

VIMSS and the NABIR FRC update



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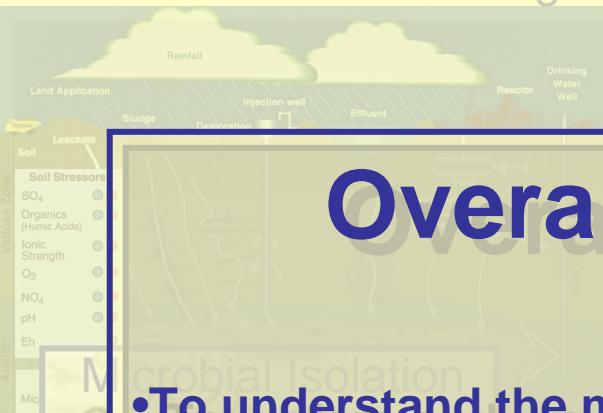
tchazen@lbl.gov

<http://vimss.lbl.gov>



Virtual Institute of Microbial Stress and Survival

Environmental Monitoring



Pathway Models

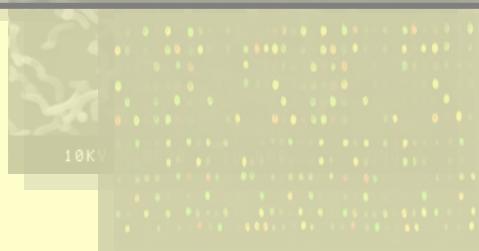


Pathway Inference

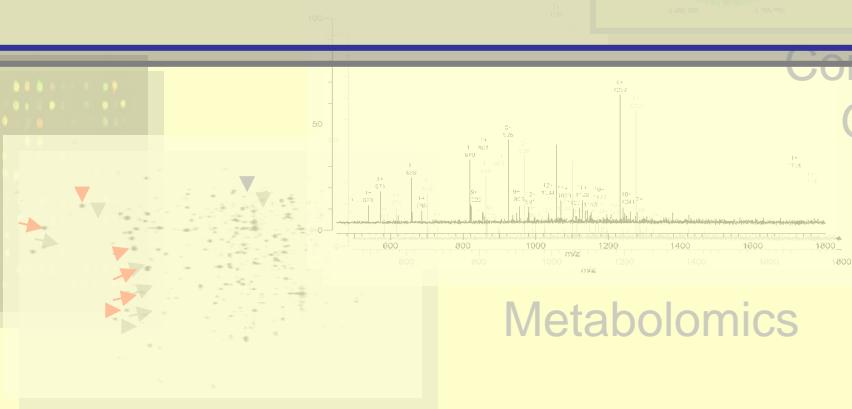
Overall VIMSS Goals

- To understand the mechanisms by which microbes adapt and survive
- To elucidate how they carry out mission critical processes
- To rapidly characterize new microbes to the level of a model microbe

sFTIR
In situ
physiology



DNA Microarrays



Proteomics

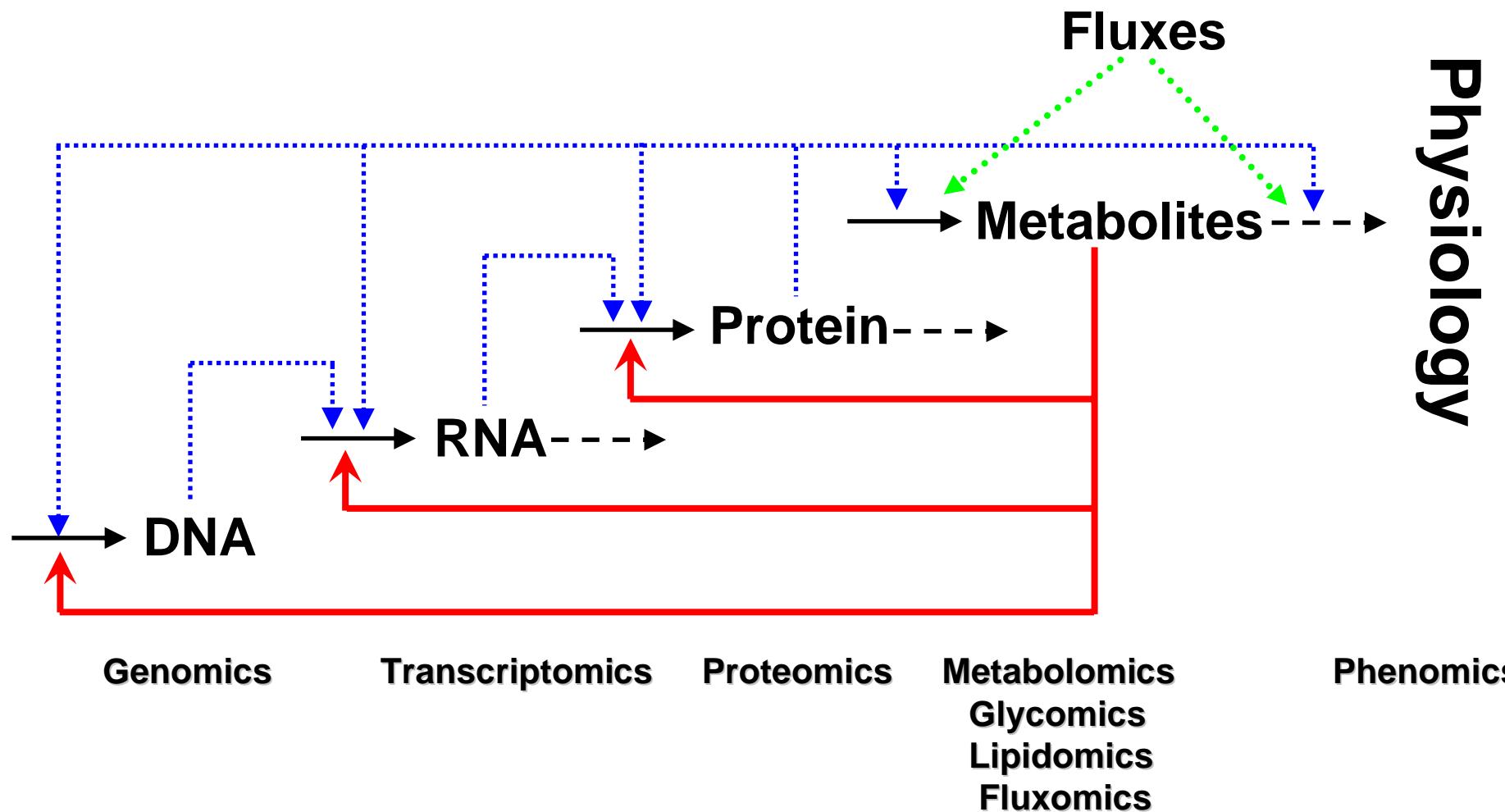
Comparative Genomics



Metabolomics

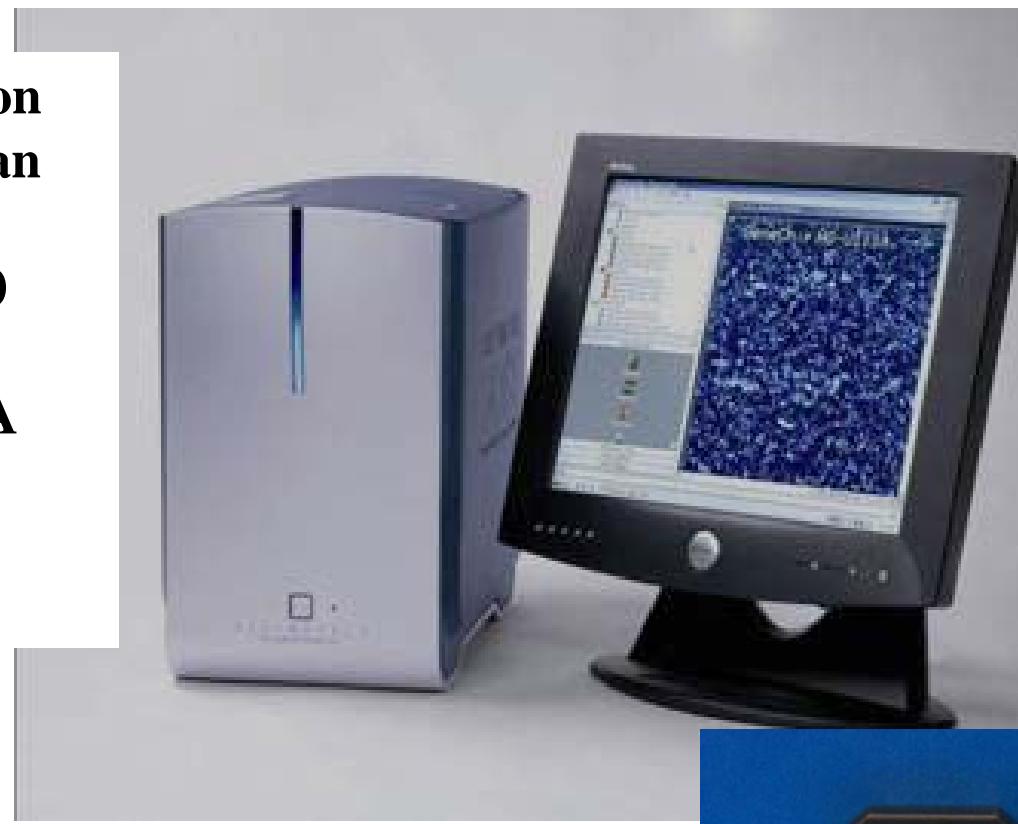
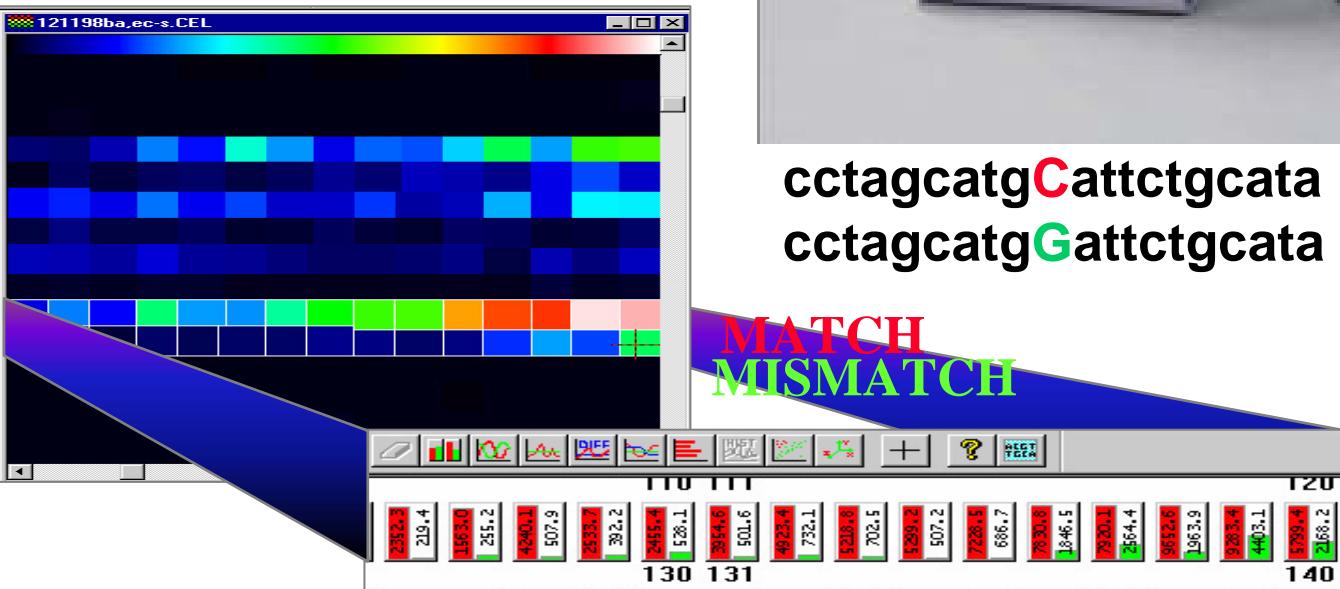


The Omics!!

