



Metagenomic Analysis of Microbial Communities in Uranium- Contaminated Groundwaters

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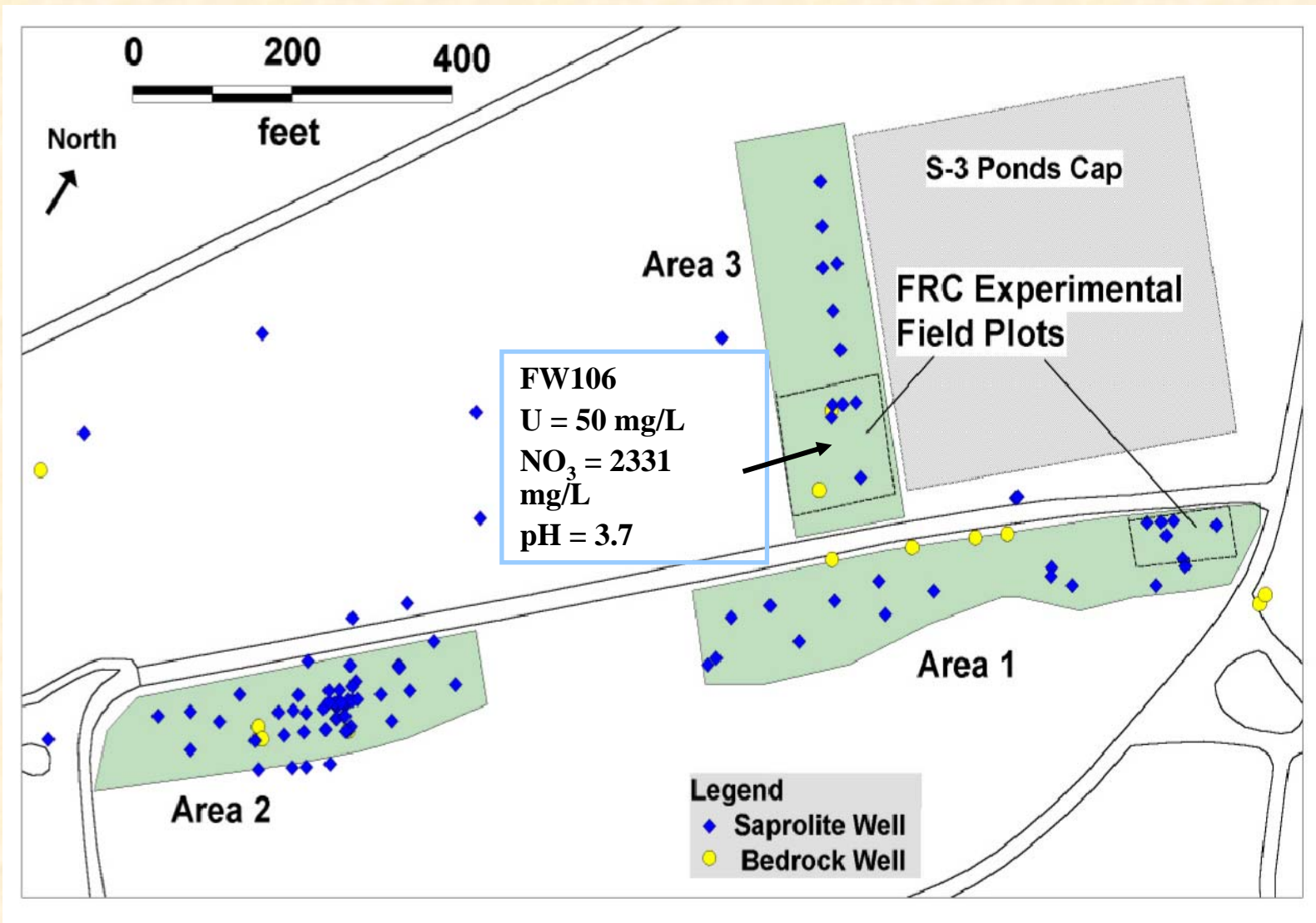


Community Genomics

Grand challenges

- Extremely high diversity, 5000 species/g soil
- 99% of the microbial species are uncultured

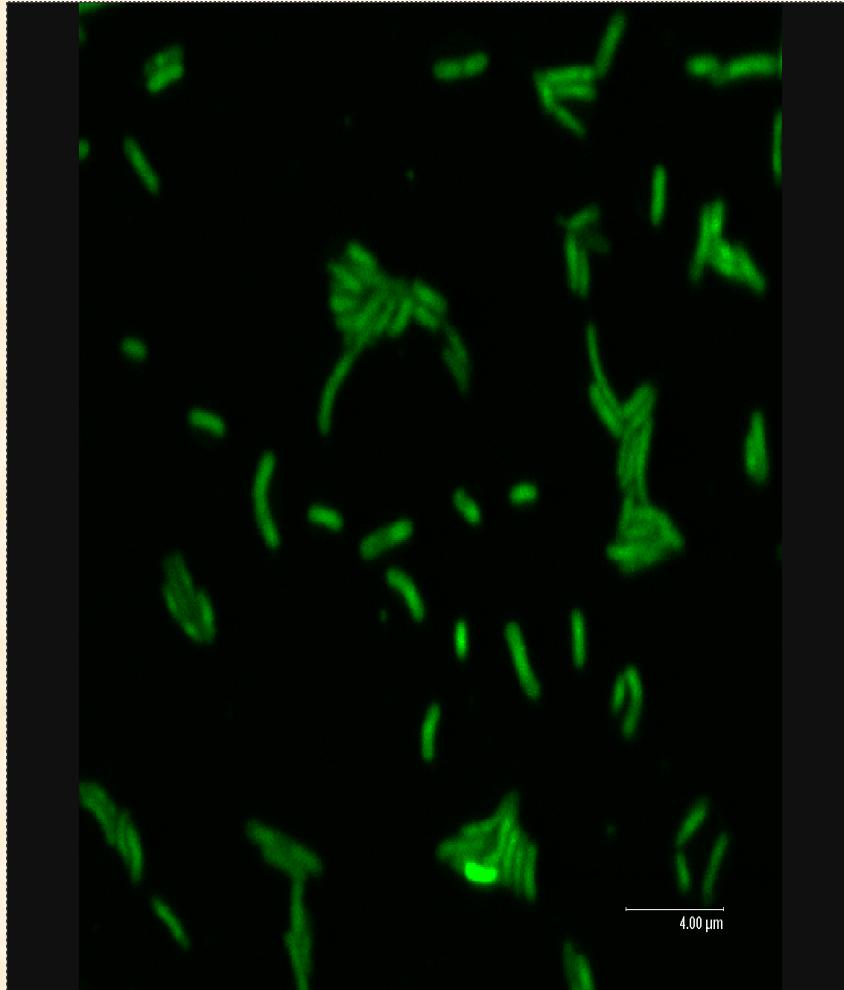
Field Research Center



FW106 Groundwater Geochemistry

- **pH - 3.7**
- **Uranium - 51 mg/L (soil ~500 mg/kg): with highest U contamination in the world**
- **Nitrate – 2,331 mg/L**
- **Sulfate – 1,997 mg/L**
- **TOC - 244 mg/L (ppb to ppm, acetone, PCE, butanol, benzoic acids)**
- **TIC - 284 mg/L**
- **Several metals and organics - μg to mg/L**

