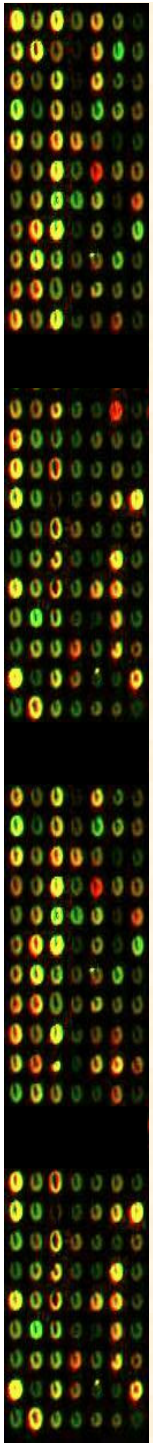

Development of Integrated Genomic Technology for Microbial Community Analysis

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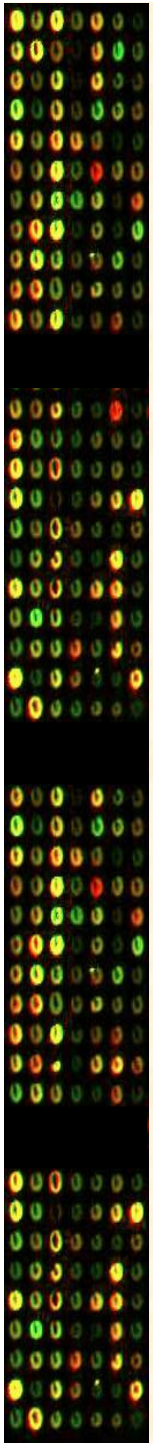
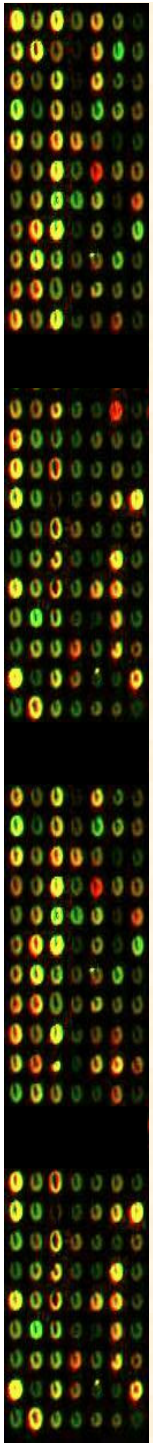


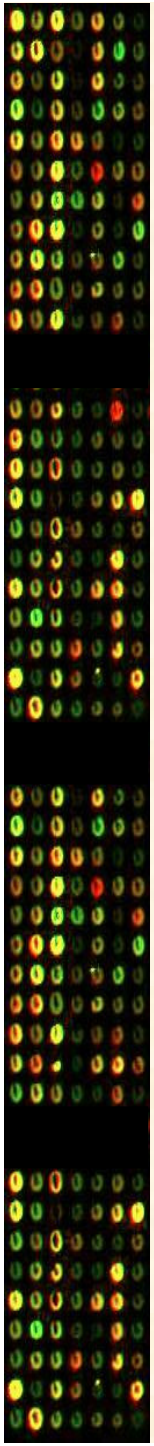
Project Objectives

- Developing microarrays-based genomic technologies for microbial detection.
- Use of the developed genomic technologies for assessing microbial community structure and dynamics related to bioremediation.



Pioneering advances in microarray-based technologies to address challenges in microbial community genomics

- Challenges:
  - Specificity: Environmental sequence divergences.
  - Sensitivity: Low biomass.
  - Quantification:
  - Existence of contaminants: Humic materials, organic contaminants, metals and radionuclides.
- Solutions
  - Developing different types of microarrays and novel chemistry to address different levels of specificity.
  - Developing novel signal amplification strategy to increase sensitivity
  - Optimizing microarray protocols for reliable quantification.