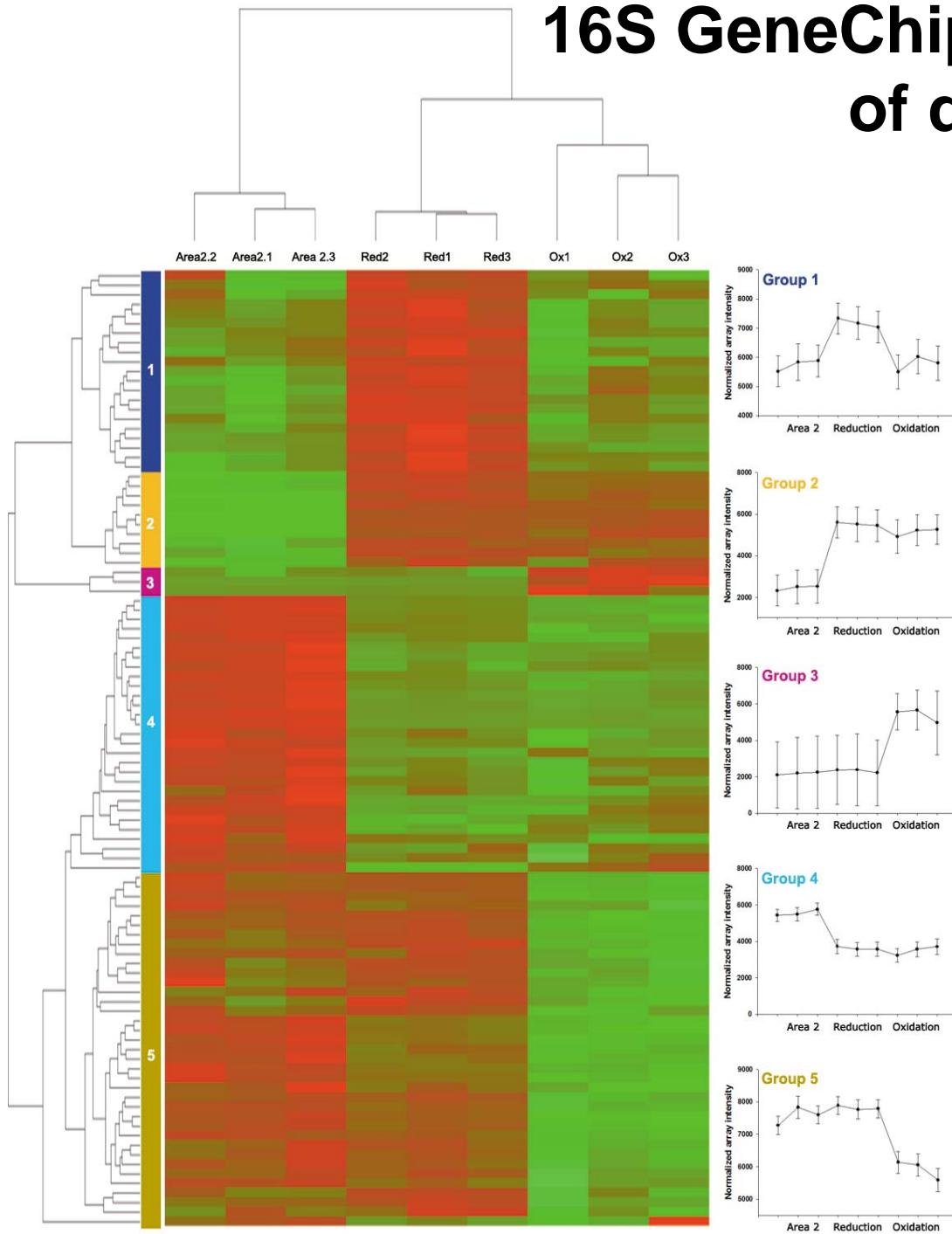


DOE 16s rDNA microarray

- Rapidly detect the composition and diversity of microbes in an environmental sample
- Massive parallelism - 550,000 probes in a 1.28 cm² array
- all 9,900 species in 16S rDNA database
- Single nucleotide mismatch resolution



16S GeneChip analysis of dynamic bacteria



- *Arthrobacter ureafaciens*
- Alpha-proteobacteria
- *Azoarcus sp.*

- *Geothrix*
- *Geobacter*
- *Anaeromyxobacter*

- *Acidobacteria*
- *Desulfovibrio*

- *Actinobacteria*
- *Firmicutes*
- Alpha-proteobacteria

- *Actinobacteria*
- Alpha-proteobacteria

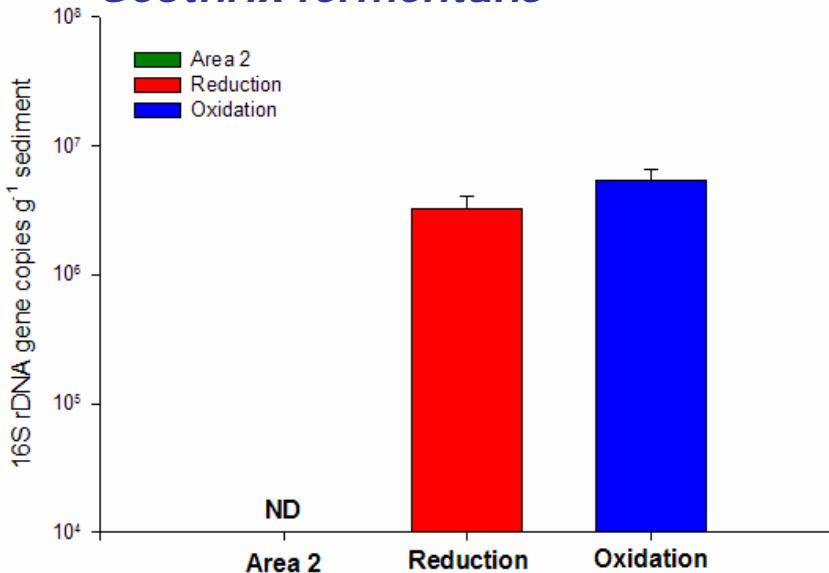
Real-time quantitative PCR confirmation – Uranium Bioremediation

(a) Array quantitation

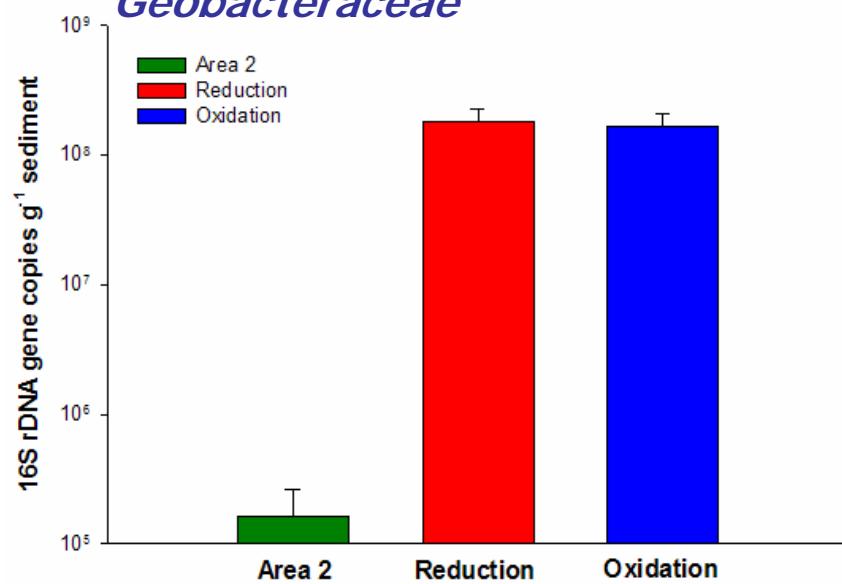
Representative organism	Phylocode	Group	Average Difference		
			Area 2	Reduction	Oxidation
<i>Geothrix fermentans</i>	2.13.8.386	Nitrospina	45	2344	2290
<i>Geobacter metallireducens</i>	2.28.4.7.4.10207	δ-proteobacteria	251	2238	2188
<i>Geobacter arculus</i>	2.28.4.7.4.10209	δ-proteobacteria	38	1412	1698

(b) qPCR quantitation

Geothrix fermentans

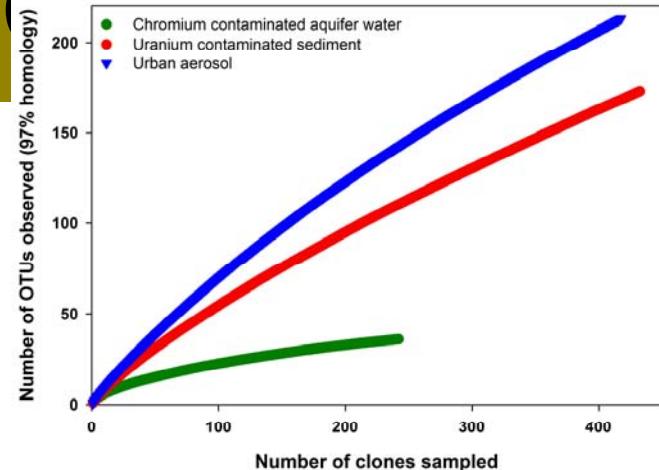


Geobacteraceae

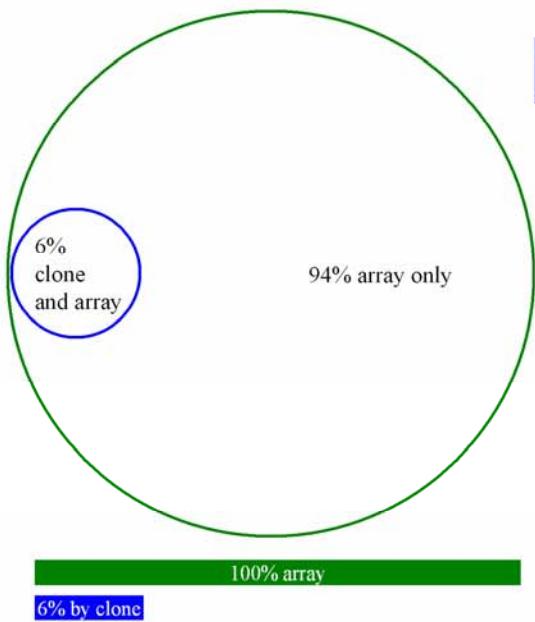


(Tokunaga et al., 2005; Wan et al., 2005)