FW106 Bacterial Cells



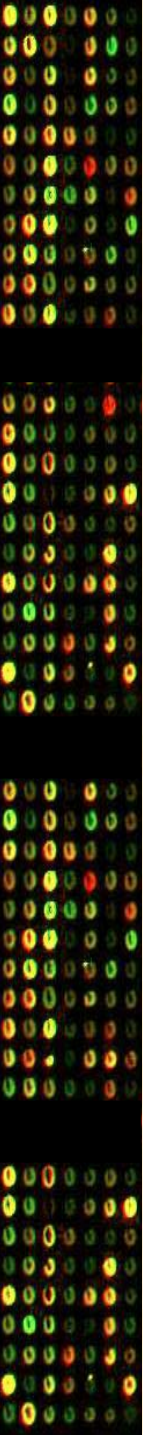
- 10^4 to 10^5 cells/ml
- Uniform morphology
- Filtered biomass from ~**1,700 L** for sequencing, Took about 6 months to optimize the protocols.
- 300 ug DNA was isolated.
- Sent DNA twice to JGI for library construction.
- **3 Libraries**
 - **40 kb fosmid**
 - **8 kb**
 - **3 kb**

Metagenome Statistics

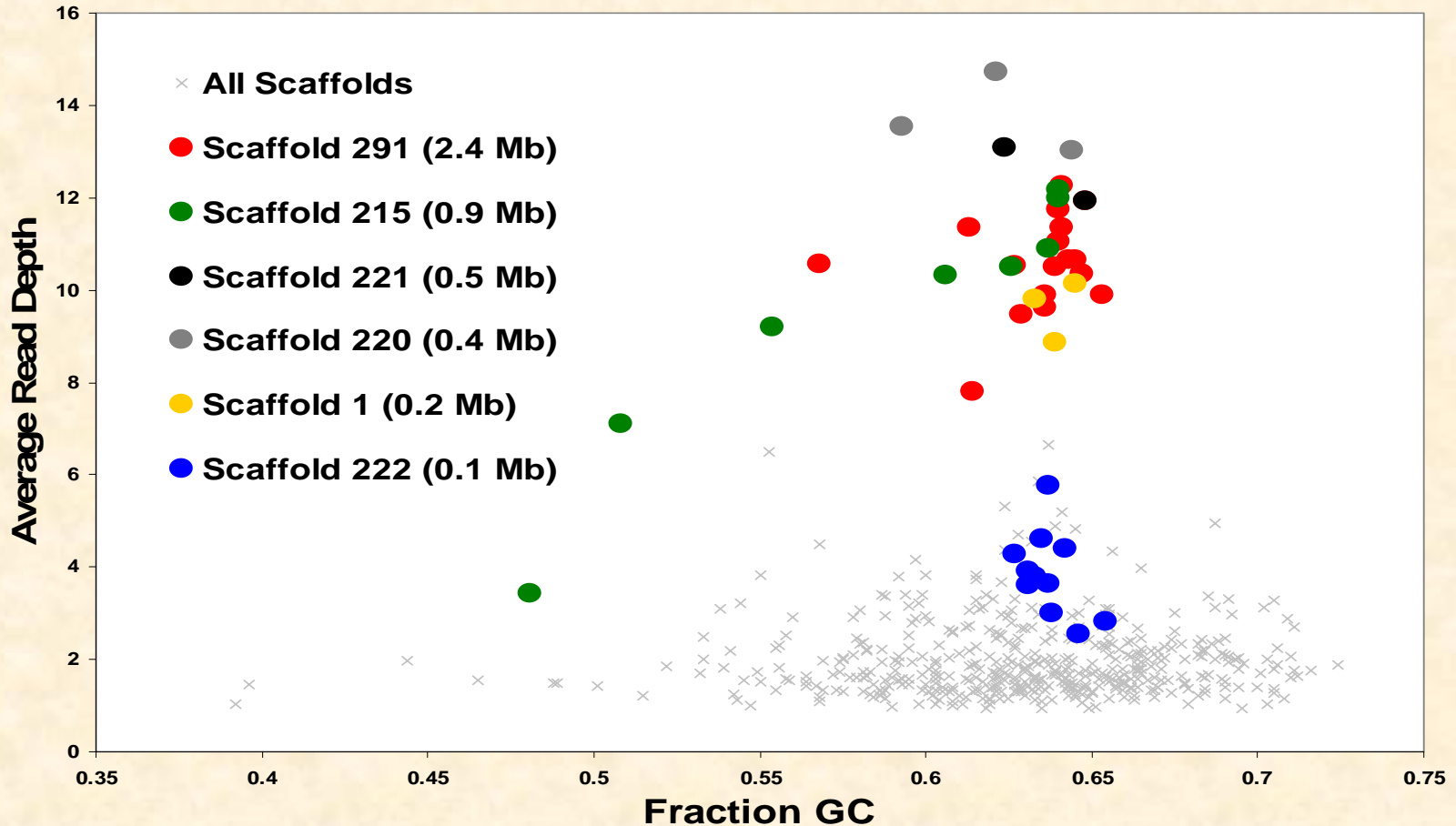
- **~52.2** Mb raw sequence data
 - ~20 Mb small-insert PUC library
 - ~23 Mb 6-8 kb pMCI library
 - ~9 Mb large-insert pCC1Fos library
- **~8.1** Mb contigs
 - 2586 total contigs (94 bp-1.24 Mb)
 - 75 major contigs
 - **224** scaffolds w/ at least 2 contigs
 - Largest Contig ~1.2 Mb
 - Largest Scaffold ~2.4 Mb

Some key questions

- **How many species are present in this community?**
- **What types of metabolic capacity does the community have?**
- **Whether do these populations respond to biostimulation?**

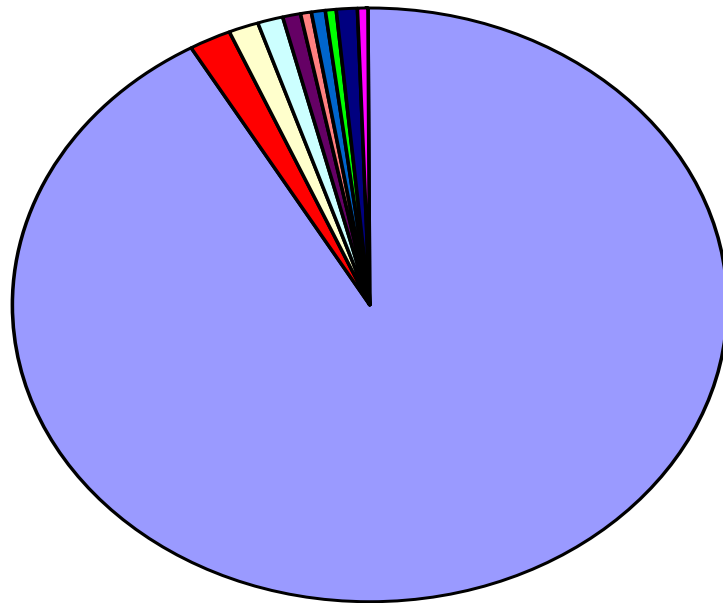
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- **How many species?**
 - **GC content**
 - **16S analysis**
 - **Metabolic gene analysis**

