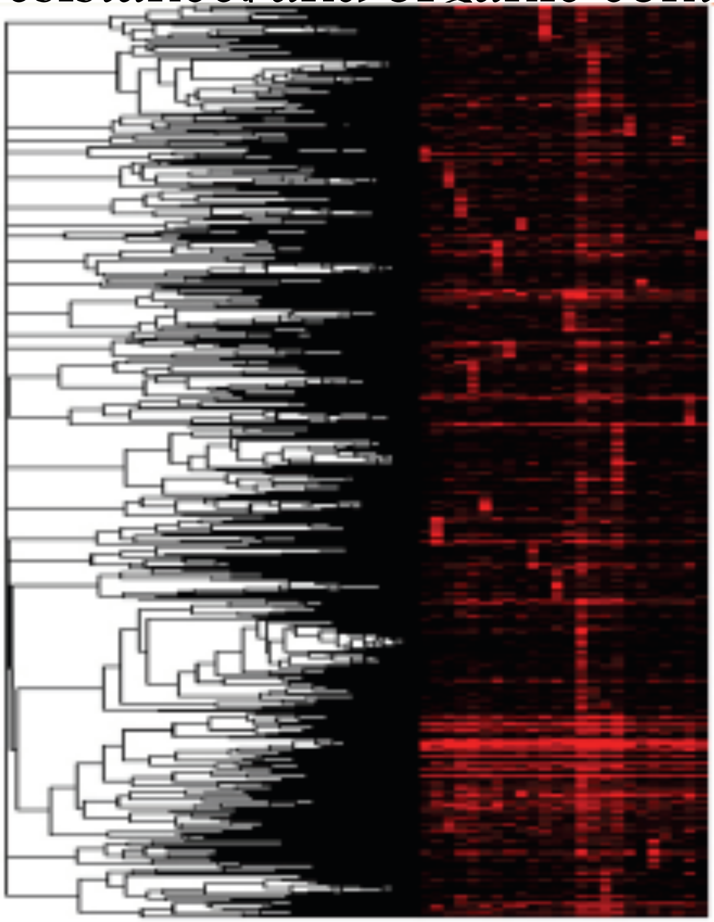
Presence of methanogens indicates strongly reducing conditions

Clone libraries

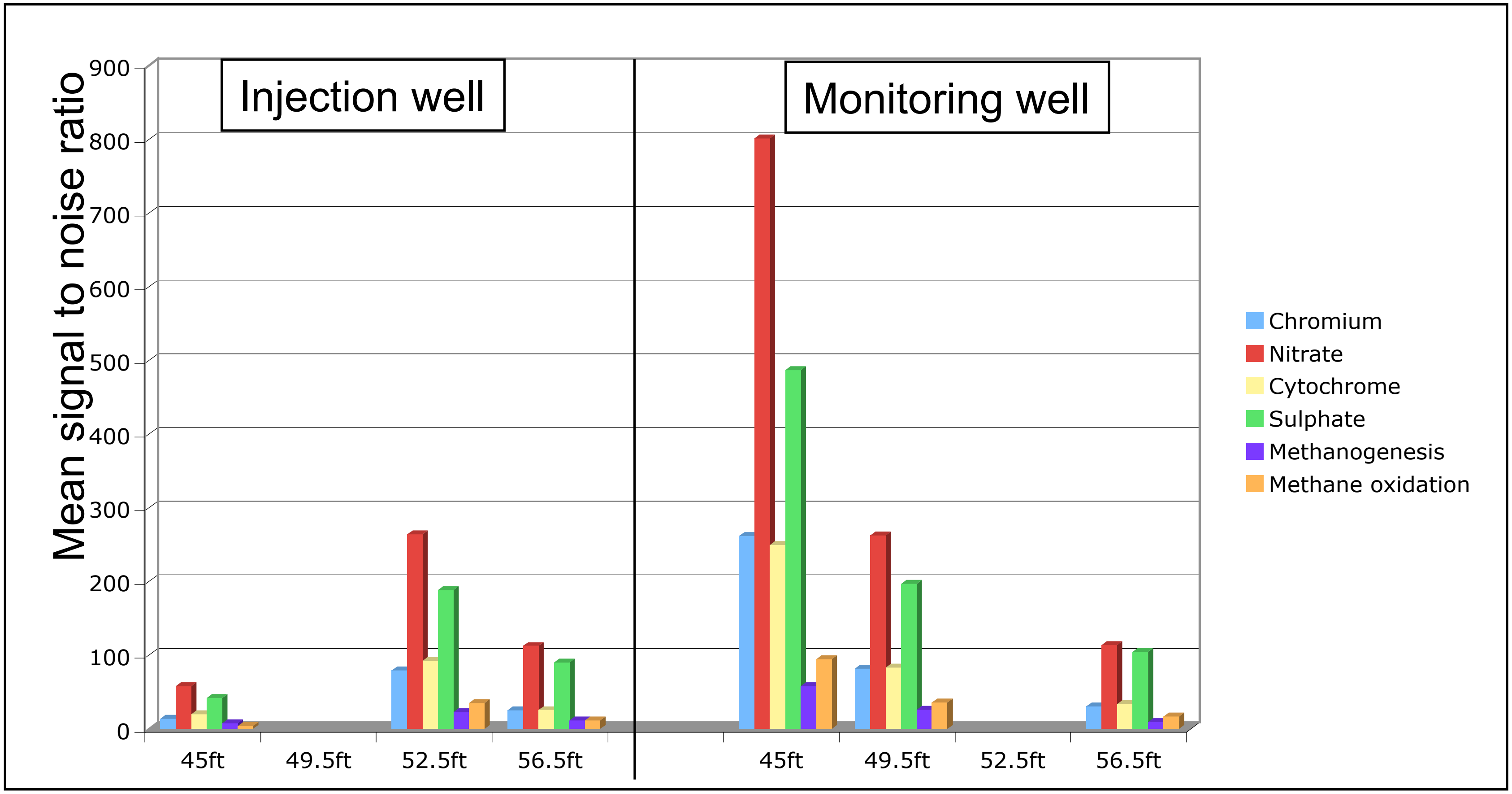
- 16S clone libraries generated by MDA
- Analysis in progress.
- Initial results implicate *Caulobacter cresentus, Psedumonas, Stenotrophomonas* and *Desulfovibrio* spp.