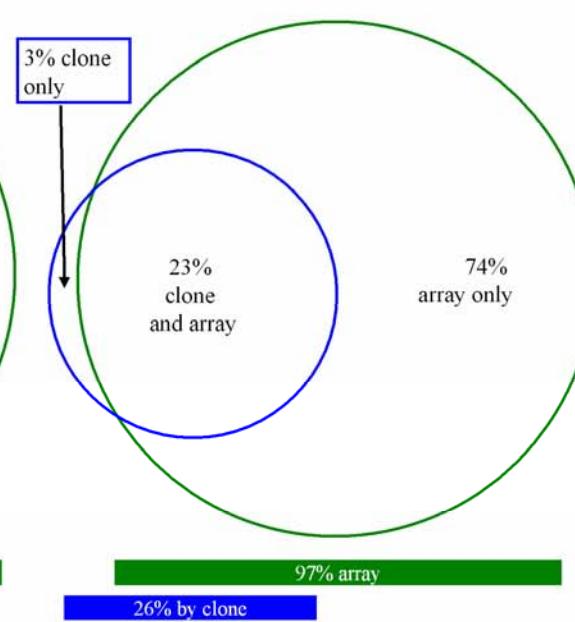
Accuracy Venn



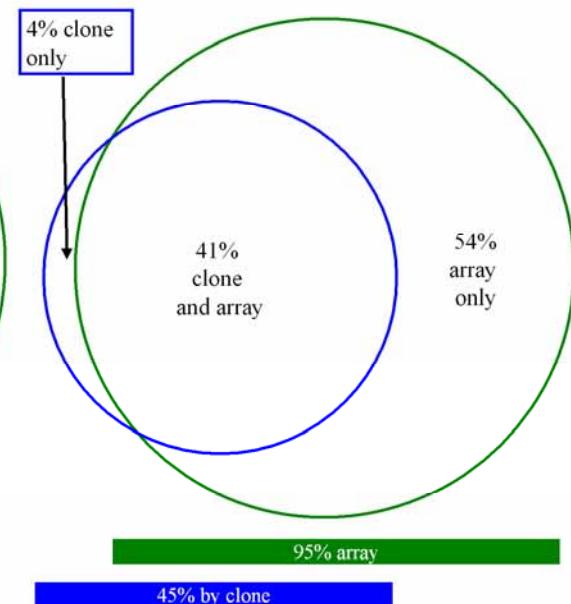
Cr(VI) groundwater



U(VI) subsurface soil



Urban aerosol

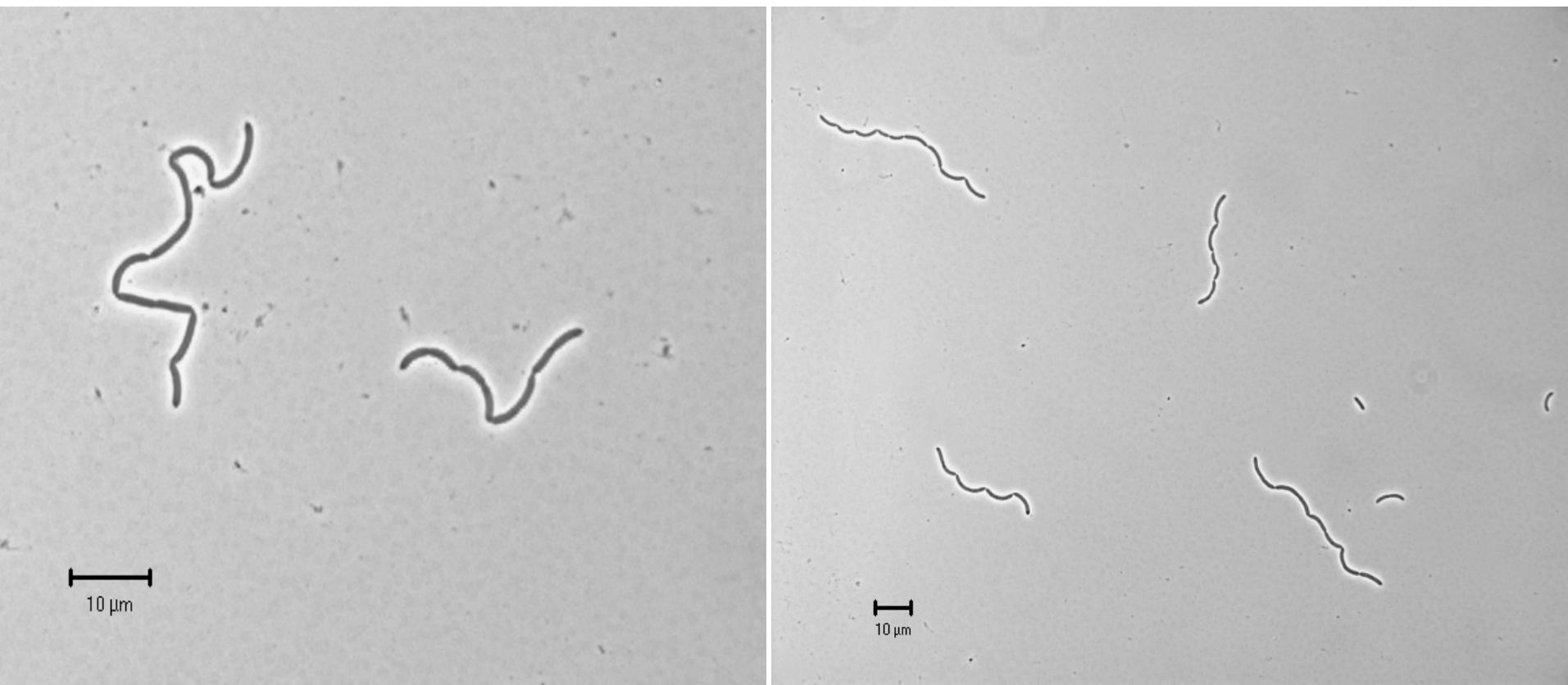


384 clones

768 clones

768 clones

Micrograph of FRC isolate growing on H₂, CO₂, acetate,
and SO₄⁼



Principal Components Analysis

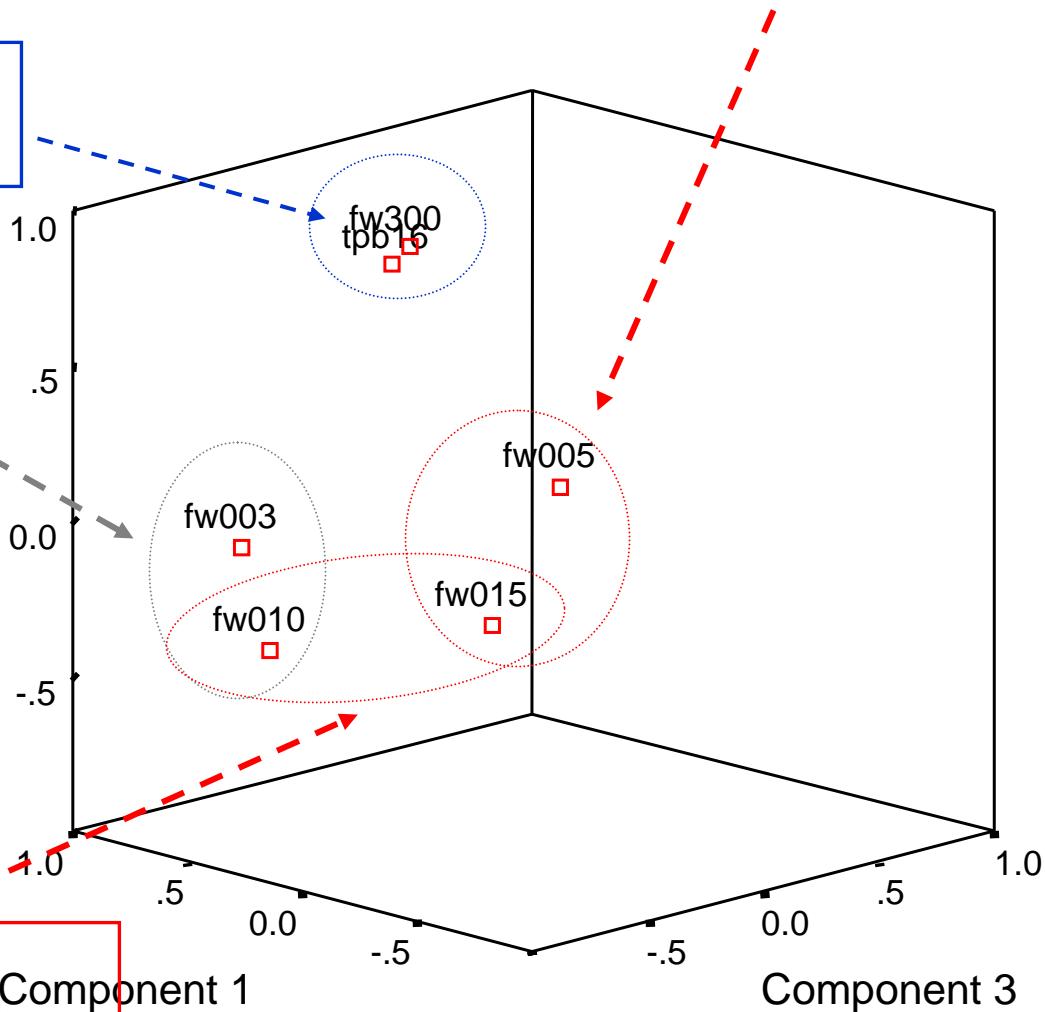
87% variance
107 compounds

* Tetrachloroethene
* ^{99}Tc , SO_4 , Na
* 1,1,2-trichloro-1,2,2-trifluoroethane

* Absence of 1,1,2-TC-1,2,2-TFE
* Absence of ^{99}Tc , NO_3 , Al, Ca

* Ca
* NO_3
* Total organic carbon

Component 2



* Tetrachloroethene
* ^{99}Tc , NO_3 , Al, Nb, Zn, Sb, V, Th
* 1,1,2-trichloro-1,2,2-trifluoroethane

SSU rDNA, *nirS*, *nirK*, *amoA*, *pmoA*, *dsr*

94% of variance

* *Methylobacter* sp.

* *Aquicella* sp.

* uncultivated *Acidobacteria*

* uncultivated δ -*Proteobacterium*

* predominant *pmoA*, *amoA*, *nirS*

Component 2

* *Diaphorobacter* sp.

* *Rhizobium* sp.