GC content of the sequences



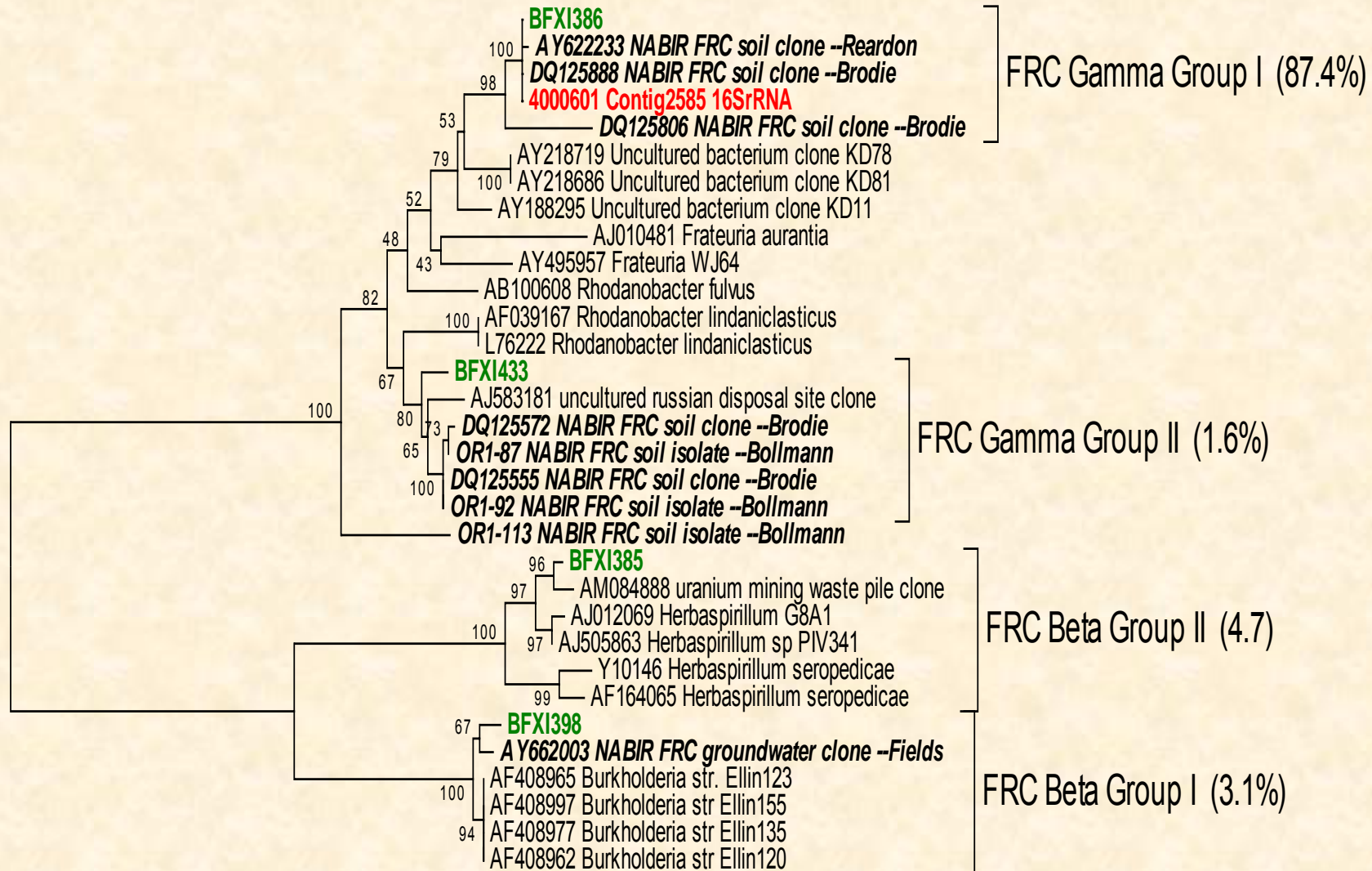
- Two types of sequences

Abundance of different OTUs



- 90% OTUs from one Frateuria group, like a pure culture.
- The populations are extremely unevenly distributed

16 phylogenetic tree



0.02

Estimated Genome Coverage

- FRC Gamma Group I – 11X
- FRC Gamma Group II – 0.2X
- FRC Beta Group I (*Burkholderia*) – 0.2X
- FRC Beta Group II (*Herbaspirillum*) – 0.4X

Estimated 2.1 Gb sequence data required for
8X coverage of all known phylotypes

Genome size estimates based on *Xanthomonas* (4.2 Mb), *Herbaspirillum* (6.8 Mb) and *Burkholderia* sp. (7.0 Mb)



Metabolic capacity of this community

FW106 Energy Metabolism Genes

- 30 Cytochromes
 - 23 c-type
- 3 NiFe Hydrogenase Operons
- 2 Nitrate Reductase
- 2 Formate Dehydrogenase
- Multiple Cytochrome Oxidase Complexes
- 2 Lactate Dehydrogenase
- 2 Complete Complements of TCA Cycle Genes
- Hexose Transporters
 - 2 Glucose/Galactose Transporter
 - 2 ABC-Type Xylose Permease
 - Multiple PTS System Components



FW106 Nitrogen Metabolism Genes

- 1 Assimilatory Nitrate Reductase (*nasA*)
- 1 Respiratory Nitrate Reductase (*narGH*)
- 1 Nitrite Reductase Complex
- 1 Nitrate/Nitrite Two-Component Sensor (*narX*)
- ABC-Type Nitrate Transport System

FW106 Stress Response Genes

- 3 RecA
- 2 CRP/FNR Family
- Sigma Factors
 - 1 σ^{70} (*rpoD*)
 - 1 σ^{54} (*rpoN*)
 - 2 σ^{38} (*rpoS*)
 - 3 σ^{32} (*rpoH*)
 - 2 σ^E (*rpoE*)
 - 1 σ^{28} (*FliA*)
 - 2 σ^{70}/σ^{32}
- 30+ Sensor Histidine Kinase
- 40+ Cation/Multidrug Efflux Pump and General Multidrug Resistance
- 7 Na⁺/H⁺ Antiporters
- 9 Heat Shock Response

FW106 Metal Resistance Genes

- Arsenate Reductase Operon
- Mercuric Reductase Operons
- Copper Resistance
- Cobalt-Zinc-Cadmium Resistance
- Chromate Resistance Genes
- Misc. Heavy Metal Resistance Genes