Summary of 50mer-based FGAs for environmental studies

Oligonucleotide probe size: 50 bp

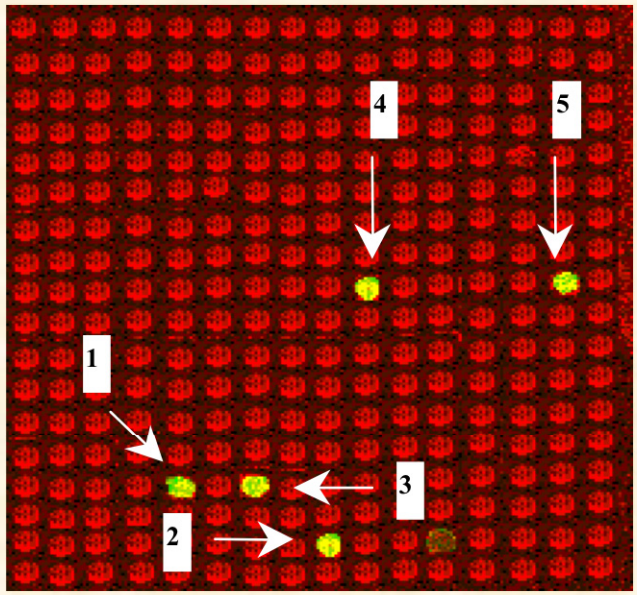
Tiquia et al. 2004. BioTechniques 36, 664-675
Rhee et al. 2004, AEM 70:4303-4317

- Nitrogen cycling: 302
- Sulfate reduction: 204
- Carbon cycling: 566
- Phosphorus utilization: 79
- Organic contaminant degradation: 770
- Metal resistance and oxidation: 85

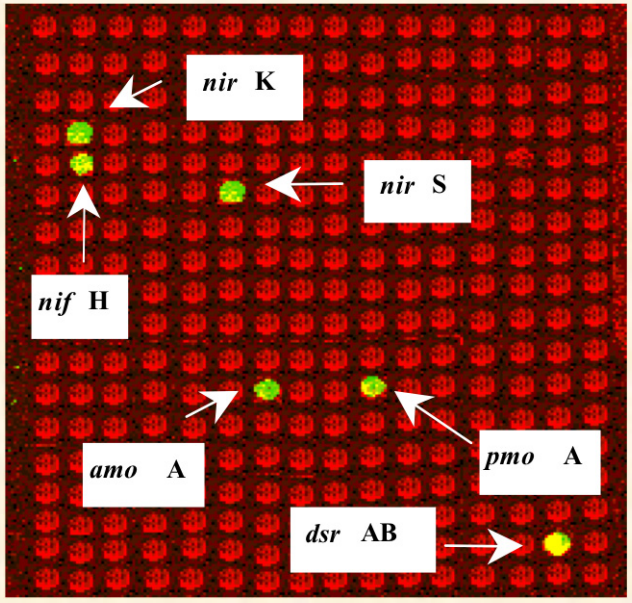
- **Total: 2,006 probes**
- **All probes are < 88% similarity**