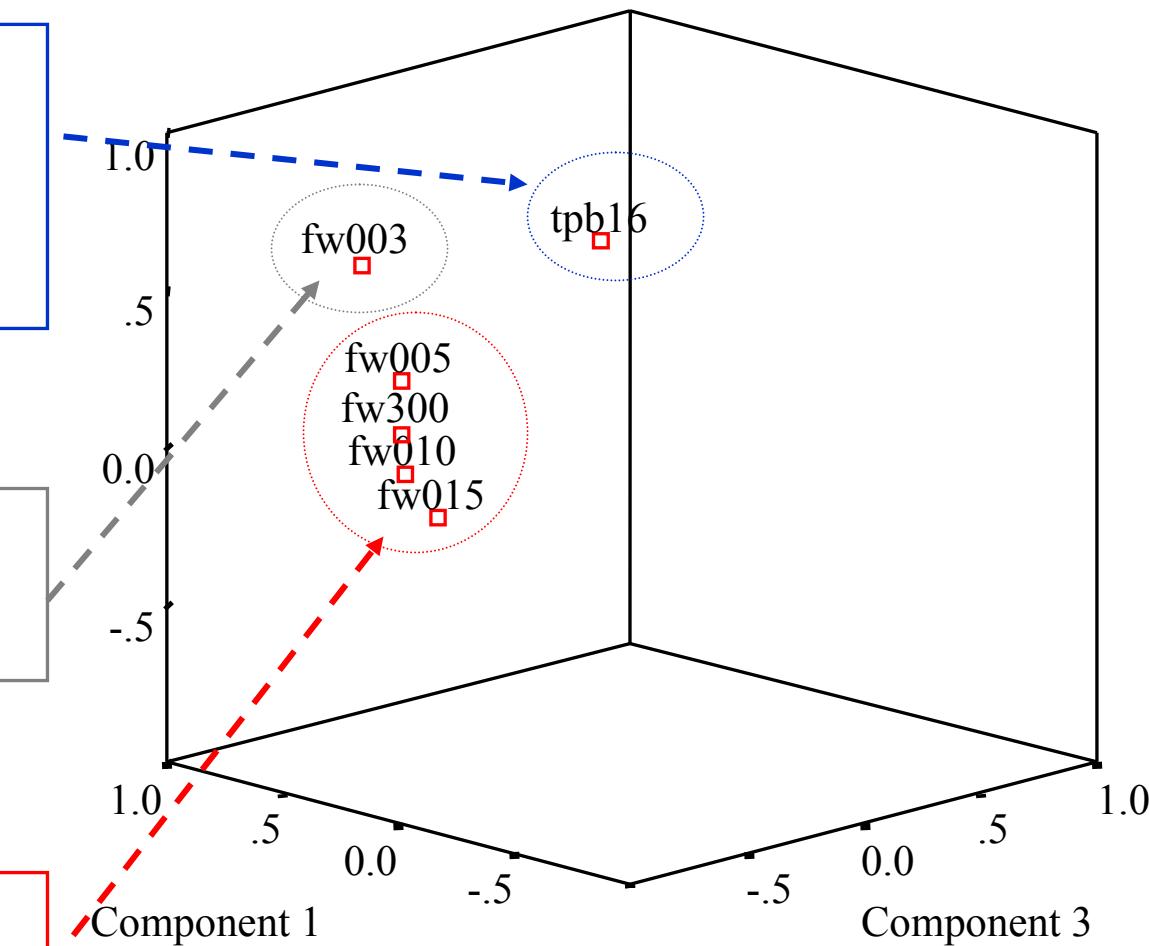
* predominant *dsr*, *amoA*, *nirS*

* *Pseudomonas stutzeri*

* *Pseudomonas marginalis*

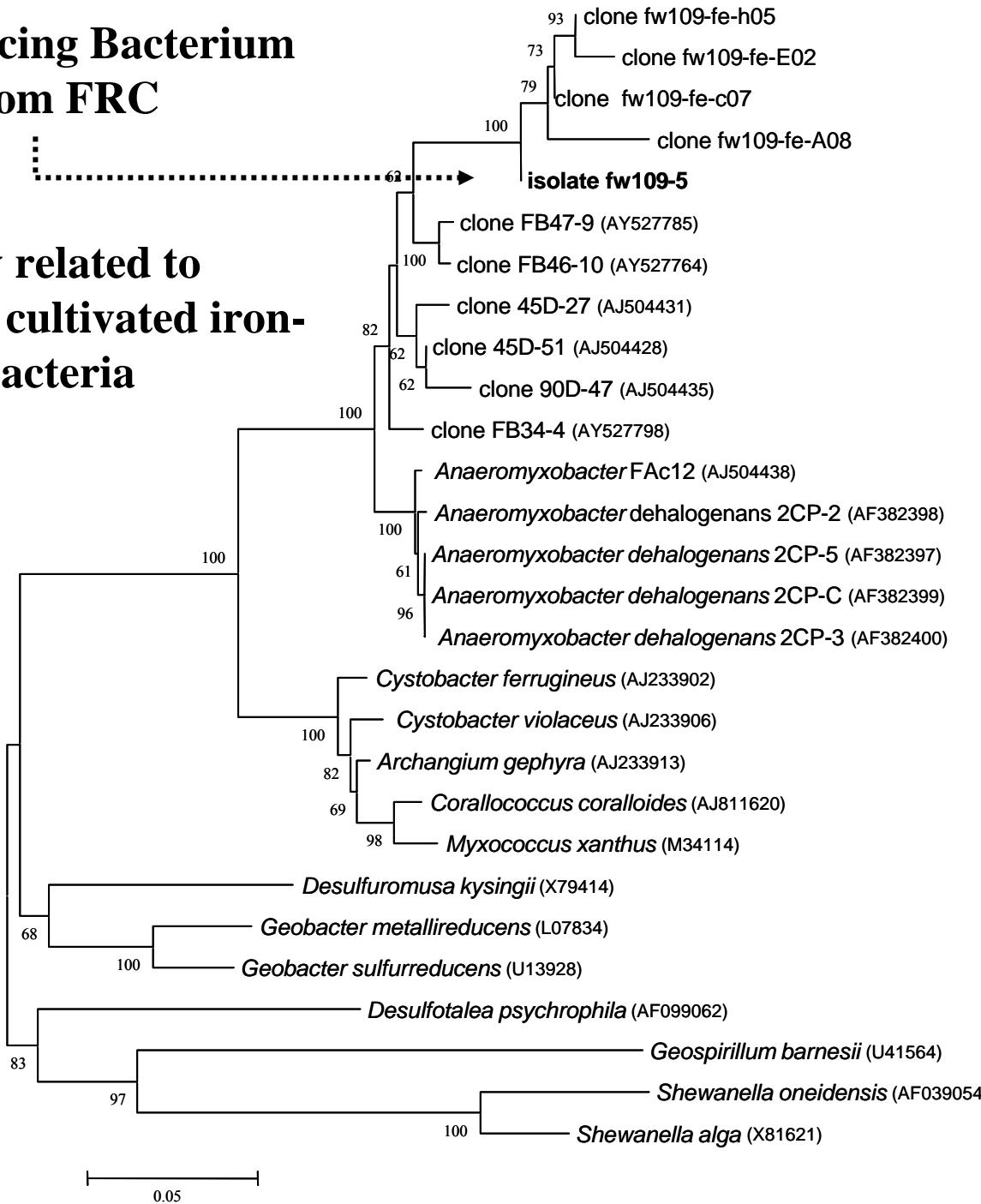
* α -*Proteobacterium*

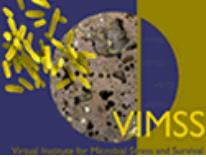
* predominant *nirS*



Iron-Reducing Bacterium Isolated from FRC Sediments

Not closely related to
previously cultivated iron-
reducing bacteria





Characterization of Field Samples

M.W. Fields, C.E. Bagwell, S.L. Carroll, T. Yan, X. Liu, D.B. Watson, P.M. Jardine,
C.S. Criddle, T.C. Hazen, and J. Zhou.

Bacterial community structure measured at different levels of resolution along a contamination plume. (internal review in process)

Work in Progress

Sample	Type	Method	Data
FB-077	S	16S of enrichment (Fe/SO ₄)	Seq. in progress
FW-106	GW	Cultivation *Metagenome/16S	In progress
FW-024	GW	16S	analysis
FW-104	GW	16S	analysis
FW-101	GW	16S	analysis
FW-026	GW	16S	analysis
FW-103	GW	16S	analysis
FW-109	S	16S of SO ₄ -enrichment	analysis
FW-109	S	16S of Fe-enrichment	analysis
FW-109	S	Cultivation	Verify isolation

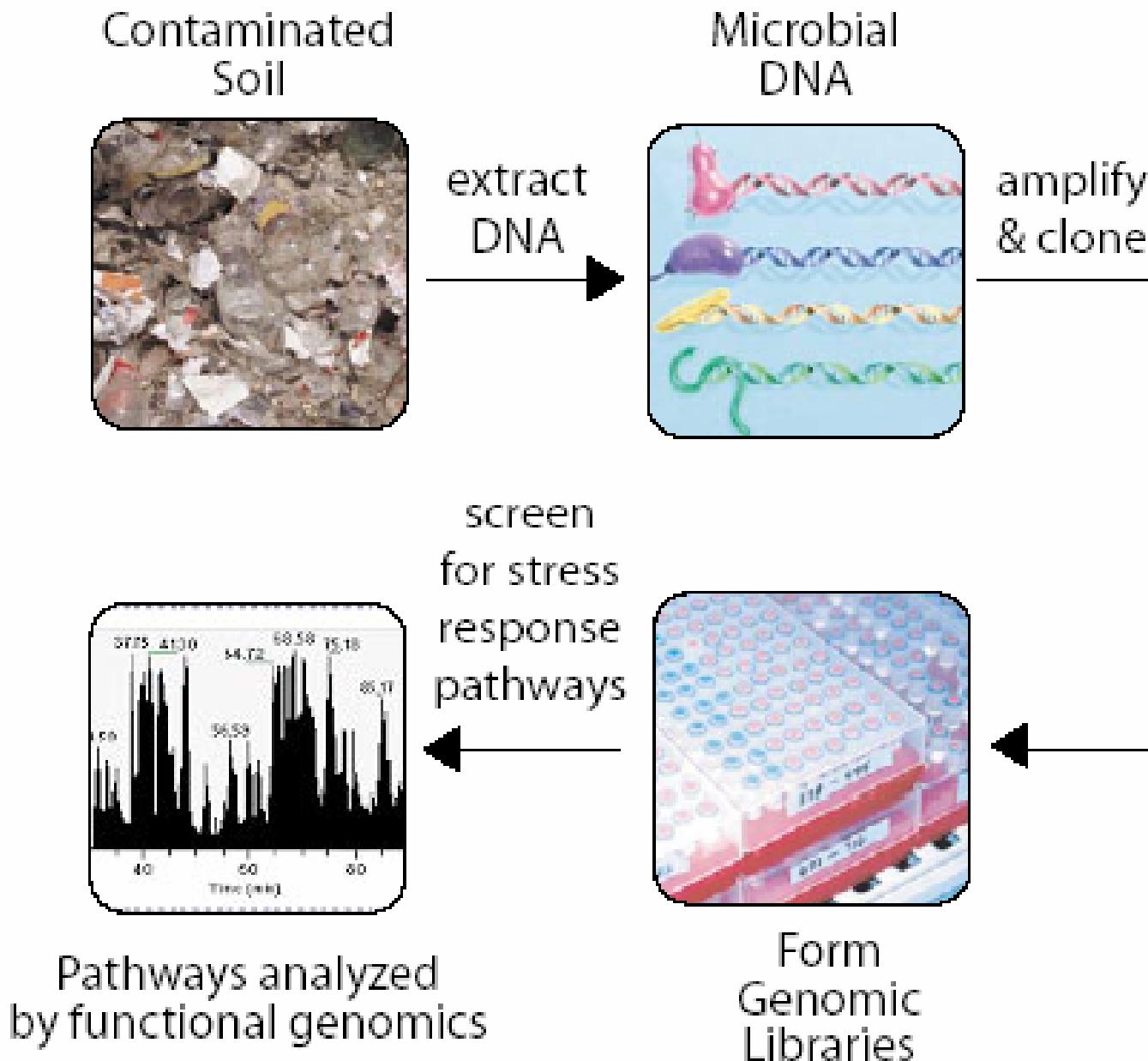
Use 16S and metagenome data to isolate “predominant” organisms *Azoarcus* and *Frateuria*