Responses to biostimulation

Probes Designed for a Second Generation FGA

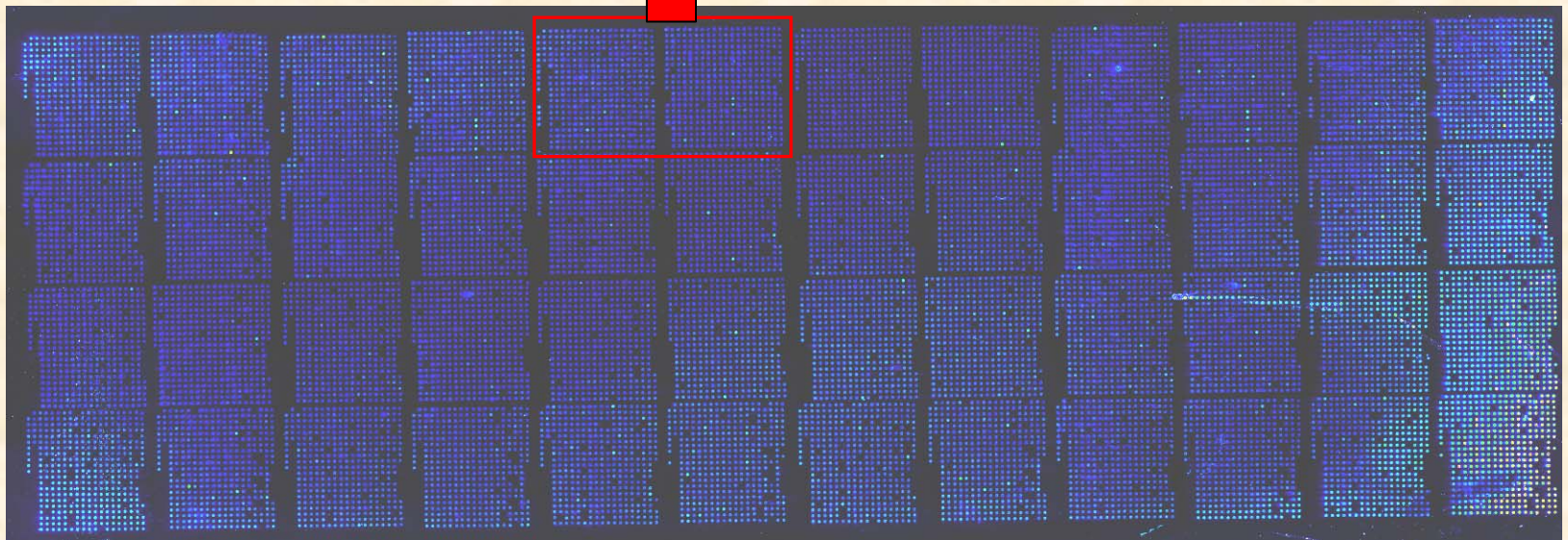
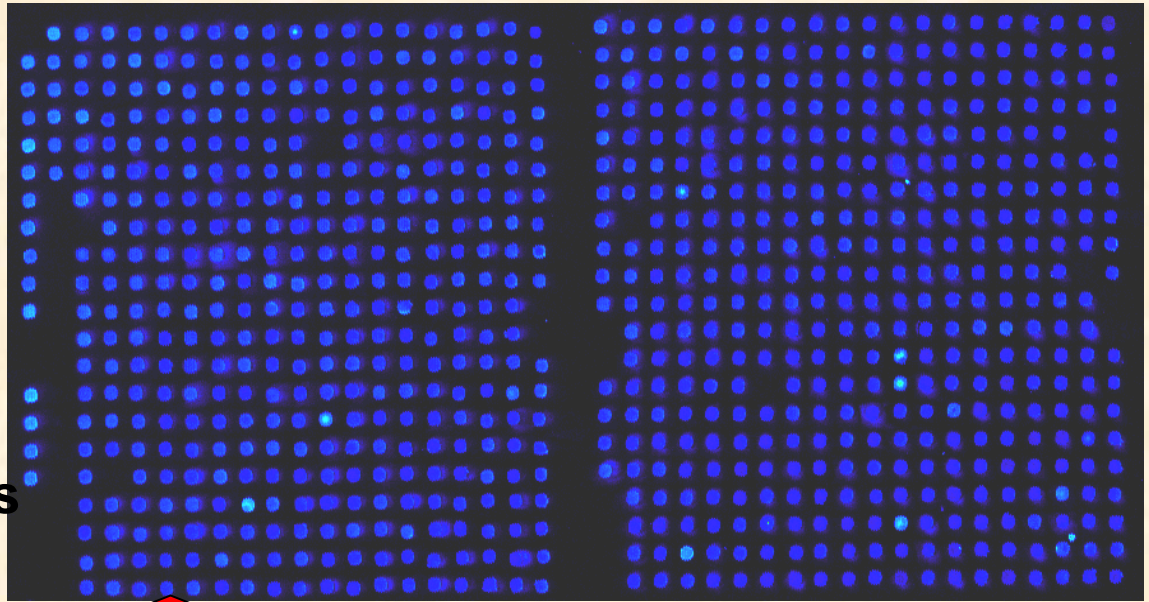
- Nitrogen cycling: **5089**
- Carbon cycling: **9198**
- Sulfate reduction: **1006**
- Phosphorus utilization: **438**
- Organic contaminant degradation: **5359**
- Metal resistance and oxidation: **2303**
- **300 probes from community sequences**

Total: **23,408 genes**

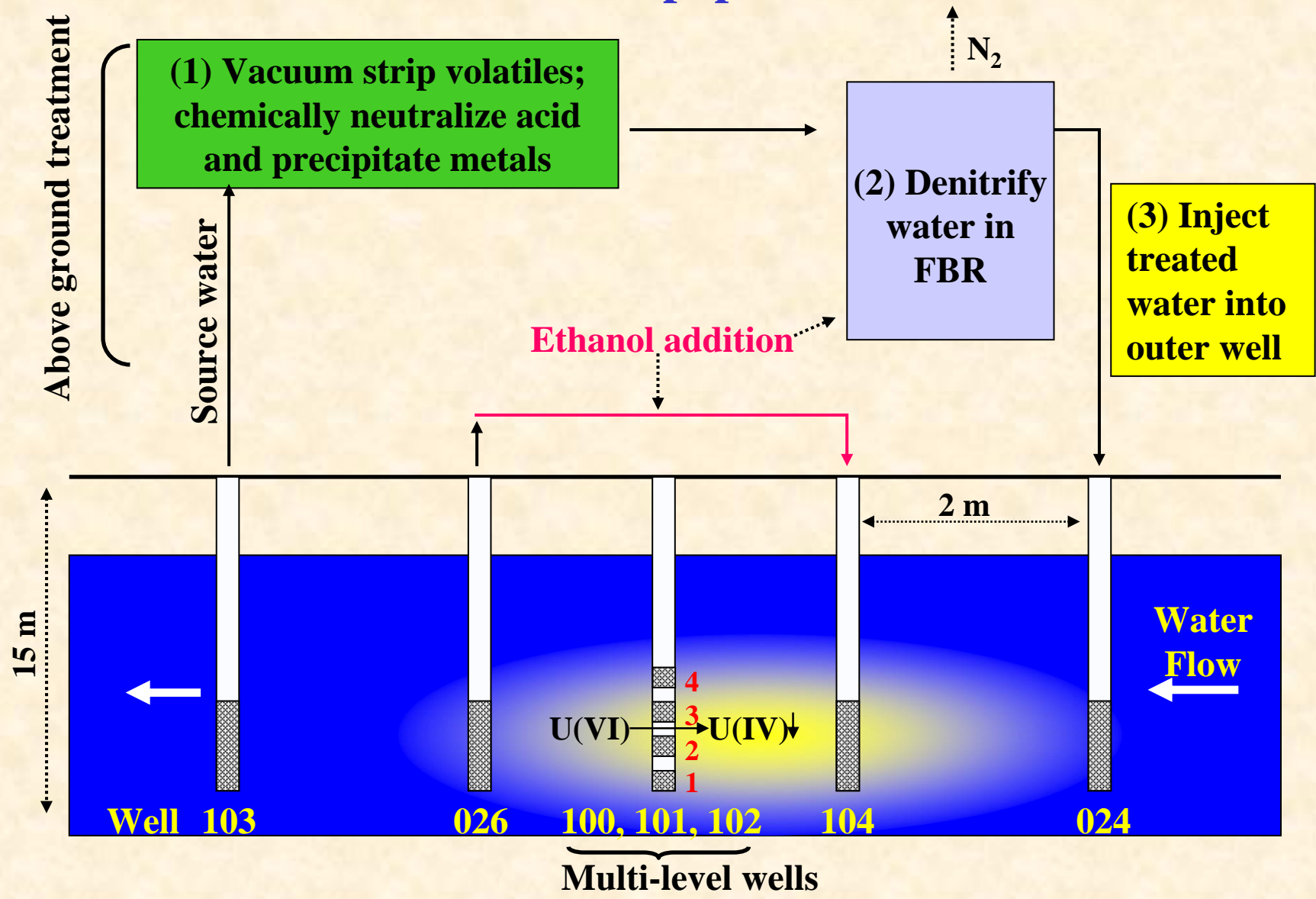
- **23,000 probes designed**
- Will be very useful for community and ecological studies