Geochip

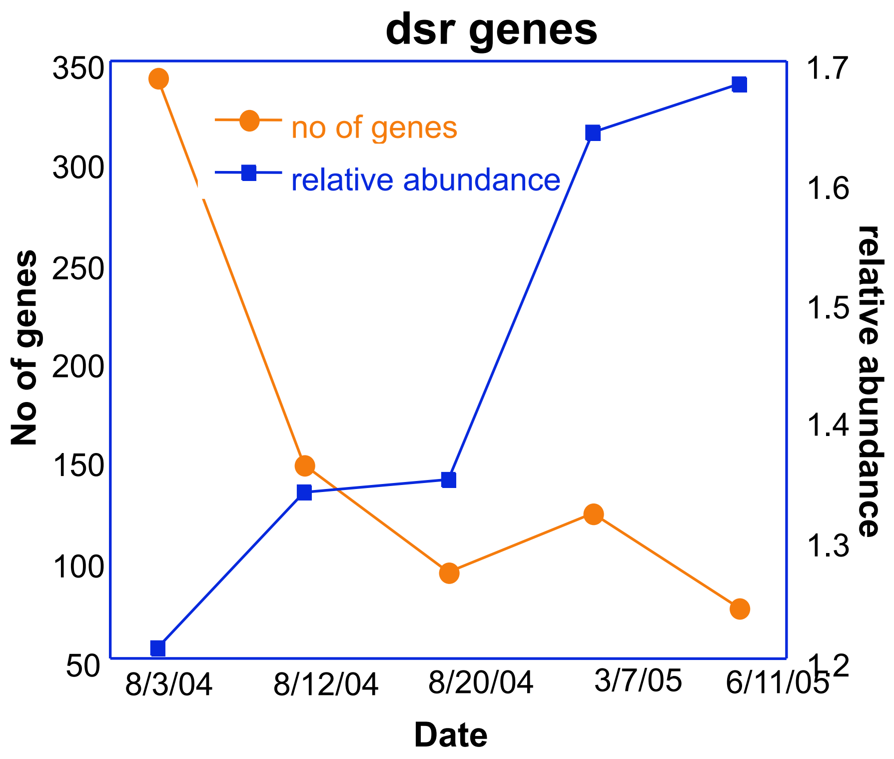
- Approx 25000 oligonucleotide (50 mer) probes
- covering >10000 genes in >150 functional groups
- Genes for nitrogen, carbon, sulfur and phosphorus cycling, metal reduction and resistance, and organic contaminant degradation.