16S sequences in enrichment similar to organism isolated at UW

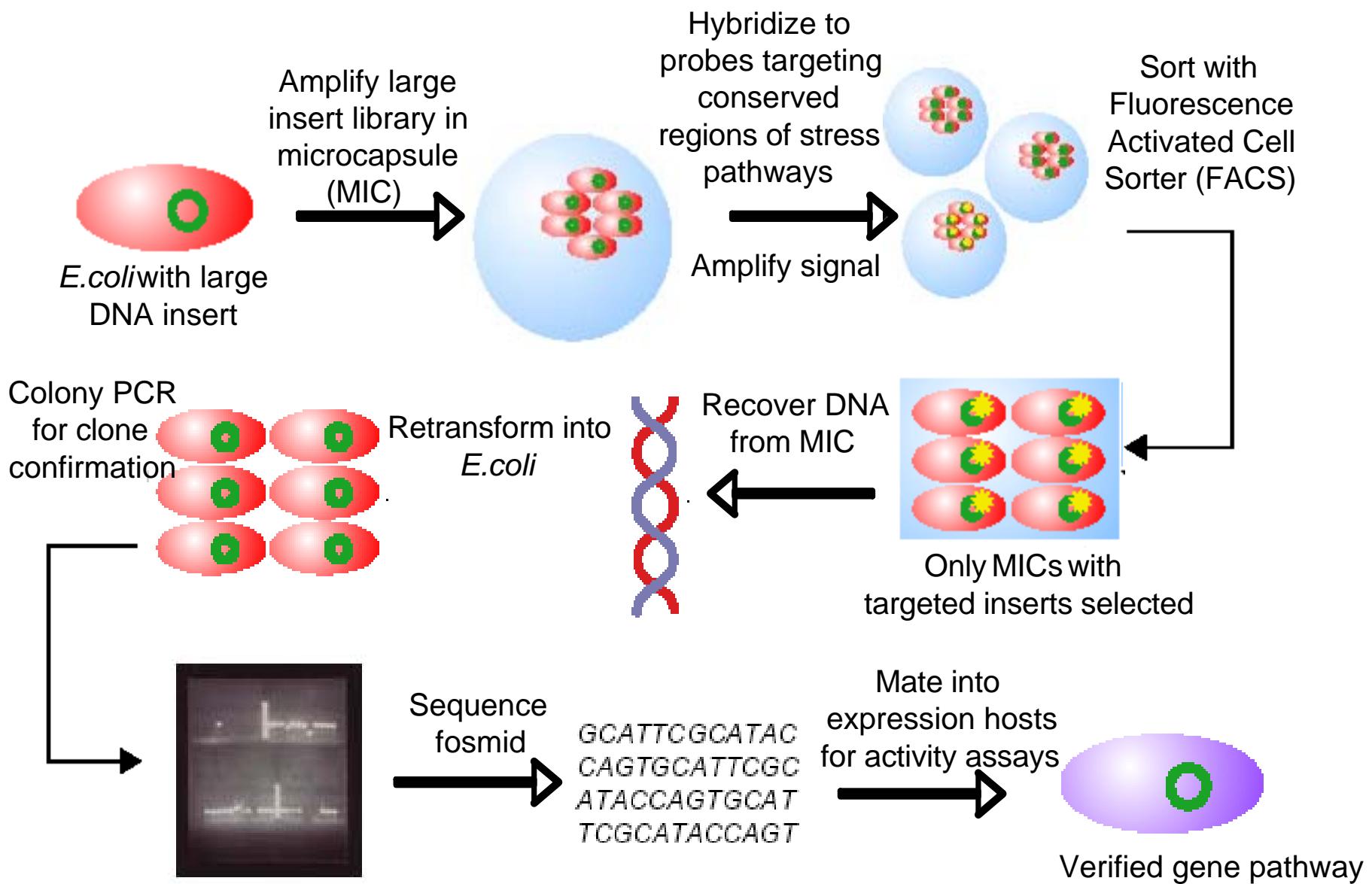
Predominant field-site organism, *Anaeromyxobacter*, submitted for genome sequencing

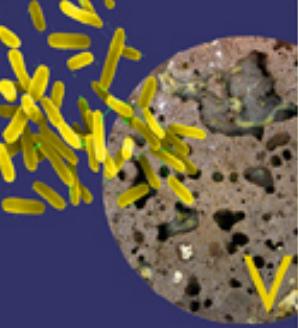
Compare communities between enrichments and groundwater samples across geochemical gradient

Diversa Technology Flow for VIMSS

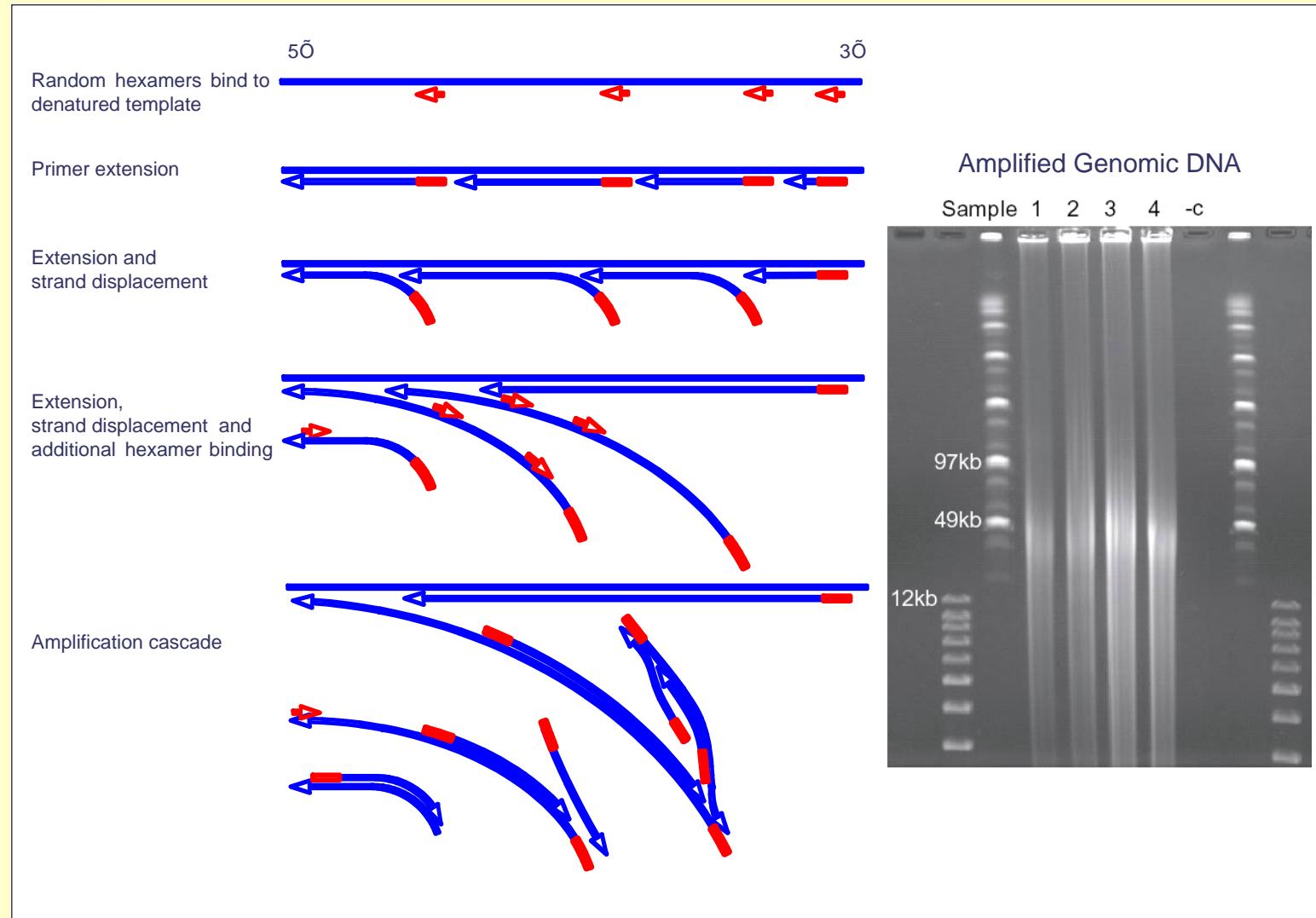


High Throughput Technology for Enriching Low-Copy Number Pathways





Multiple Displacement Amplification



Large Fragment Cloning

Amplified DNA



Partially digest



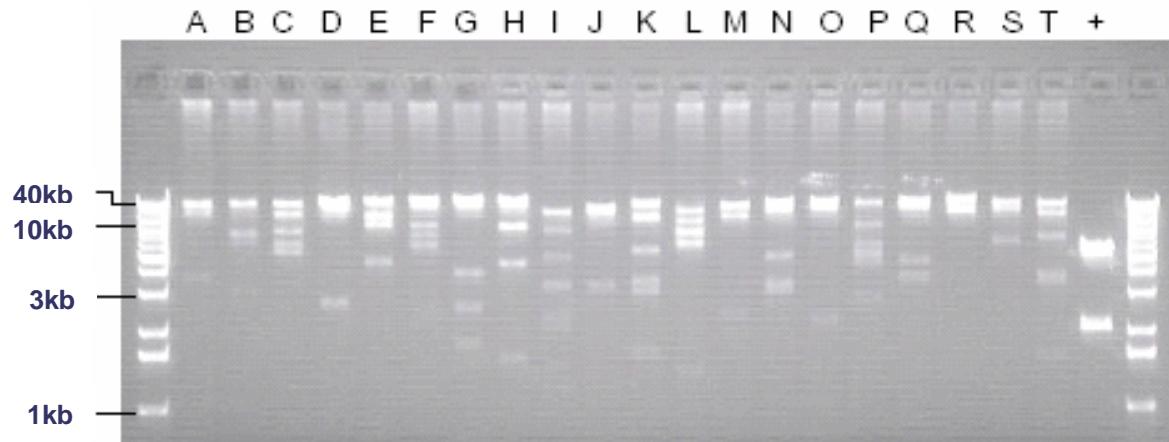
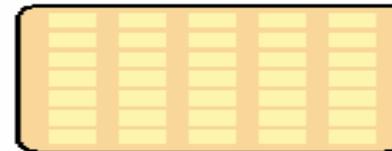
Ligate to
fosmid arms