**Version A contains
~9000 experimental
probes in duplicate
on each slide**

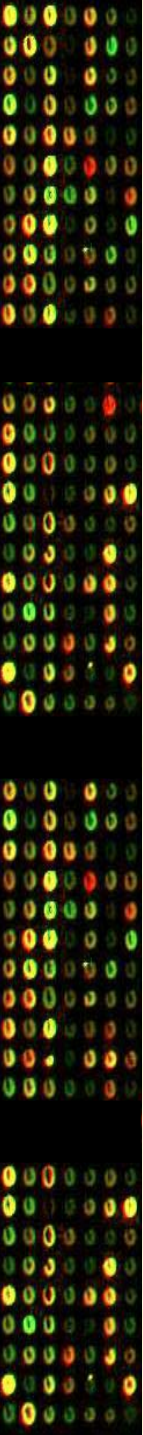
**All Probe Version
contains ~24500
experimental probes**



Biostimulation of microbial populations for Ur removal

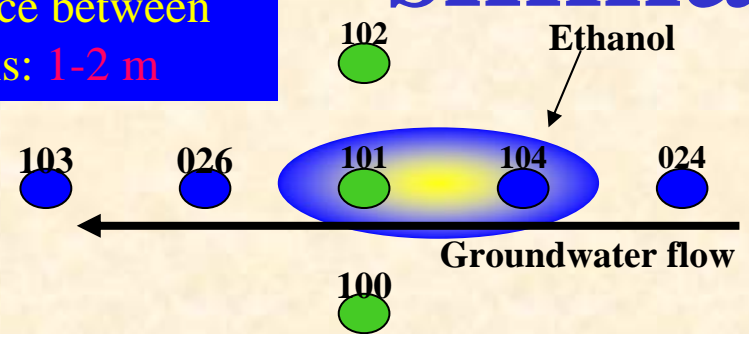


- Above ground denitrification and neutralization of groundwater
- *in situ* biostimulation with ethanol and reduction of U(VI)

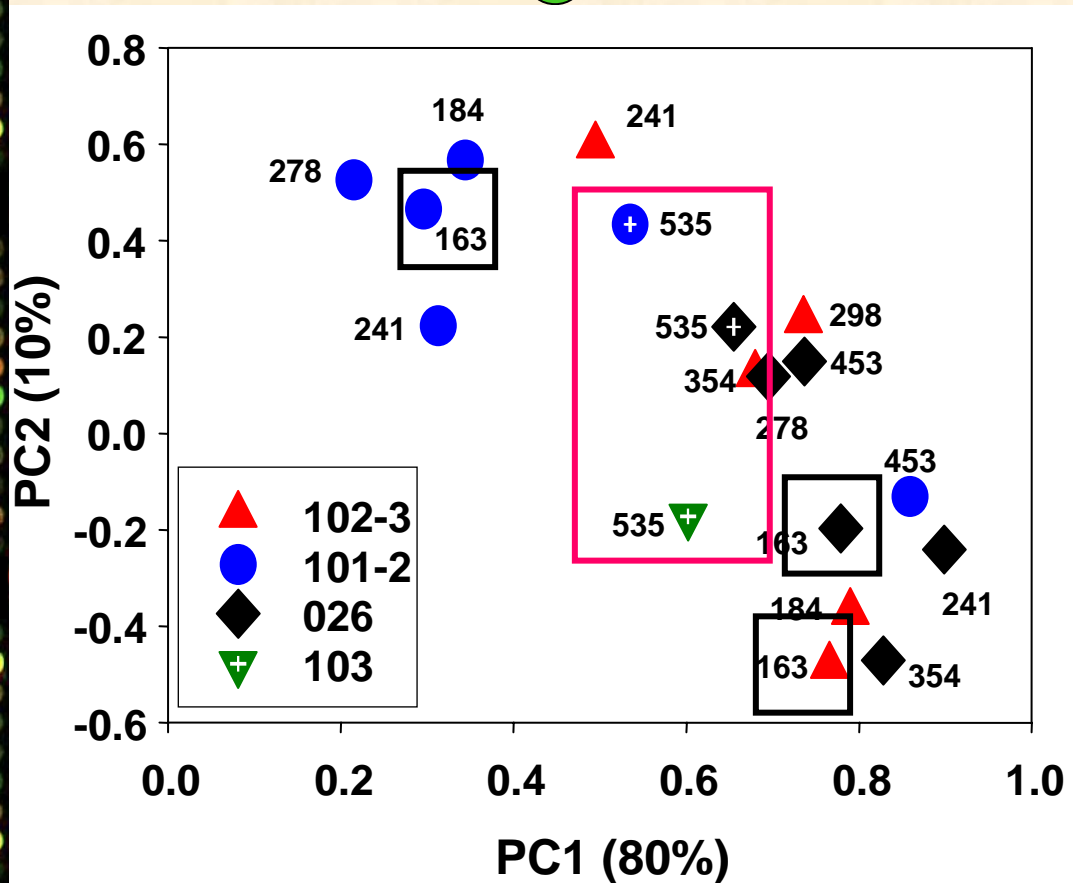
- 
- **Microarray analysis**
 - **1-2 L of groundwater**
 - **Extraction of DNAs**
 - **100 ng for amplification, 3x**
 - **Hybridization, 3x hybridizations**