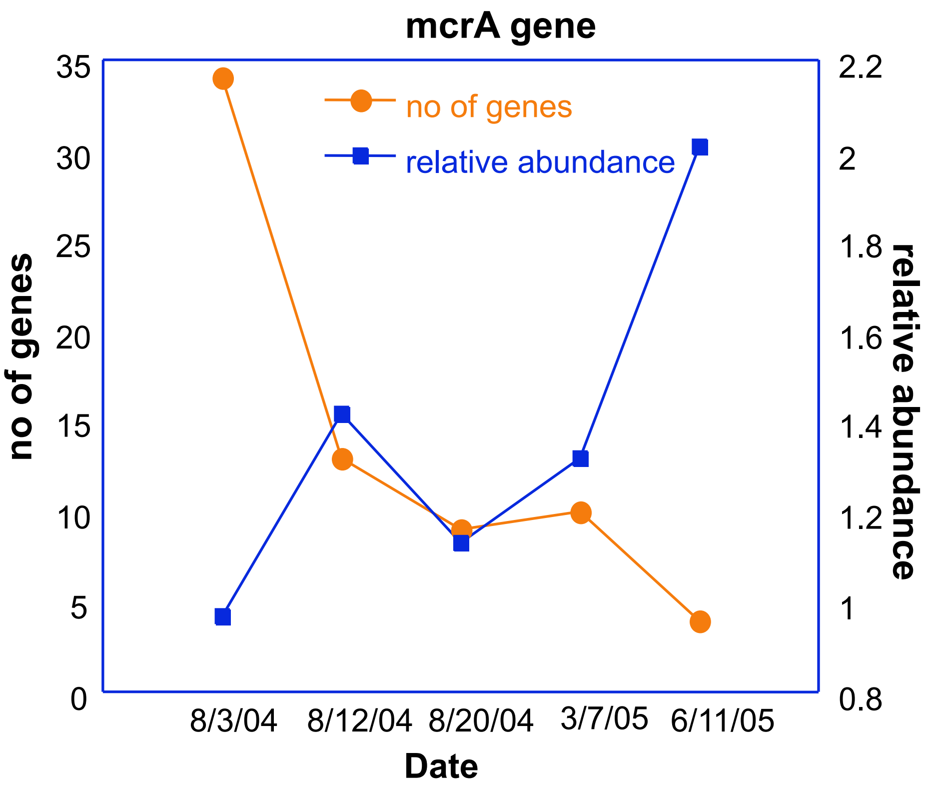
Geochip microarray results:



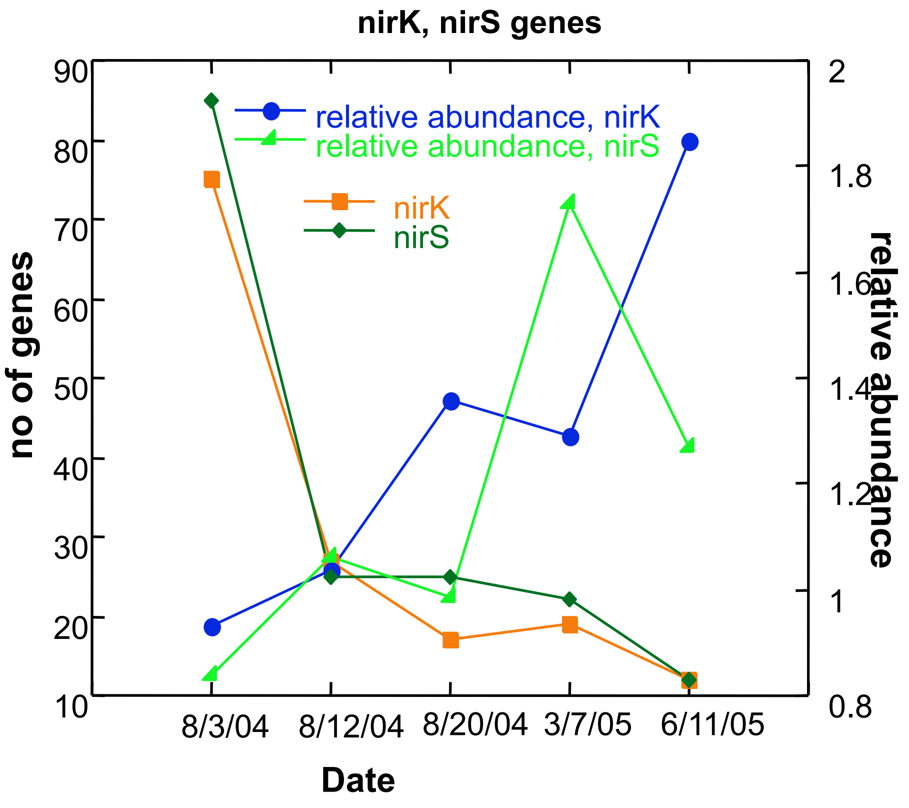
Nitrate, Sulfate, Iron reduction. Methanogenesis, Methane oxidation, Sulfur oxidation. Many chromium tolerance/reduction genes.