Package into phage λ
particles and
transfect *E.coli*



Array clones on an
agarose plate





Summary of Soil Samples Evaluated

Sample ID	Sample Name	Core Segment	Core Depth	Comments	Small Insert DNA Conc. Pre-Ampli	Small Insert DNA Conc. Post-Ampli	Small Insert Library	Large Insert Library
Background								
FB609-01-00	B-Top	24-43" long	180-234"	Background,	5ng/ μ l	278ng/ μ l	Yes	Yes
	B-Bottom			Anaerobic	4ng/ μ l	209ng/ μ l	Yes	Yes
Area 1								
FB060-01-27	1-Top	27-46" long	180-228 "	<25ppm U238	5ng/ μ l	140ng/ μ l	Yes	Yes
	1-Bottom			Anaerobic	5 ng/ μ l	206ng/ μ l	Yes	
FB060-01-00	1aL	0-25" long	180-228"		~	~	Yes	Yes
Area 2								
FB052-01-12	2-Top	12-36" long	245-300"	<200ppm U238	5ng/ μ l	246ng/ μ l	Yes	
	2-Bottom			Anaerobic	5 ng/ μ l	212ng/ μ l	Yes	
FB052-01-00	2aL	12-36" long	245-300"		~	~	Yes	Yes
	2bL				~	~	Yes	Yes
Area 3								
FB056-01-34	3-Top	34-58" long	144-198"	<25ppm U238	2ng/ μ l	179ng/ μ l	Yes	Yes
	3-Bottom			Anaerobic	2ng/ μ l	263ng/ μ l	Yes	Yes
	3aL				~	~	Yes	Yes
	3bL				~	~	Yes	Yes

Notes: Pre-Ampli = DNA conc., pre-amplification with DNA polymerase
Post-Ampli = DNA conc., post-amplification with DNA polymerase

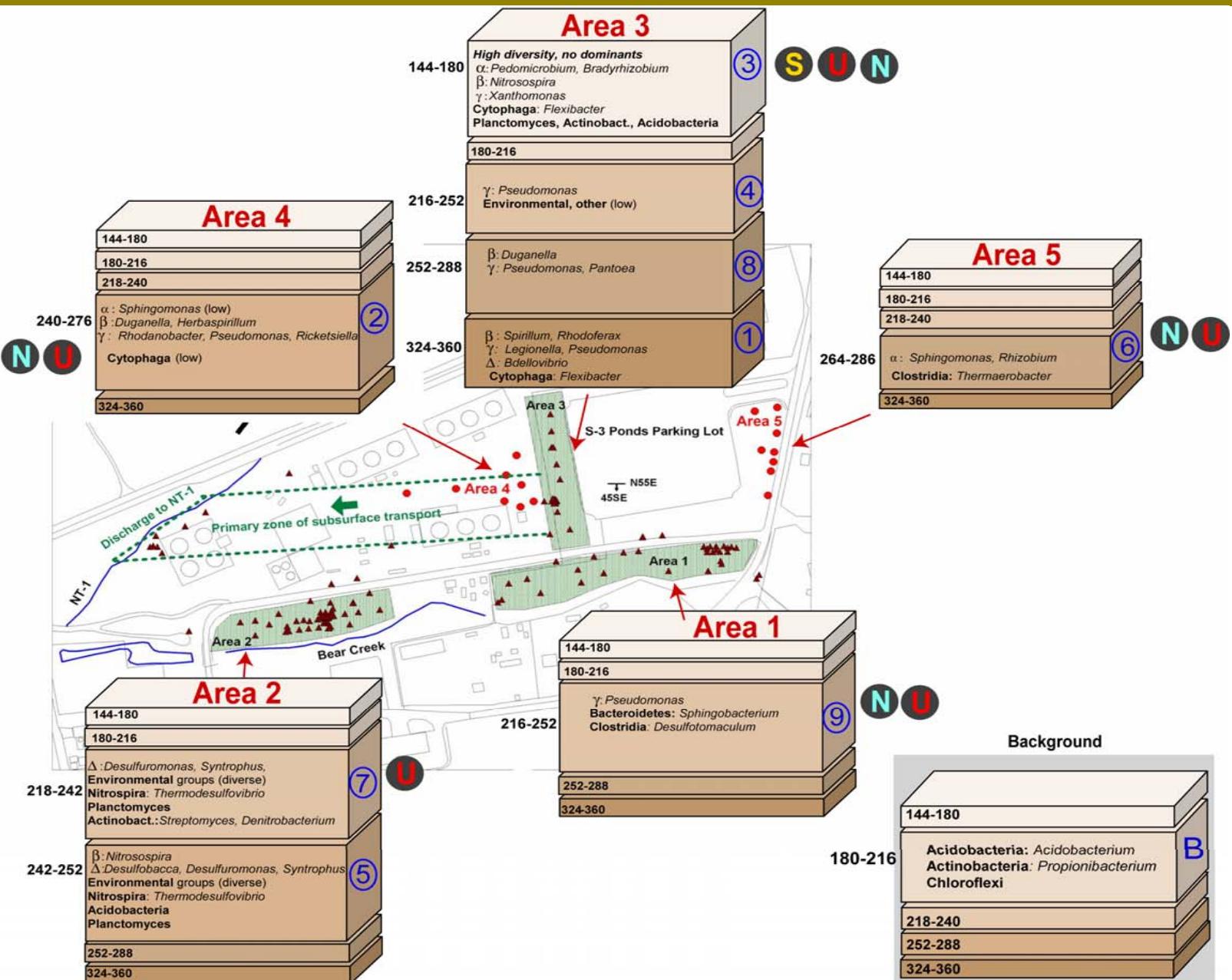
Library 3868, Sample 1, Area 3, FB-075-04-07; Depth: 324-360 inches

Total Clone-ends Sequenced	1270	
Identical Clones	52	4.1%
Clones with no Blast hits	155	12.2%

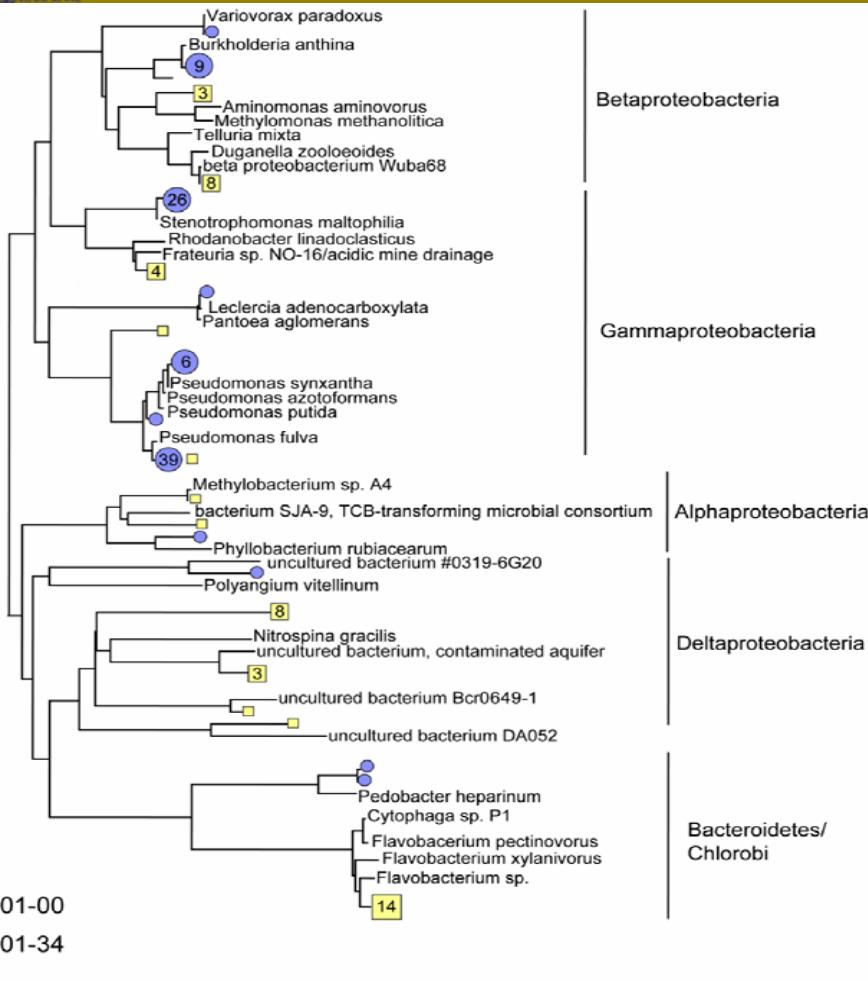
Library 3875, Sample 3, Area 3, FB-075-04-07; Depth: 144-180 inches

Total Clone-ends Sequenced	1119	
Identical Clones	8	0.7%
Clones with no Blast hits	206	16%

Microbial Diversity at the NABIR FRC Sampling Sites



Sequence Analysis



Sample of blast hits from clones in a library constructed from amplified gDNA from sample #FB052.

Value	Functional category based on blast hits
	<u>Transporters and small molecule binding</u>
1.00E-33	lead, cadmium, zinc and mercury transporting ATPase
4.00E-39	Permeases of the drug/metabolite transporter (DMT) superfamily
2.00E-15	ABC-type multidrug transport system, ATPase subunit
3.00E-18	extracellular solute-binding protein
5.00E-50	putative cation-diffusion-facilitator; metal tolerance, zinc, cadmium
8.00E-99	cobalt/cadmium/zinc transporter
	<u>Toxicity response</u>
5.00E-28	Radical activating enzymes
2.00E-33	Organic radical activating enzymes
	<u>Intermediate central metabolism</u>
2.00E-47	glucose-1-phosphate thymidylyltransferase
2.00E-33	Glycine/D-amino acid oxidases (deaminating)
5.00E-27	Glycosyltransferase
6.00E-67	NAD synthase
5.00E-15	acyl-CoA dehydrogenase
	<u>DNA, RNA and protein synthesis</u>
1.00E-15	DNA repair protein RAD51
2.00E-45	ATPases involved in chromosome partitioning
1.00E-34	DNA-directed RNA polymerase beta subunit/160 kD subunit

Diversity analysis of genomic DNA from soil samples #FB052 and #FB056 based on rRNA genes. The neighbor-joining phylogenetic tree was generated using genetic distances calculated with DNADIST in the PHYLIP package.

Representative related database sequences were included for reference. Clusters of sequences with higher than 95% identity were represented by symbols indicating the number of those sequences.