Overall community similarity

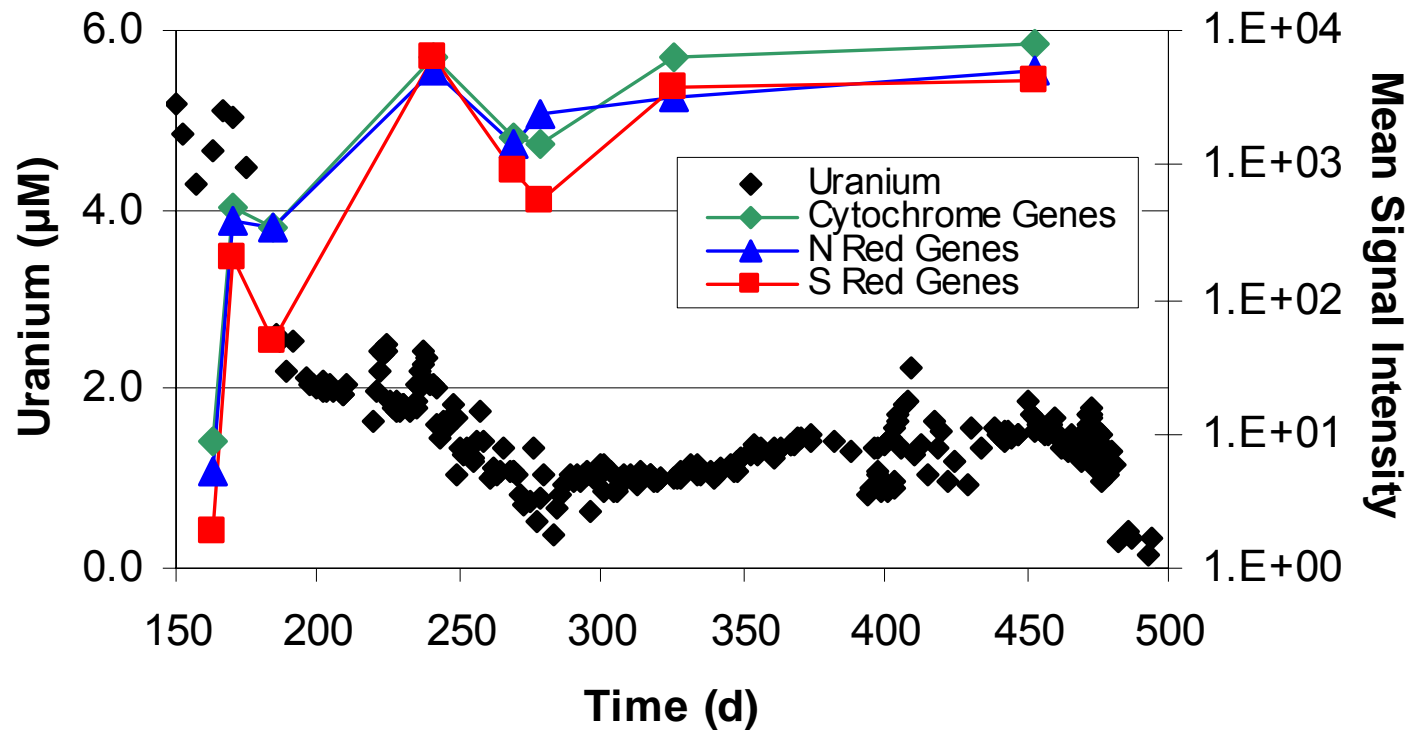
Distance between wells: 1-2 m



- Initially, 026 & 102-3 were similar but distinct from 101-2 although they are only a few meters away (Black box), indicating heterogeneity in the microbial populations
- Over time, the populations in the different wells became more similar to each other (Red box), possibly due to continual influx of injected groundwater.
- These results suggest bioremediation treatment significantly altered community compositions.