Specificity of 50 mer microarrays

Very specific hybridization was obtained



- 5 nirS genes were mixed together
- Only corresponding genes were hybridized

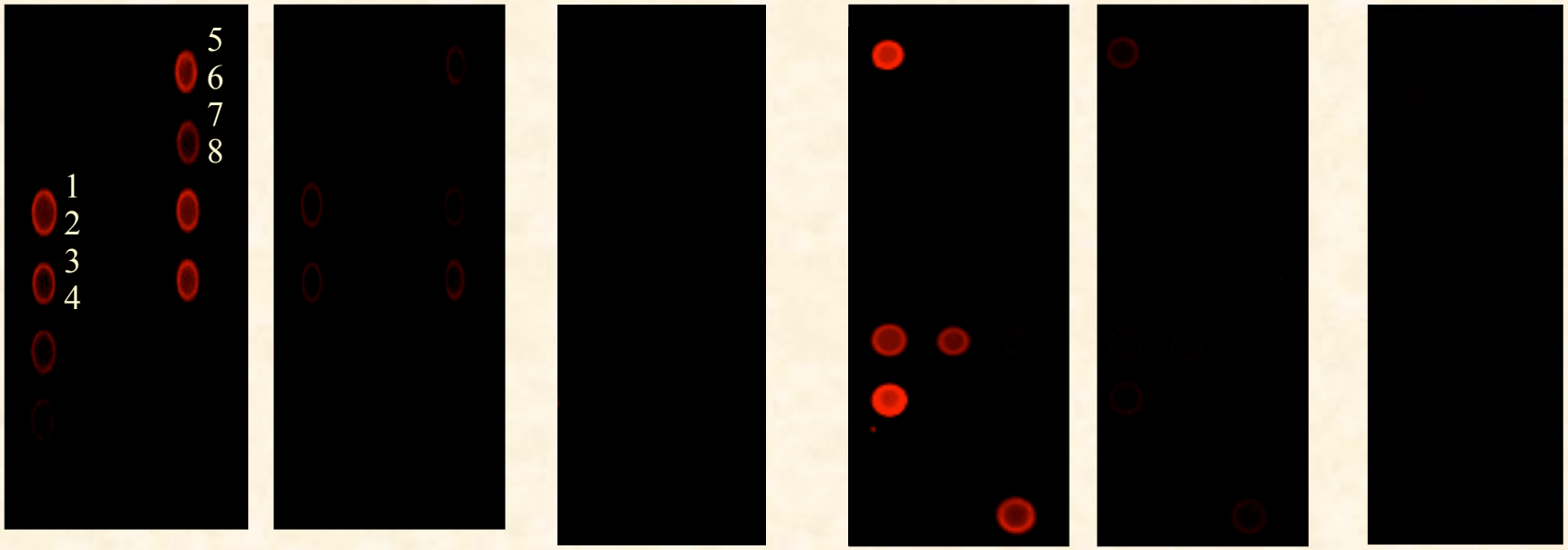


- 6 types of genes were mixed together
- Only corresponding genes were hybridized

Sensitivity

Genomic DNA

Cells



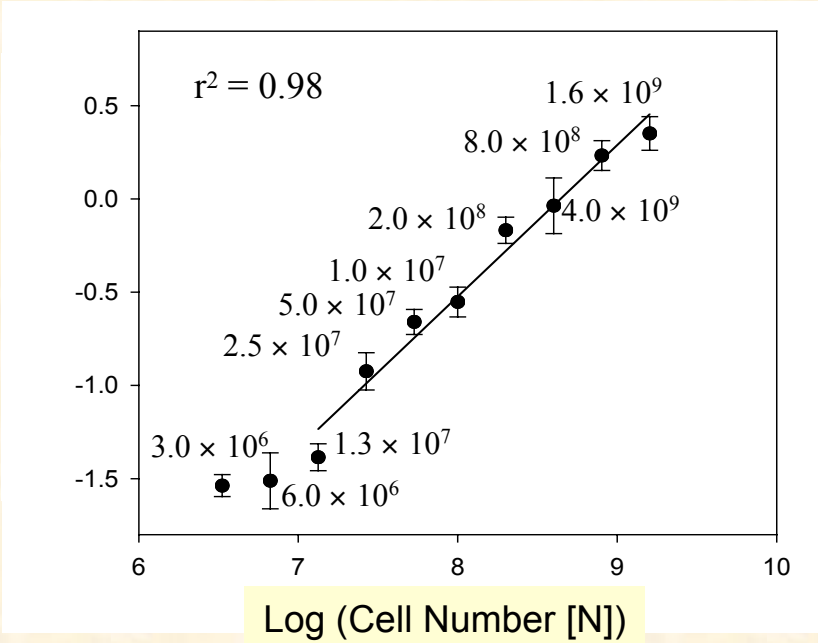
500 ng gDNA | **50 ng** | 25 ng | 1.6×10^9 | **1.3×10^7** | 3.0×10^6