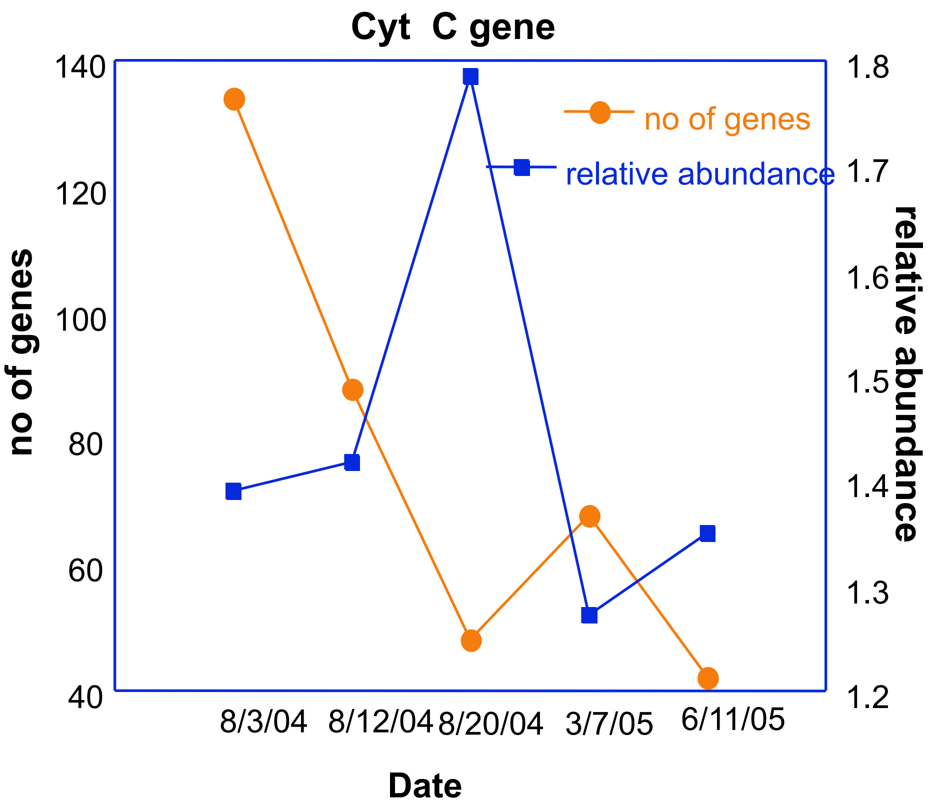
Sulfite reductase



Methyl coenzyme-M reductase

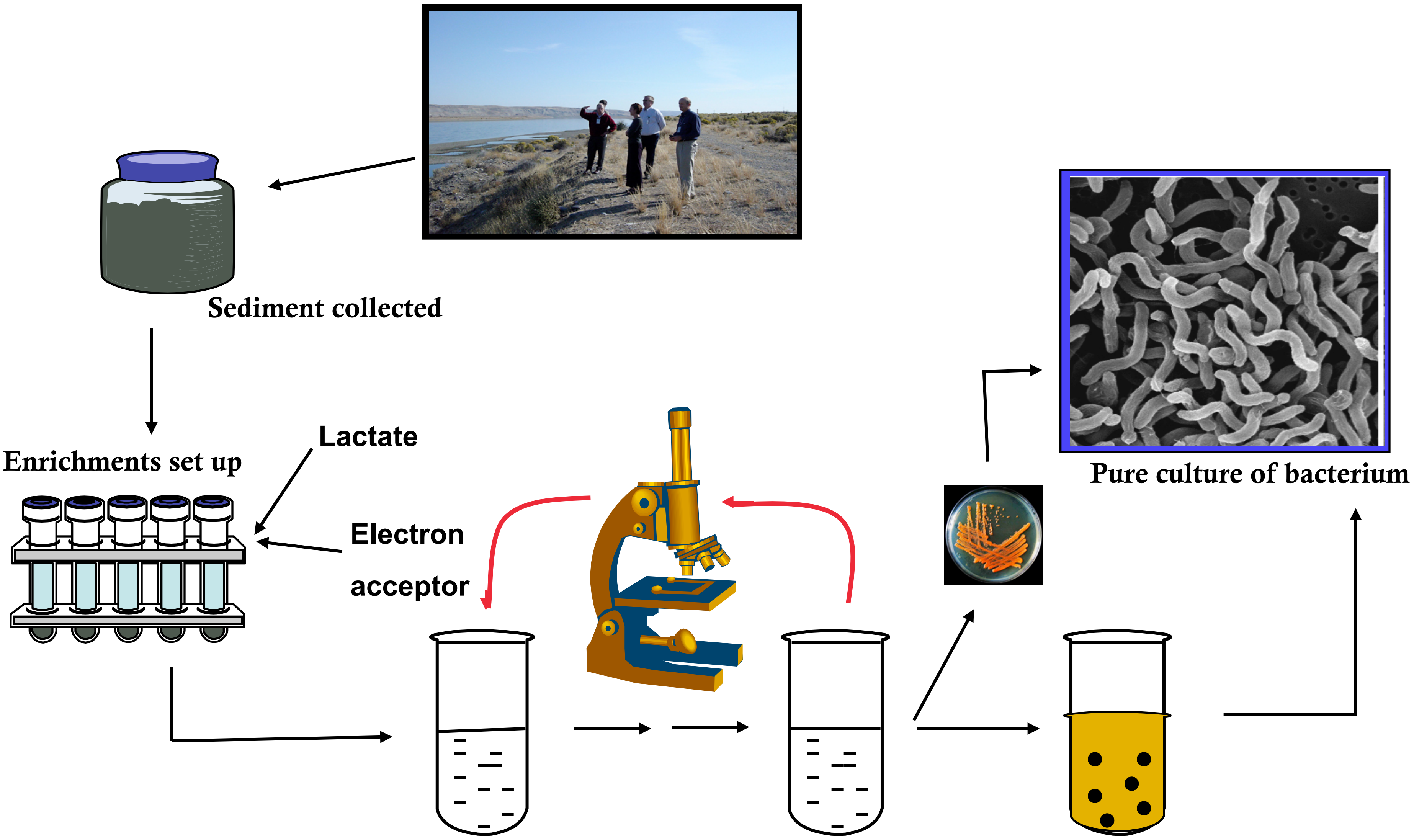


Nitrite reductase



Cytochrome C

Isolating microorganisms:



Sediment collected

Enrichments set up

Lactate

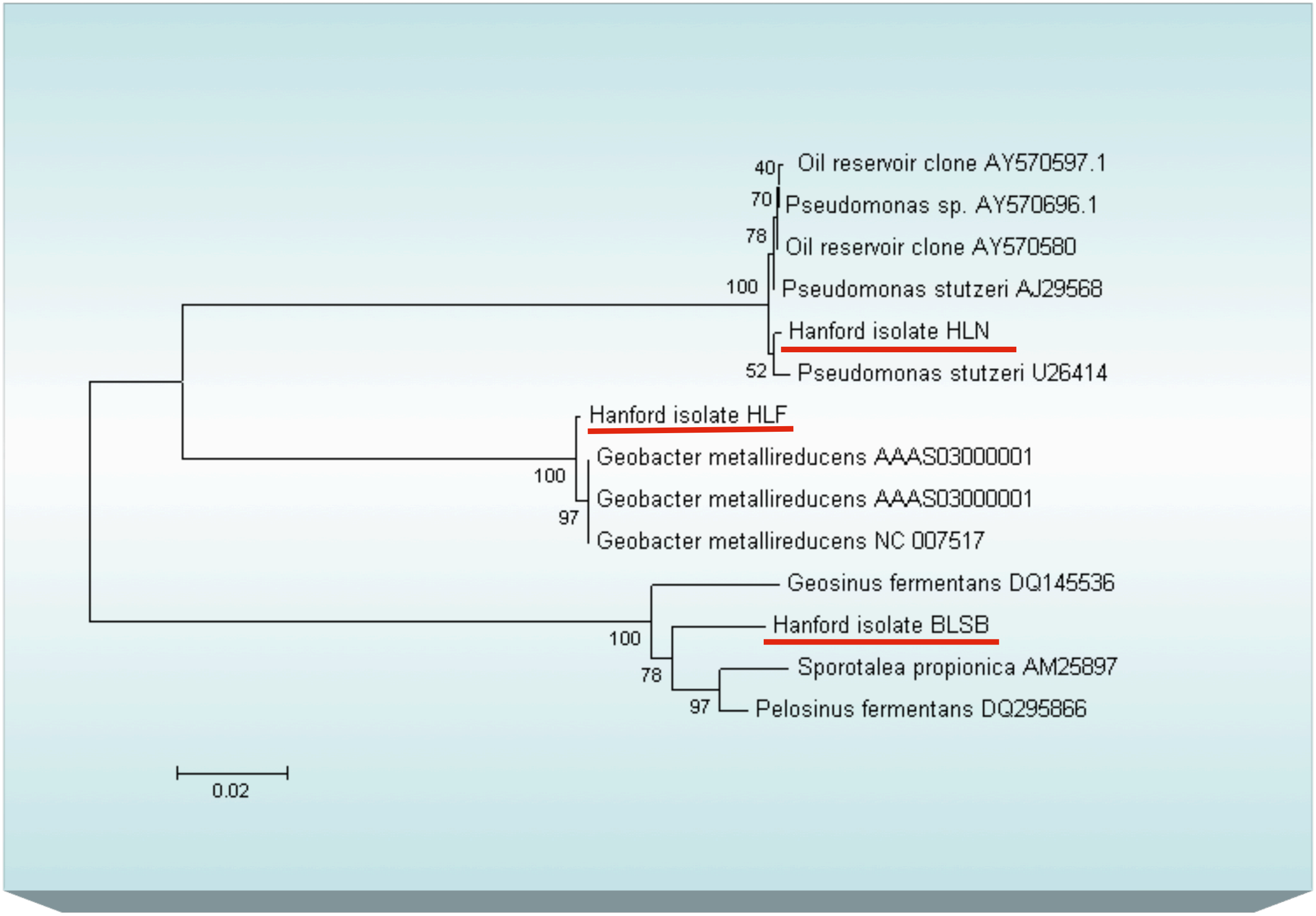
Electron acceptor

Periodic transfers and microscopic counts

Isolated colonies developed by agar shake tubes method

Pure culture of bacterium

16S-rDNA based Phylogenetic tree



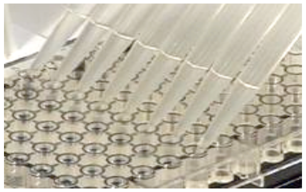
Characterization using the OMNILOG phenotypic microarray