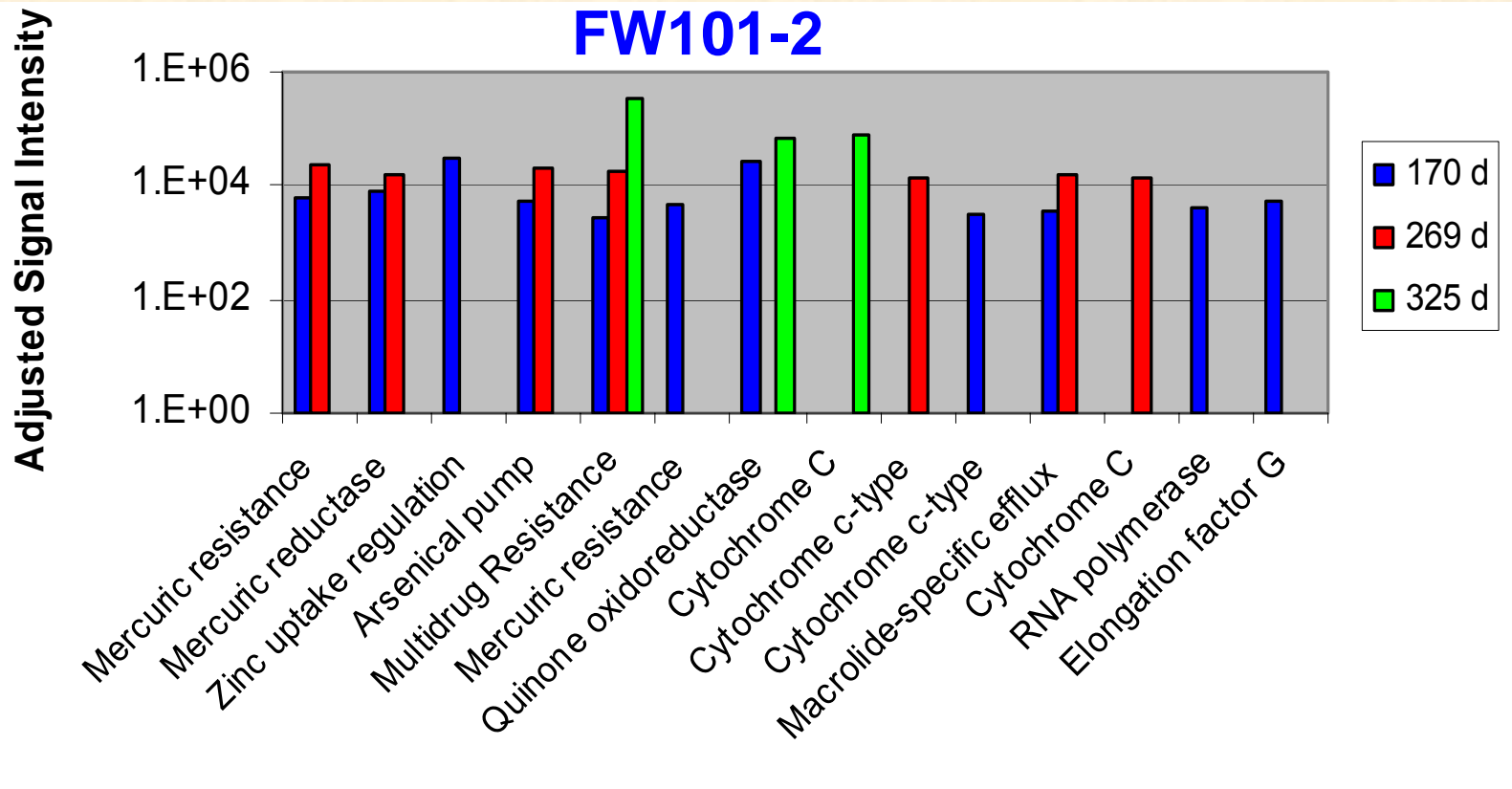


FW101-2 Denitrification, Sulfate Reduction & Cytochrome C Genes



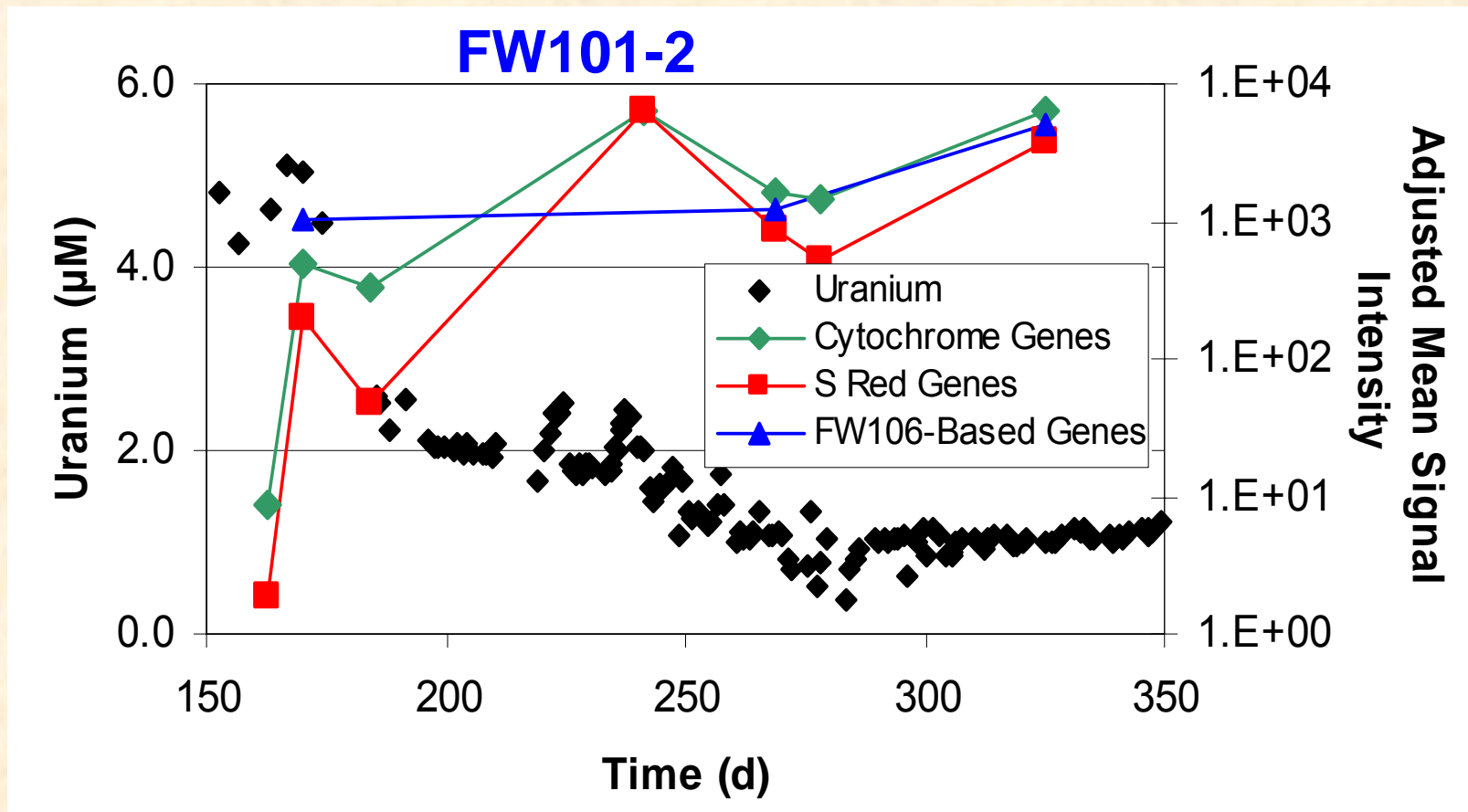
- Increased levels of cytochrome C genes correlated with lower uranium levels

FW106 Genes in Biostimulated Groundwater



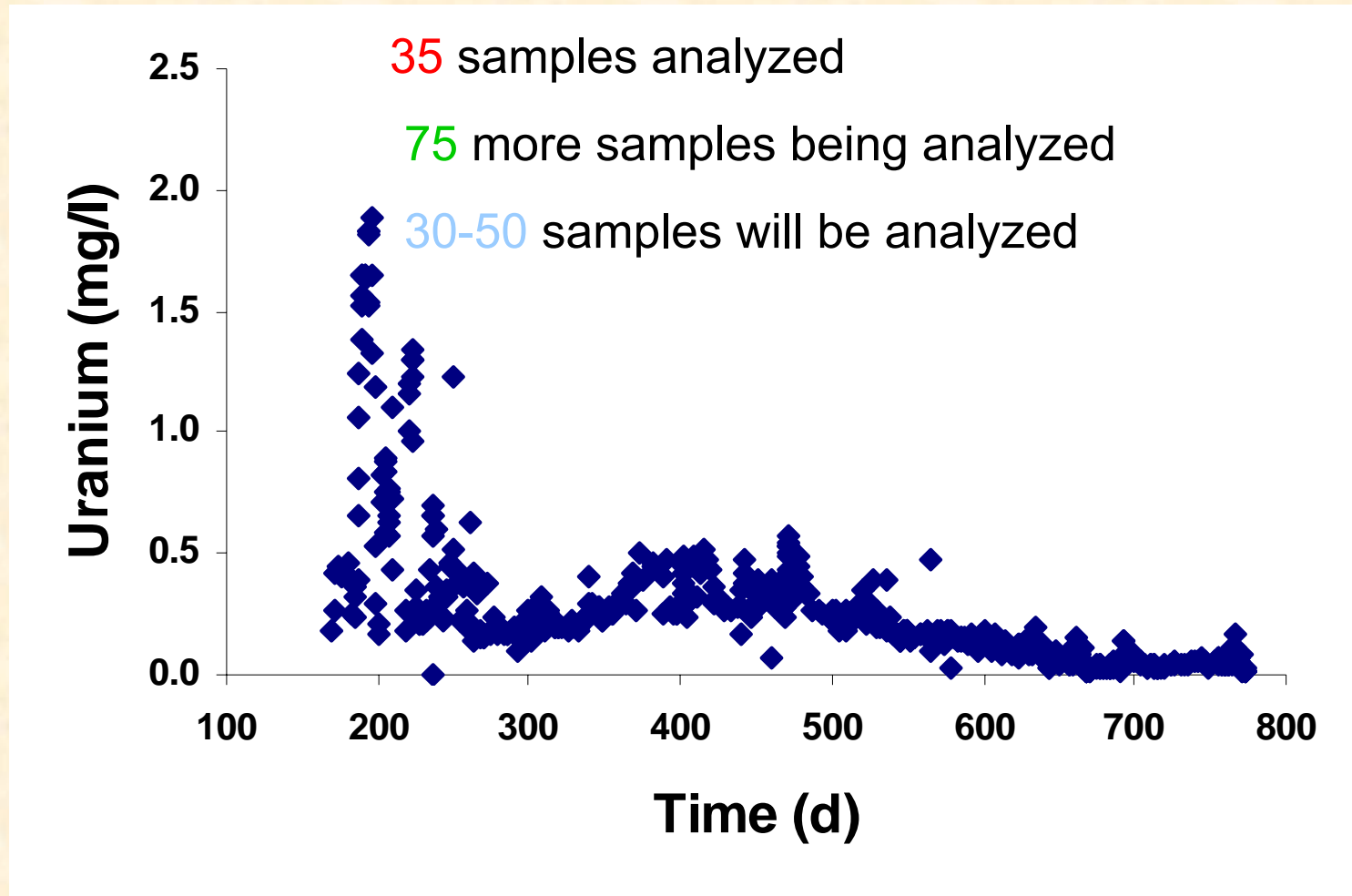
- **More genes detected at earlier timepoints but overall signal intensity increased at later timepoints**

FW106 Genes in Biostimulated Groundwater



- **Signal intensity from hybridization to FW106-based probes increased during biostimulation and U-reduction**

Functional Gene Array Analysis until the end of October



Functional Gene Array Analysis

Day	Sample Well																
	24	26	103	104	105	100(1)	100(2)	100(3)	100(4)	101(1)	101(2)	101(3)	101(4)	102(1)	102(2)	102(3)	102(4)
163	G	G	G	G			G	G			G	G			G	G	
166	G	G	G	G			G	G			G	G			G	G	
170		G									G	G			G	G	
174																	G
184		G					G				G	G			G	G	
191																	G
198																	G
212																	G
219																	G
226																	G
233																	G
236		G													G		
241		G									G			G	G	G	G
248											G				G	G	
255		G									G				G	G	
262				S													G
269											G				G		G
278		G M									G M				G M		
285											G						
298		G									G				G		G
303				S													
304											G						G
325		G									G				G		G
354		G M									G M				G M		G M
388											G						
453		G M		S							G M						G M
535	G S M	G S M	G S M	G S M							G S M						
558											G S	G S					
591											G						
622		G S		G S							G S				G		G
641		G		G							G				G		G
670		G S M	G	G M							G M	G			G		G
698											G						G
705											G				G		G
710			G		G	G			G	G			G	G			G
712	G			G			G	G			G	G			G		G
713		G	G		G	G			G	G			G	G			G
719											G				G		G
726											G				G		G
733											G				G		G
740											G				G		G
746	G	G		G			G	G			G	G			G		G
747			G		G	G			G	G			G	G			G
754		G		G							G	G			G		G
761											G				G		G
768											G				G		G
774	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