Detection limit

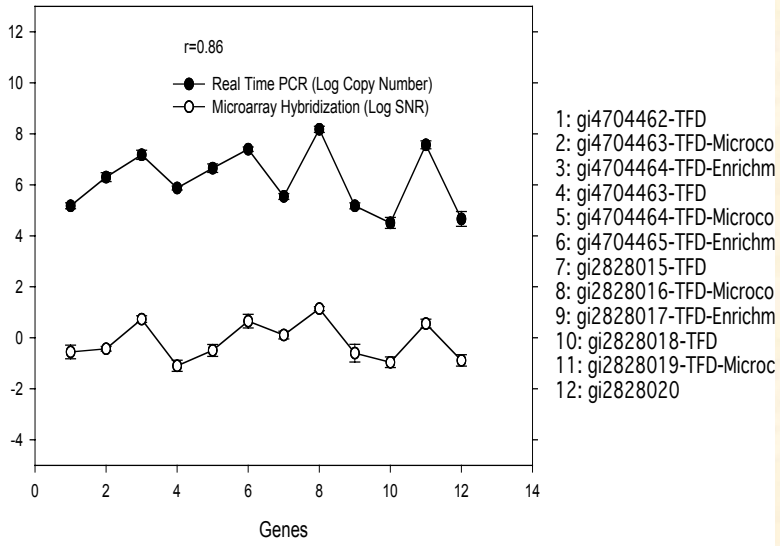
- 50 ng pure DNA in the presence of non-target templates
- 10^7 cells