Cells grown to
mid log phase

Centrifuged at

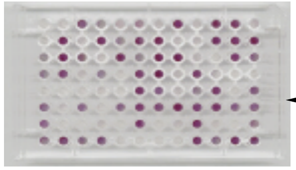
6000rpm
for 10min

Cell pellet
resuspended
to obtain density
of 10^7cells/ml^{-1}



100μl of cell suspension
inoculated each well

Plates sealed and incubated
anaerobically for 96 hours

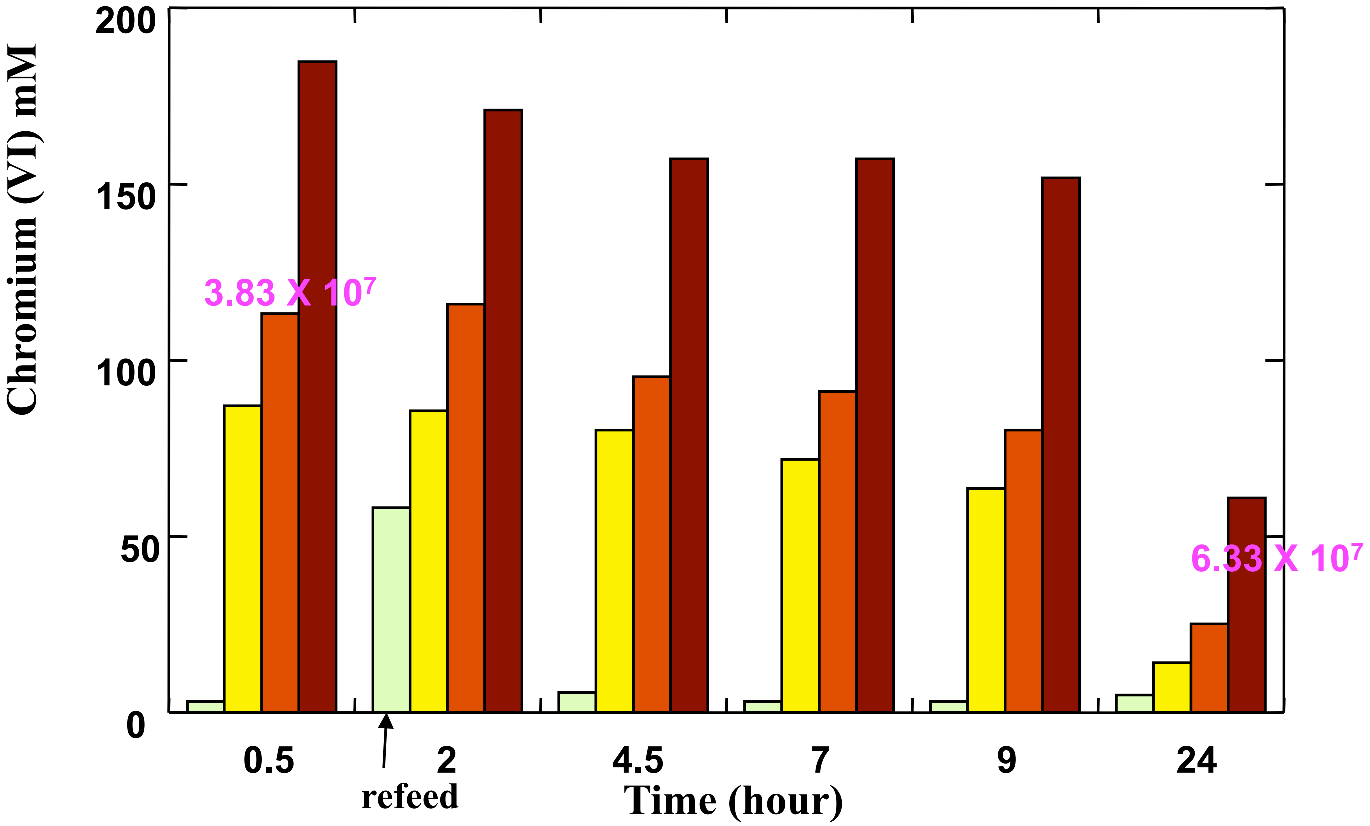