Histidine Kinase Sequence-Based Screen

DNA Probe Generation

Degenerate PCR primers designed from conserved regions

Sense Primer

5'-GGCSCAYGARATSAACAAACCC-3'

1		50
(1)	-----QTEKMMMSVGG ^G LAAG ^A MAHE ^M NNPLGAILLG ^G QNIQRRLD ^P AL	
(1)	-----QTEKMLSVGG ^G LAAG ^A MAHE ^M NNPLG ^G GILQGAQNIMRRI ^D PTL	
(1)	-----QSEKMLSIIG ^G GLAAG ^A MAHE ^M NNPLGAILQGAQN ^I QRRLD ^P AL	
(1)	-----QSEKMSVSG ^G ALAAG ^A MAHE ^M NNPLGAILQGVQNLR ^R RLTADL	
(1)	-----QTEKMHSVGG ^G VAAG ^A MAHE ^M NNPLS ^A IAQ ^A QN ^I ERRRLATDL	
(1)	-----VVQNEKMLSIIG ^G GLAAG ^A MAHE ^M NNPLAGVLQGVQNVR ^R RFSP ^L	
(1)	-----QTEKMLSLIG ^G GLAAG ^A MAHE ^M NNPLAVILQG ^G DTVQRRLAPGL	
(1)	-----QTEKMISVGGIAAG ^A IAHE ^M NNPLG ^G IIQLQAAHNL ^A QRT ^R PDF	
(1)	-----QTEKLATIGQLAS ^G VAHE ^M NNPLGGVILCFRN ^I LT ^E GGMDEA	
(1)	-----QSERMATVGKLS ^G AGLAHE ^M NNPLGVILCY ^A ELL ^R -QGATAE	
(1)	-----QKEKLASVGR ^G LAAG ^A LAHE ^M NNPLGVIMCY ^A ELL ^R QTQVGDSQ	
(1)	-----QSQKME ^I GKLAGG ^G VAHE ^M INTPLG ^I LY ^A QLLQDDV ^P PQGQ	
(1)	-----MGQLATIGELAAG ^A VAHE ^M INSPANAIINC ^A DLIMEDAFDSA	
(1)	-----KLA ^S MGR ^M RAE ^G VAHE ^M NNPV ^N U ^I I ^A NAAGWIED ^L LEEPD	
(1)	-----EAGKLGALGEM ^M AA ^G IAHE ^M NNPV ^N AIMMEE ^E AGWIED ^L ILADLG	
(1)	-----QKLSSIGRLAAG ^A VAHE ^M NNPLAIINEKAGLAADLIALSQ	
(1)	-----QSSKMAALGKLAAG ^G VAHEVN ^N PLMLIRENAGWIKDLLWEED	
(1)	-----QAGKMATL ^G EMATGV ^M VAHELNQPLTVIKAASGFLLRKARKGE	
(1)	-----QAAKMKSLGEMS ^A GV ^M VAHELNQPLN ^A IRMGSDYLN ^M ALEQGL	
(1)	-----QKEFQAQKFHQAEKMATV ^G MLAAG ^A VAHEVN ^N PLTAINGFAEGILR ^R V ^K RLR	

201		250
(175)	PRVT ^T LRTRREGDMAR ^I D ^V E ^D NGPGMTPDIRKRV ^F EP ^F FTTKDVGVGT ^G LG	
(175)	PRITV ^T RG ^M ALEGDR ^I R ^L D ^V A ^D NGPGMVEDV ^R RRVFEP ^F YT ^T KNV ^G EGT ^G LG	
(176)	PELHV ^T TRVL ^M PSEVR ^V D ^V VID ^D NGPG ^I SPAV ^R KRIFEP ^F FT ^T R ^D AG ^K GVGMG	
(174)	PRI ^T ITTRSEENGIR ^I LEIAD ^D NGPGM ^T AEQ ^R RRAFEP ^F FT ^T REVGMGT ^G LG	
(179)	IHI ^A VT ^T EE ^S YDGL ^R LAIS ^D NGPGM ^H HEAL ^R RRVFEP ^F FT ^T KPPGKGT ^G LG	
(175)	PTITLRSRR ^T EKG ^V R ^I EVE ^D NGPGM ^S EDV ^R RKV ^F EP ^F FT ^T KAP ^G KGT ^G LG	
(175)	PTIHLQ ^T RCVG ^R LLIE ^I RT ^D NGPGV ^H PHEL ^A R ^F IFEP ^F FT ^T KPPGMGT ^G LG	
(175)	PAIHLRV ^R R ^R Q ^D E ^T V ^V ID ^D Q ^D NGPG ^I IP ^V ALQ ^F R ^I FEP ^F YT ^T KPPGVGT ^G LG	
(141)	--LTITARRAGDDV ^R IEVQ ^D T ^G SGV ^A EE ^H R ^E R ^I FDP ^F FT ^T KPAG ^S GT ^G LG	
(139)	-LIRIRLRLSHGLNVR ^I D ^V E ^D NG ^G L ^V ETDALQ ^R VFD ^P F ^T TKETG--T ^G LG	
(140)	-TIA ^I ST ^C G ^K GYGV ^T V ^T I ^R D ^D NGPG ^I PEAH ^R DR ^I FDP ^F FT ^T KETGRGT ^G LG	
(142)	--IRTRLDIAHM ^T V ^T A ^E FADT ^G T ^G V ^A AENLRK ^I FDP ^F FT ^T KPV ^G KGT ^G LG	
(152)	EPR ^A AG ^S CVSGPVVS ^S FRDT ^G GG ^I DPS ^I FR ^D RV ^M EP ^F FT ^T KGEGRGT ^G LG	
(152)	-RIMHGQ ^D EEDH ^A LHWAITDT ^G HG ^I AP ^A LLP ^R VFD ^P F ^T TSRVGRGMGLG	
(154)	-GVAAGN----MV ^F V ^T V ^A D ^T NGPG ^I PEA ^I LQ ^R IYD ^P F ^F TT ^T KPV ^G KGT ^G LG	
(150)	-FAADG----GMVG ^V SIQ ^D NGKG ^M SEEVQ ^R HIFE ^F PF ^F TT ^T KKT-A ^G T ^G LG	
(154)	-GAAPDR---SE ^V F ^V S ^V SDT ^G EG ^I P ^P ELLS ^R I ^F D ^P F ^T TKKV ^G EGT ^G LG	
(152)	-VITLRTETDG ^R EV ^V MEVC ^D S ^G PG ^I P ^R ALQ ^R I ^F E ^P FT ^T KKV ^G GT ^G LG	
(153)	-RITITTSHEGDT ^T V ^T LSVK ^D NGCG ^I P ^A T ^L RE ^Q V ^F QP ^F FT ^T KATGQGMGLG	
(180)	AVITV ^A P ^F R ^D ERHAG ^F TV ^R DT ^G SG ^I P ^Q E ^H LHS ^I FE ^P FT ^T KKV ^G GT ^G IGIG	

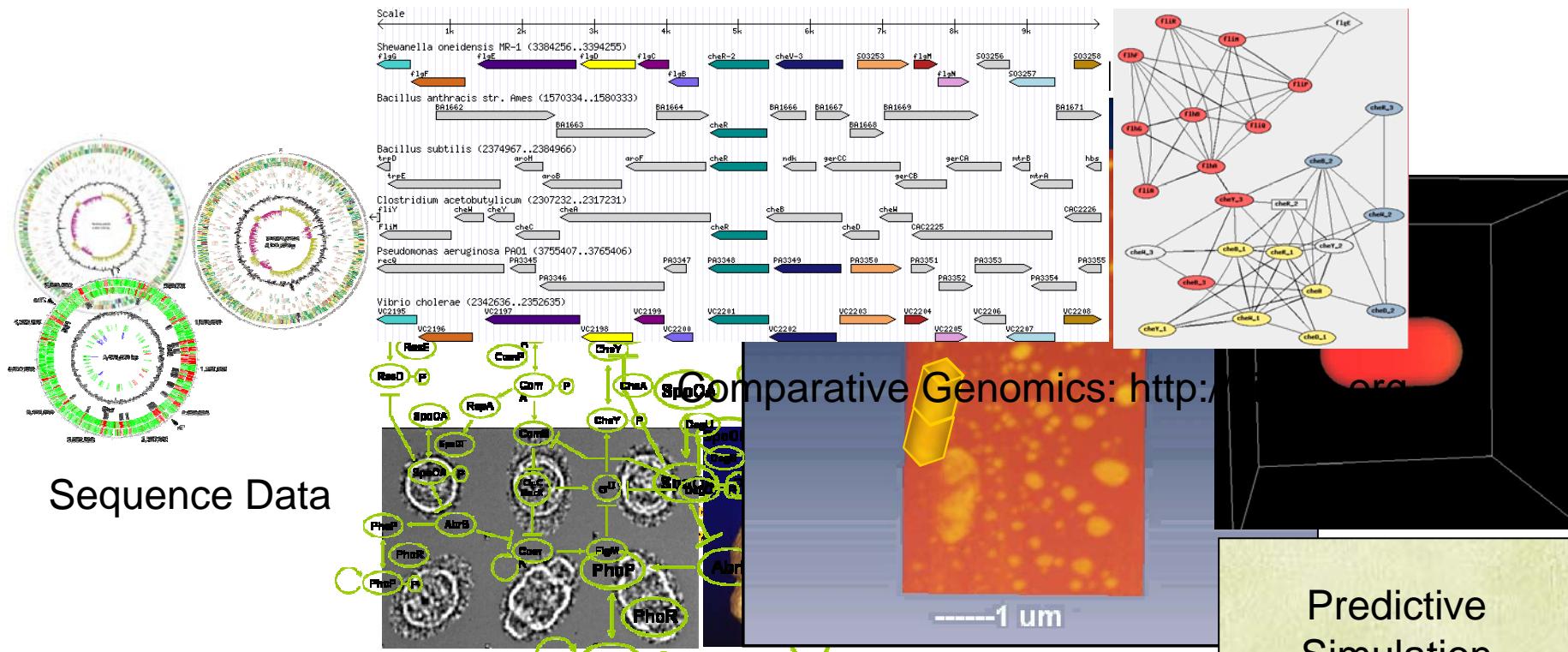
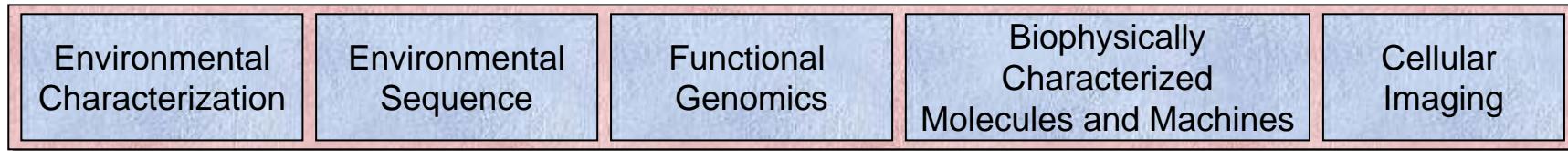
Antisense Primer

5'-GGTSGTGAAGAACGGYTCGAA-3'