Quantification and validation

Microarray hybridization



Real-PCR

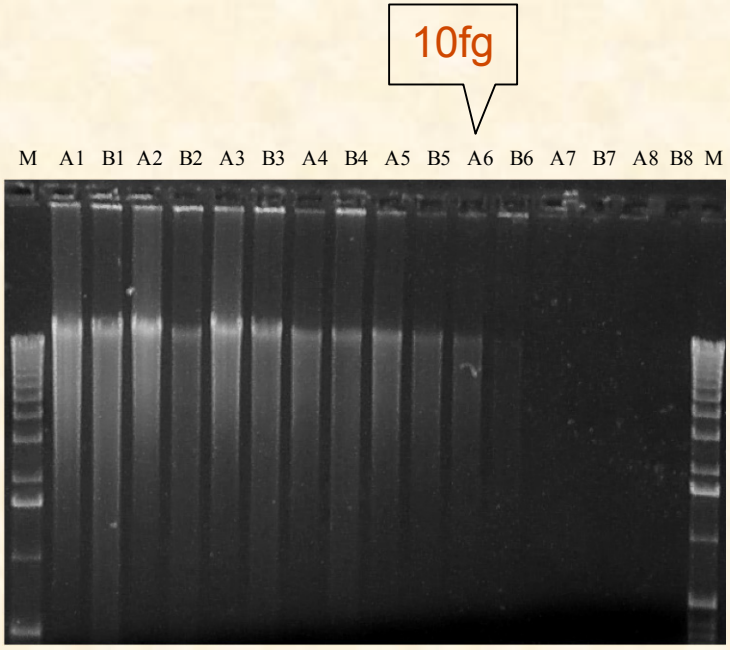


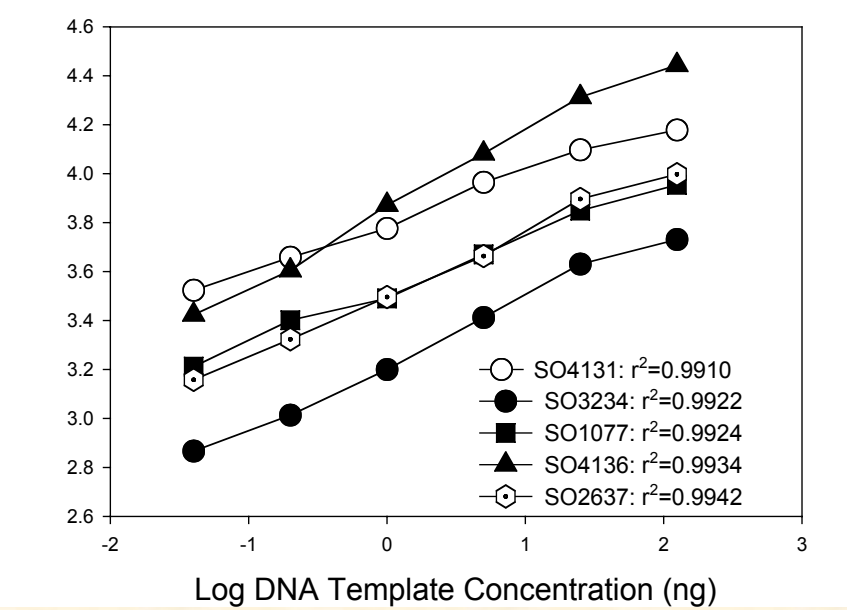
Quantification

- **Good linear relationship**
- **Quantitative**

- Microarray result is consistent with real-time PCR

Novel amplification approach for increasing hybridization sensitivity





As low as 10fg (2 cells) can be detected

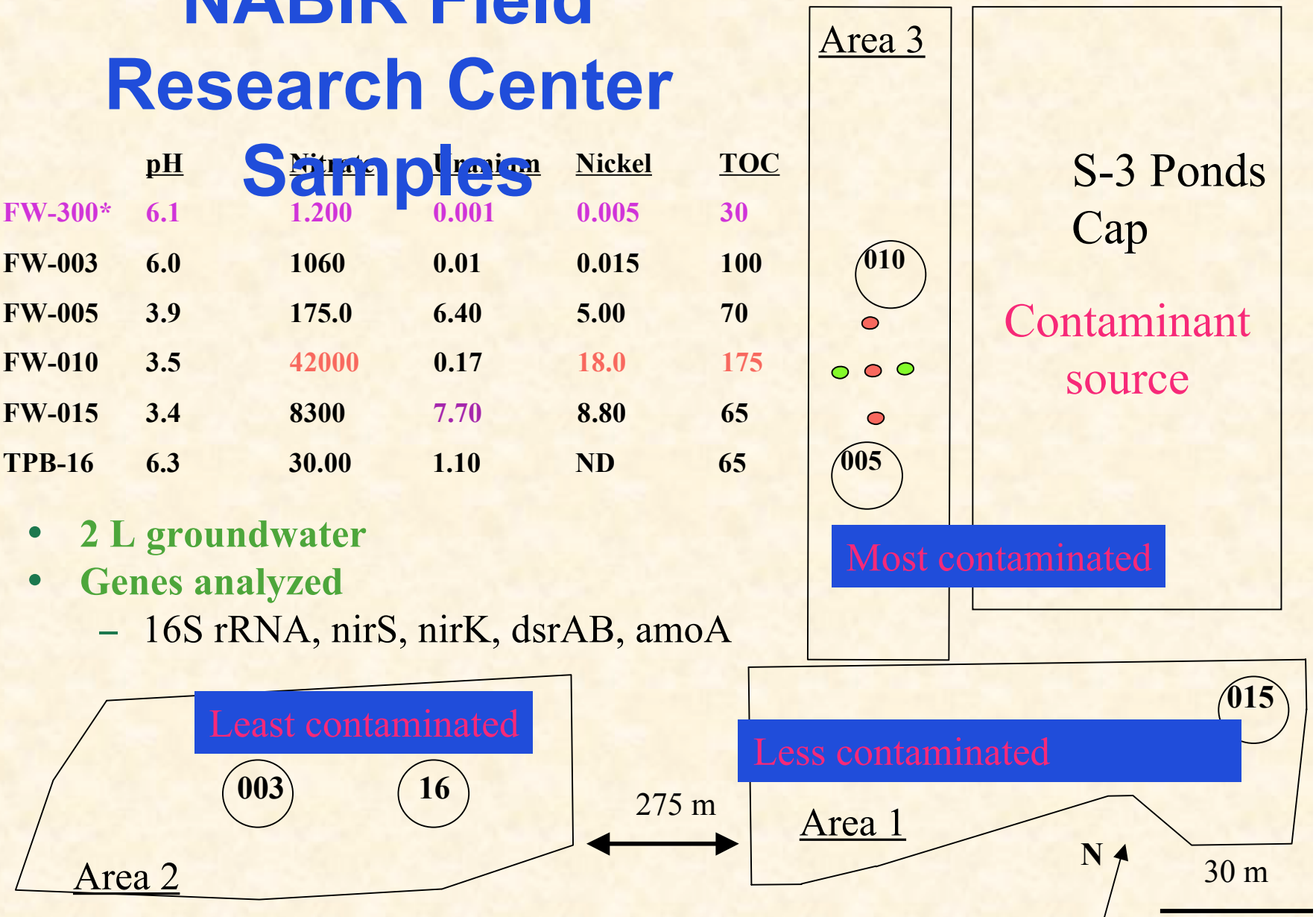
Amplification is quantitative for majority of the genes