Growth results obtained and data
analysis

Electron Donors and Carbon source

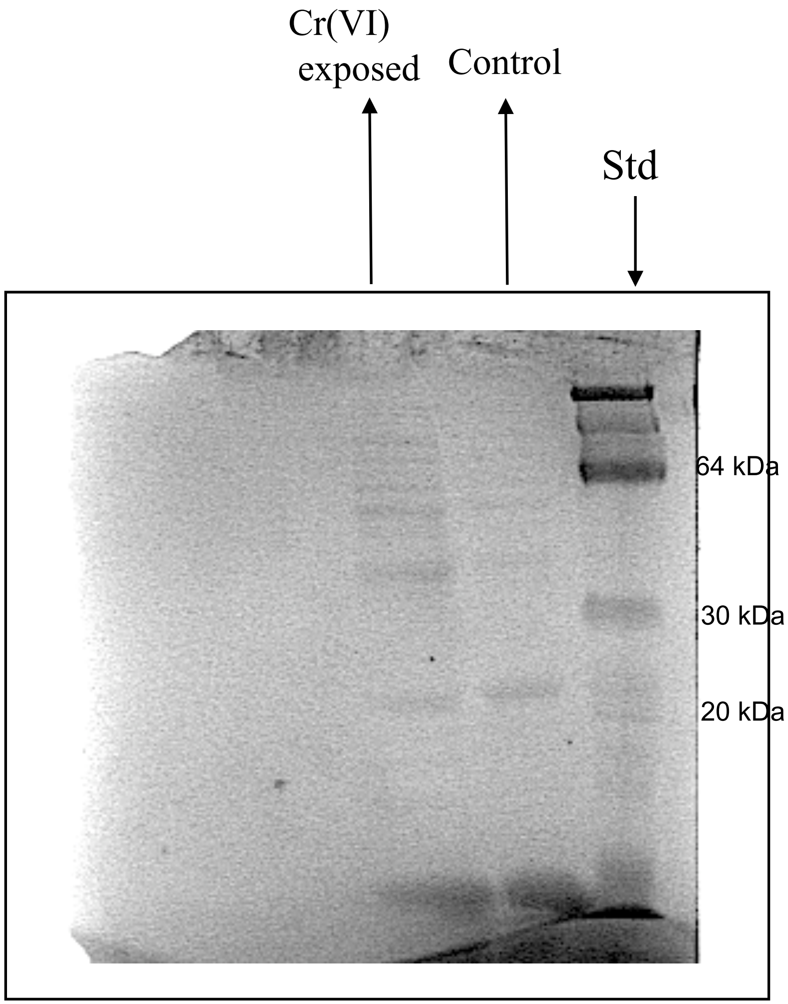
	HLN	RCH1	RCH2
Butyrate	-	-	-
Propionate	-	-	-
Ethanol	-	-	-
Pyruvate	+	+	+
Fumarate	+	-	+
Lactate	+	+	-
Acetate	+	-	+
Citrate	+	-	+
Glucose	+	-	-
Formate	+	-	-
Succinate	-	-	-
Benzoate	-	-	-
Glycerol	+	-	+