A subfamily from *D. vulgaris* of Histidine Kinases

Bioinformatics Critical Path

Centralized, Cross-Referenced Databases



Molecular Machines

Deduction of pathways

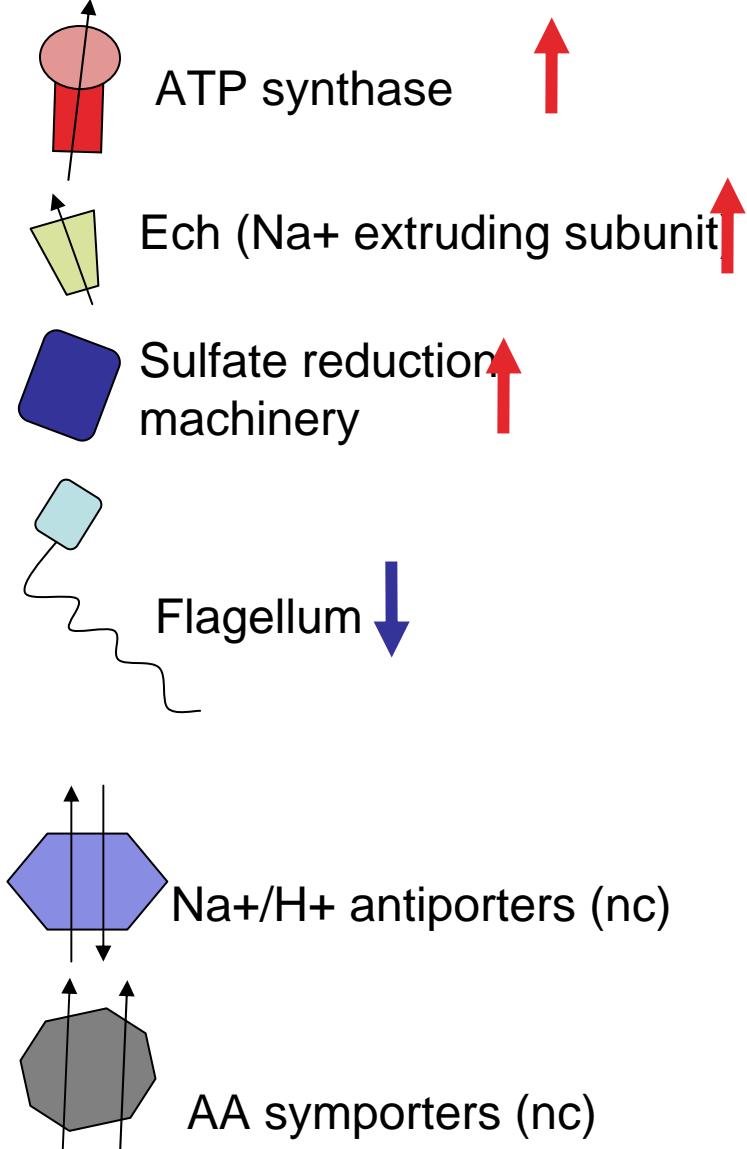
Cell Imaging

modules and dynamics

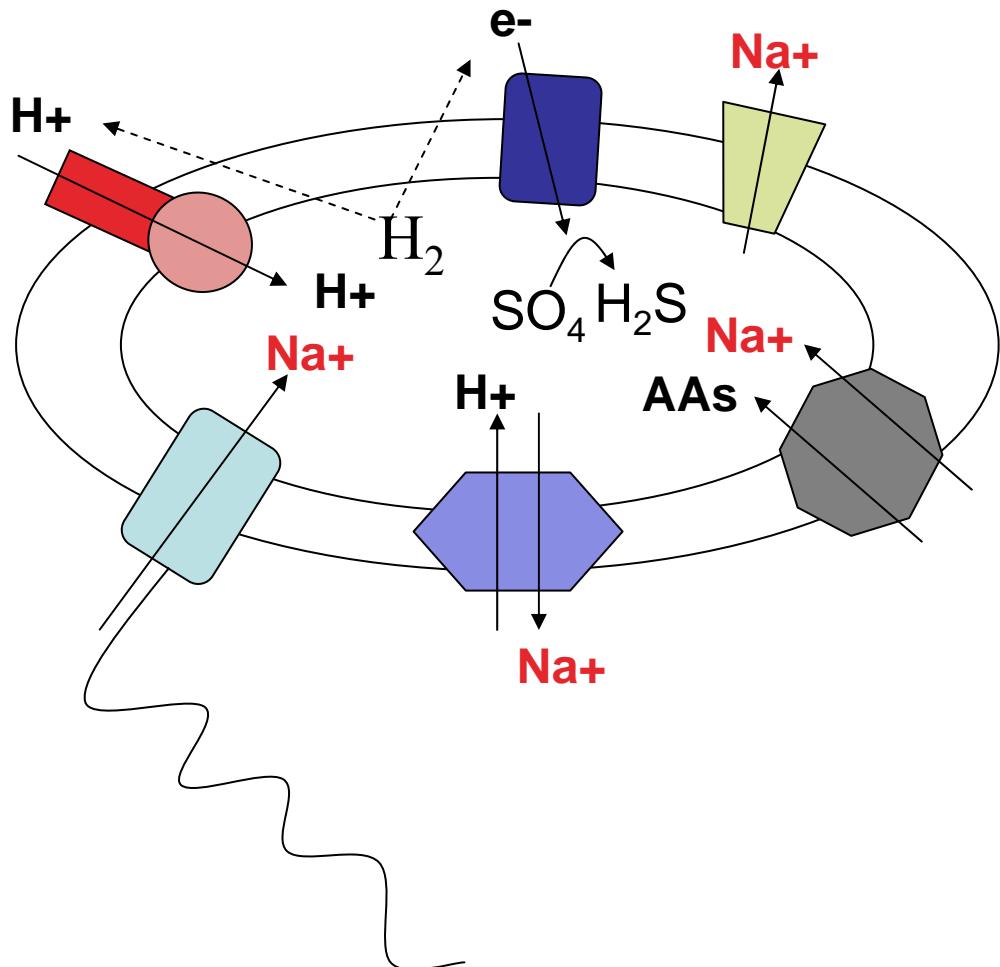
Pipeline Stressors

- NaCl, KCl - complete, paper submitted this week
- NO₂ - complete, paper submitted
- NO₃ - complete, paper in preparation
- pH - complete, paper in preparation
- Heat shock - complete, paper submitted
- O₂ - complete, main paper in preparation, FTIR paper submitted
- Contaminated Site Stress Simulation - in progress, FRC groundwater, e.g. high nitrate
- Cr - in progress
- U - in progress
- Knockout mutations - Fur & Zur stress pipelines in progress

Stress Response Pathway Pipeline NaCl example

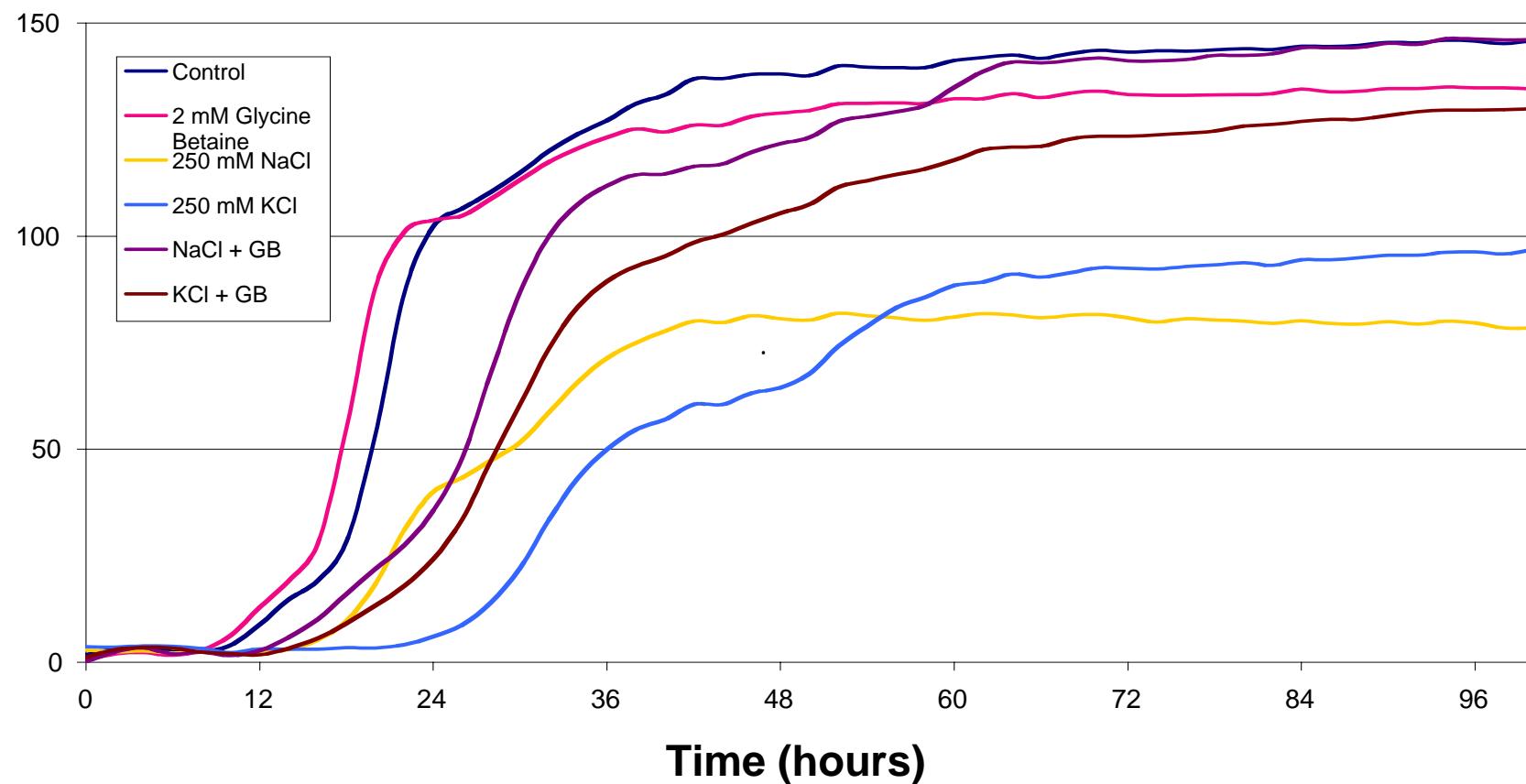


Overview of transcriptomics



Osmoprotection

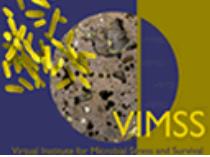
D. vulgaris : Osmotic Stress and Growth with Glycine Betaine



NOTE: mean concentration ($n=6$) simultaneous runs using same cell preparation, sampling at 2 h interval, LS4D medium, DvH-ATCC:29579_3128 (File: DvH-GBppt2-030605.xls)

Supportive Projects

- ***Microbes online*** - Comparative Genomics Database, Genome Browser, Operon Browser, Regulon Browser, Pathways - <http://www.microbesonline.org>
- ***DvH mutations*** - >50 knockouts and tagged
- ***Sequencing***
 - *D. vulgaris* DP4 - phageless strain, in progress
 - *D. vulgaris* plasmidless strain, in preparation
 - need more Dv strains from DOE sites
- ***Stress expression and SRB roles*** - analysis of FRC and Hanford using enrichments, FGA, phylochip, metagenome analyses, clone libraries
- ***Community Analysis (Phylochip)***
 - Criddle DNA in comparison to FGA
 - Kostka DNA in comparison to enrichments, TRFLP etc.



Acknowledgments



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Pacific Northwest National Laboratory
...delivering breakthrough science and technology

Phil Long, et al.



Steve Koenigsberg, Ana Willet



Paul Richardson, Phil Hugenholtz



Judy Wall, et. al.



Mathew Fields, et. al.



Stephen Sutton, Matthew Newville



Sandia National Laboratories

Anup Singh, et. al.



David Stahl, et. al.

D I V E R S A Martin Keller, et. al.



Indiana University Lisa Pratt, et. al.

Princeton T. C. Onstott, et. al.



Contacts



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Center for Environmental Biotechnology

<http://www-esd.lbl.gov/CEB>

Virtual Institute for Microbial Stress and Survival

<http://vimss.lbl.gov>

<http://www.microbesonline.org> - Comparative Genomics Database, Genome Browser, Operon Browser, Regulon Browser

Environmental Remediation Technology Program

<http://www-esd.lbl.gov/ERT>

Ecology Department

<http://www-esd.lbl.gov/ECO>

DOE Natural and Accelerated Bioremediation Research Program

<http://www.lbl.gov/NABIR>

DOE Genomics:GTL Program

<http://doegenomestolife.org>

NASA Indiana Princeton Tennessee Astrobiology Initiative (IPTAI)

<http://www.indiana.edu/%7Edeeplife/homepg.html>