G = GROUNDWATER; S = SEDIMENT; M = MPN

RED = ANALYZED BY FGA; BLUE = BEING ANALYZED BY FGA

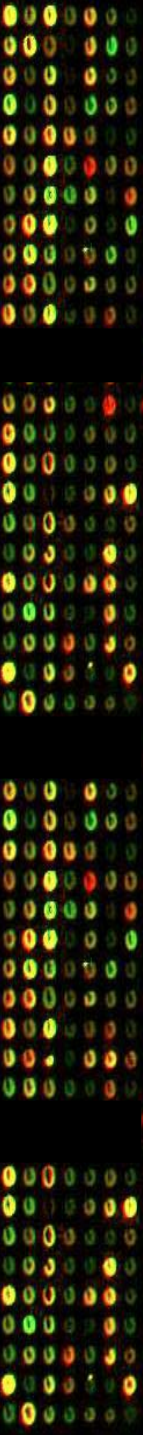


Plans for microbiology at Criddle Site

- 16S rRNA gene clone libraries, T-RFLP
- 16S arrays
- Functional gene arrays
- Isotope analysis
- Real-time PCR for *Geobacter* spp. and sulfate reducers
- Investigate microbial community under potential uranium re-oxidation conditions
- Batch experiment

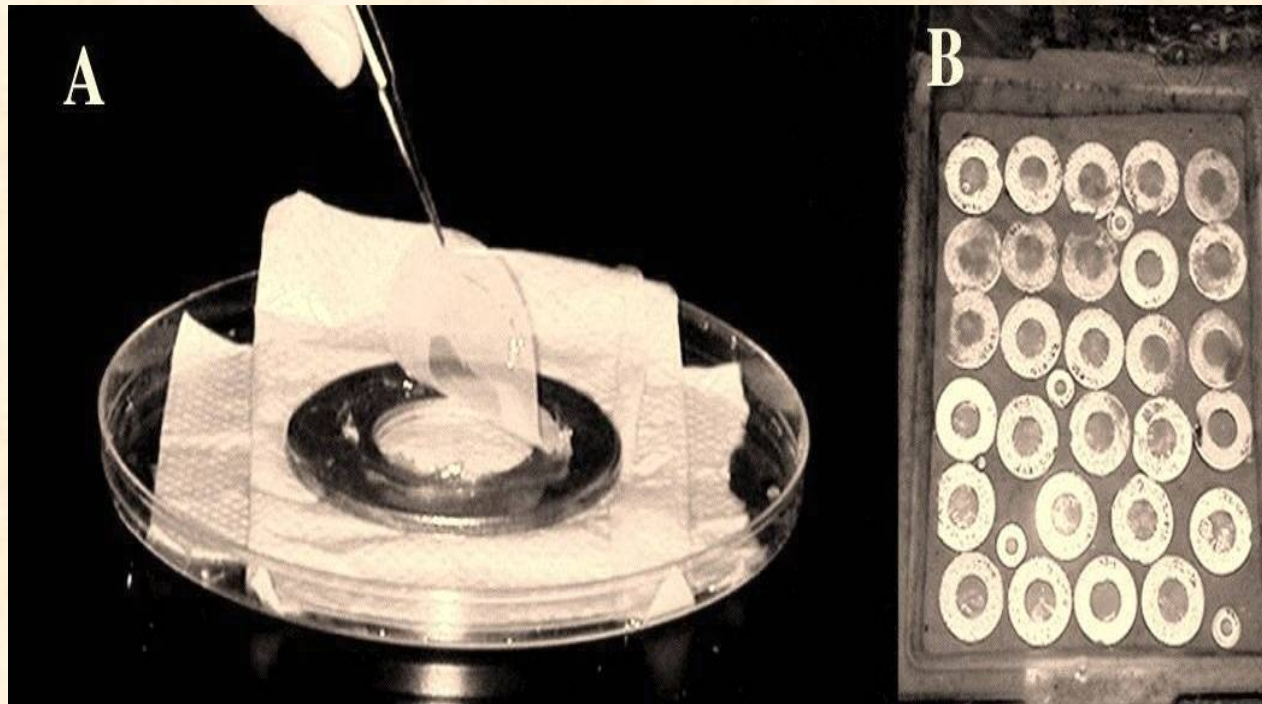
Batch Experiment

- Which populations are responsible for U reduction?
- Sediment from Area 3
- Electron donor: ethanol or acetate – 1.5 mM
- Sulfate level: 0 or 1.5 mM
- pH: 6.0 or 7.2
- Batch feed once per week
- Sample weekly for chemical analyses and every three weeks for microbial analyses



Bacterial isolation and characterization

Experimental set up



Diffusion growth chamber for *in situ* cultivation of environmental microorganisms.

A. General view.

B. Growth chambers incubated on the surface of marine sediment.

Isolates versus metagenome sequences

	<i>R.f.</i>	<i>F.a.</i>	gamma A40	OR1 87	OR1 92	OR1 113	BFXI 386	BFXI 557
<i>R.fulvus</i>								
<i>F.aurantica</i>	95.2							
Gamma-Proteo A40	95.1	95.6						
OR1-87 (isolate)	98	94.8	95.7					
OR1-92 (bac in mix)	97.2	94.6	95.3	99				
OR1-113 (isolate)	94.8	94	94.7	95.6	95.2			
BFXI386	96.8	95.1	95.1	96.4	96.1	95.5		
BFXI557	96.5	94.8	94.8	96.1	95.8	95.2	99.7	
BFXI433	97.3	94.1	94.9	98.5	99.2	95.1	96	95.7



Further Work on Metagenomics

- Sequence 1-2 more communities with higher diversity by JGI
- Use targeted approaches to obtain sequences from less abundantly organisms.
- Examine strain diversity and population genetics
- Isolate selected organisms from community and sequence genomes
- Examine evolution including horizontal gene transfer, gene family expansions, etc.
- Construct microarrays to probe community dynamics
- Understand their ecology and evolution

Additional key questions

- **What are the carbon and energy sources for the community as a self-sustaining ecosystem?**
- **Whether are they active?**
- **Whether do the different populations interact each others? How?**
- **Why are only these two major groups there?**
- **How do stresses affect population structure, adaptation and evolution?**



Summary and Conclusions

- Extremely low genome diversity was observed near S3 pond
- 4 species in Gamma and Beta subdivisions are identified.
- Despite dominance of metagenome sequence by *Frateuria*-like organism, many important metabolisms map to the *Azoarcus*-like organism.
- Metal resistance genes are highly abundant.
- Sequence analysis indicates that this community has appropriate capability to deal with such extreme stress environments.
- The dominant populations are widely spread at FRC and do respond to biostimulation but their physiological roles are unclear.

Acknowledgement

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