Chromium reduction by active cells



Pseudomonas strain HLN

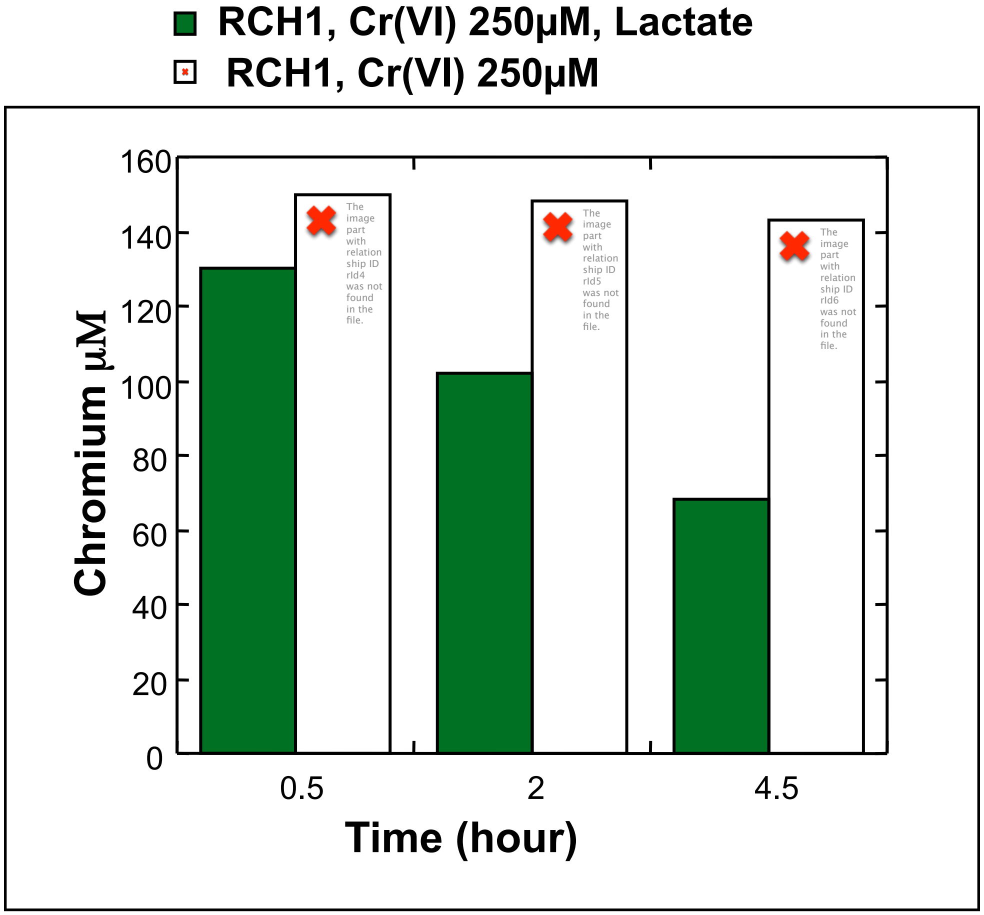
🟩 **Cells, 50uM Chrom, Lactate**
🟨 **Cells, 75uM Chrom, Lactate**
🟧 **Cells, 100uM Chrom, Lactate**
🟥 **Cells, 175uM Chrom, Lactate**



Heme staining

Pseudomonas stutzeri
strain HLN

Desulfovibrio vulgaris strain RCH1



To Conclude:

- Phylochip suggests that increased Cr(VI) immobilization coincides with the increase of the *Desulfovibrio, Geobacter, Pseudomonas* and *Dechloromonas* strains following HRC injection

- Clone libraray analysis indicated Psedumonas, Desulfovibrio spp along with others.

- Geochip reveals that following HRC injection,the richness of gene diversity corresponding to the dominant metabolisms decreased, however the relative abundance of these genes increased over time. This implies gradual dominance of each process by a few members of the population.

- Iron reducer, nitrate reducer, sulfate reducer isolated from the Hanford 100H site capable of Iron(III) reduction and Chromium(VI) reduction.
- Organisms mediate Chromium(VI) removal by direct enzymatic as well as abiotic interactions.
- Environmental *Desulfovibrio* isolate currently being sequenced by JGI

Acknowledgement

- Terry C. Hazen, Boris Faybishenko, Susan Hubbard, Gary L. Andersen, Eoin L.Brodie, Mark Conrad, Paramvir Dehal : LBNL
- Joe Zhou, Joy Van Nostrand: Univ Of Oklahoma
- Phil Long: PNNL
- Carl Abulencia, Martin Keller
- Regenesis: San Clemente, CA

ESPP2 is part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program:GTL through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

DOE GENOMICS:GTL
ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT
OFFICE OF SCIENCE
U.S. DEPARTMENT OF ENERGY

Office of Science
U.S. DEPARTMENT OF ENERGY

VIMSS