Submitted to PNAS

NABIR Field Research Center Samples

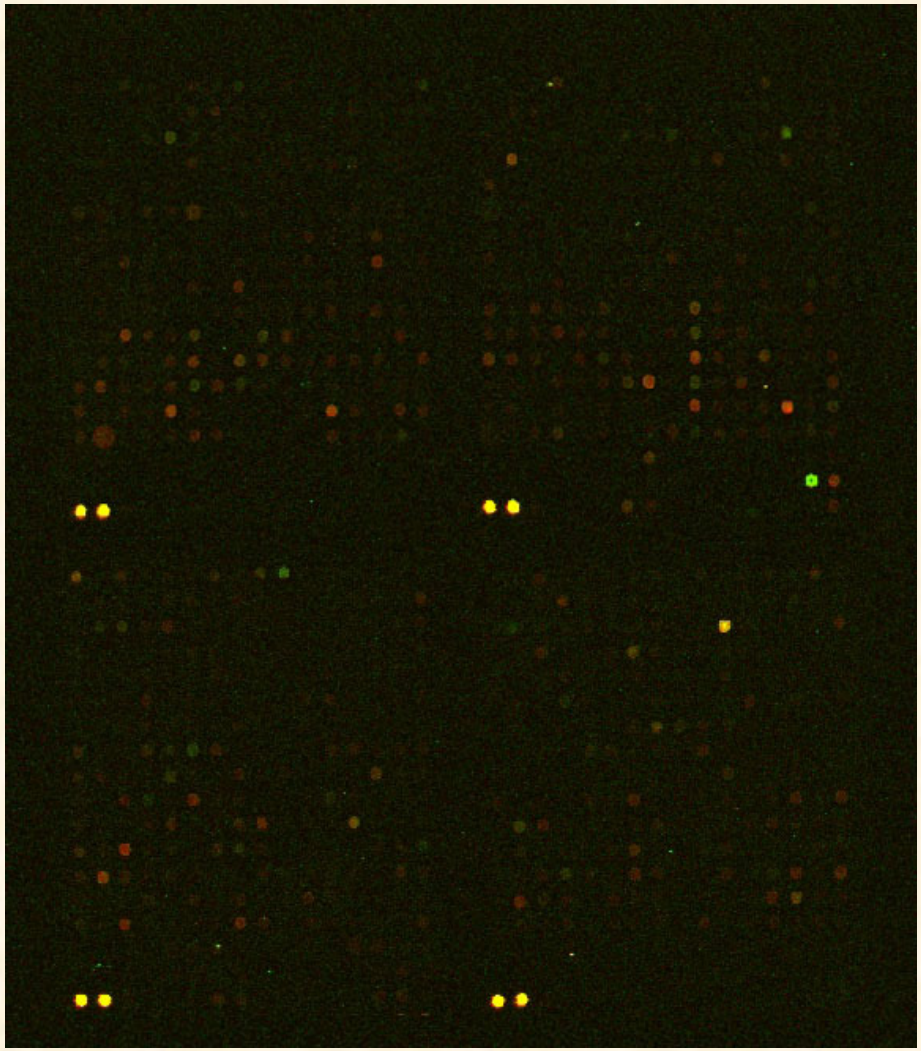
	pH	Nitrate	Uranium	Nickel	TOC
FW-300*	6.1	1.200	0.001	0.005	30
FW-003	6.0	1060	0.01	0.015	100
FW-005	3.9	175.0	6.40	5.00	70
FW-010	3.5	42000	0.17	18.0	175
FW-015	3.4	8300	7.70	8.80	65
TPB-16	6.3	30.00	1.10	ND	65

- 2 L groundwater
- Genes analyzed
  - 16S rRNA, nirS, nirK, dsrAB, amoA



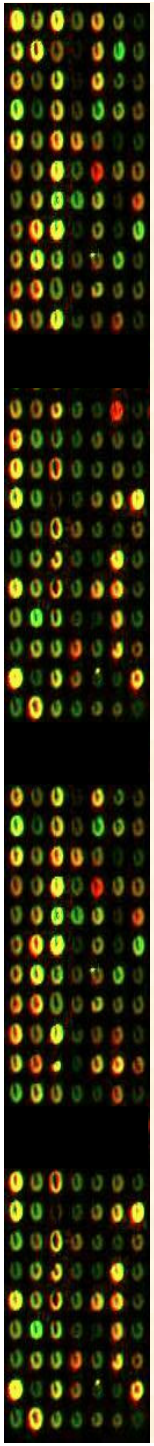
6 samples were taken to assess the effects of contaminants on microbial community structure

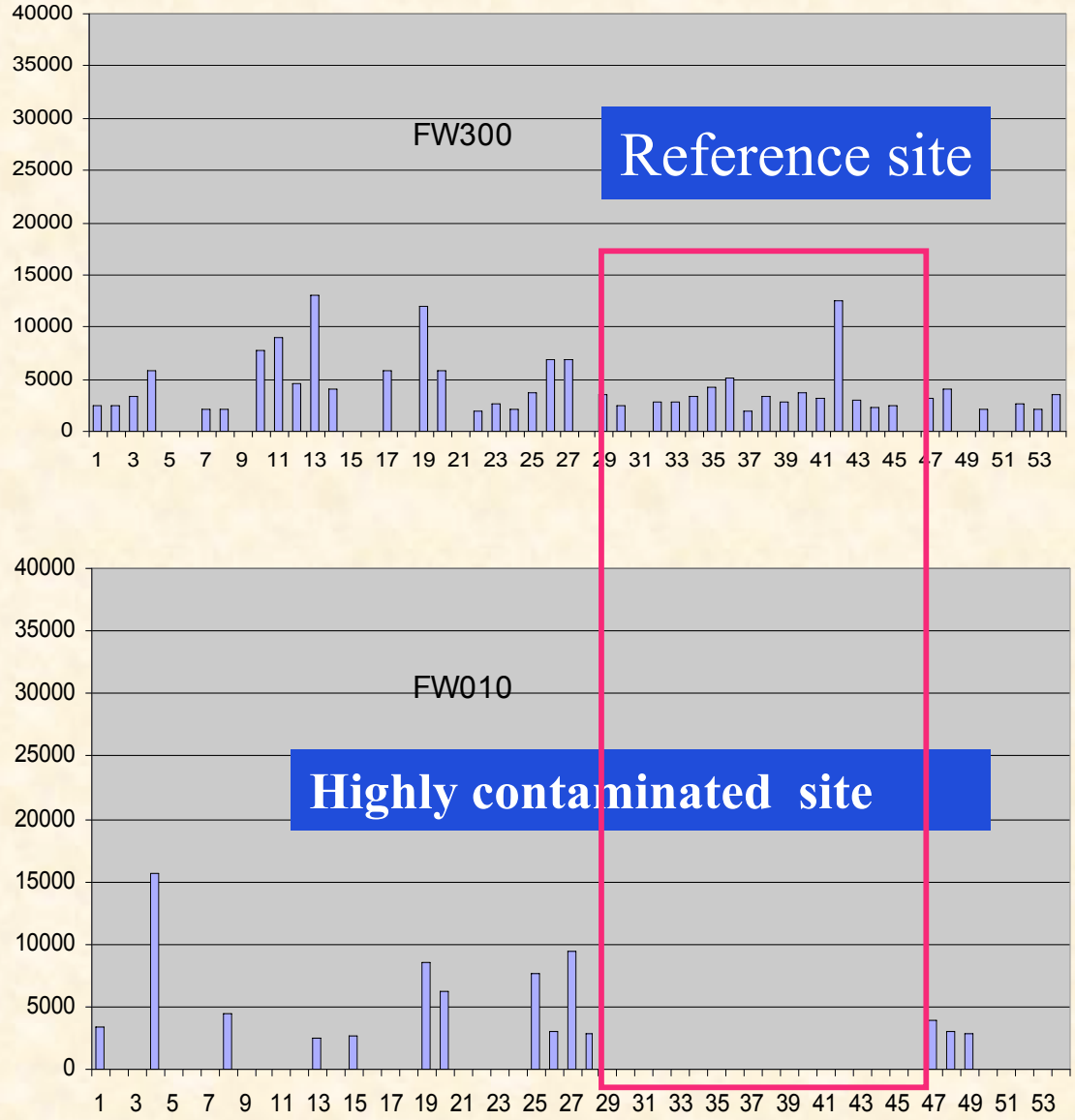
Groundwater samples with very low biomass



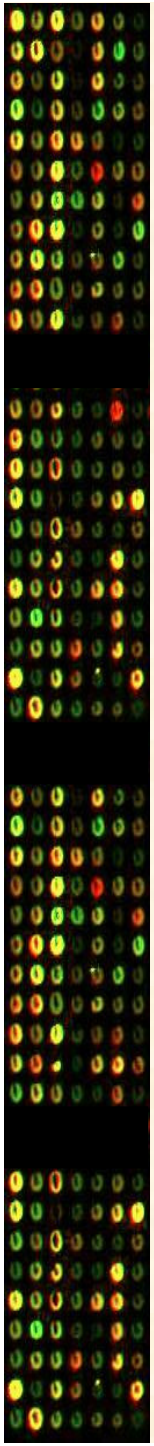
- 2L groundwater from six different sites.
- Cell counts: 1-5x10^5/ml
- DNA was isolated, 1/20 of the DNA was manipulated and used for hybridization.
- Nice hybridization was obtained with the DNA manipulated with the new method.
- No hybridization were obtained if the DNA is not manipulated.

Difference of functional genes in samples from NABIR Field Research Center





- Clear difference was observed among contaminated and noncontaminated sites.
- E.g., some genes are present in noncontaminated site but not in contaminated sites



Overall diversity among different samples

	FW300	FW003	FW021	FW010	FW024
FW300	61(20%)	189(36%)	174(35%)	80(21%)	111(23%)
FW003		25(11%)	144(35%)	61(17%)	84(20%)
FW021			10(5%)	64(20%)	90(24%)
FW010				6(5%)	118(37%)
FW024					30(16%)
Total Genes Detected	302	219	192	130	190
Genetic diversity, Simpson's (1/D)[a]	125.5	67.1	26.6	17.4	35.7

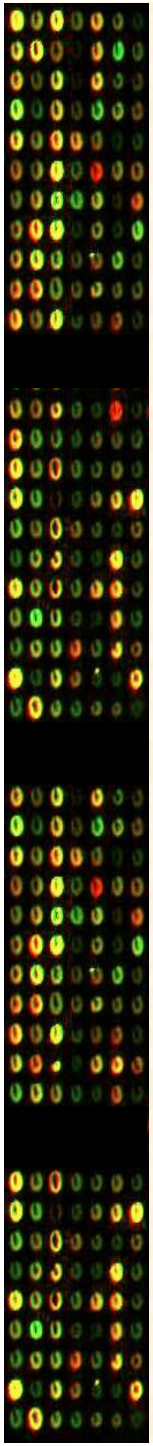
- Overall diversity correlates with contaminant level.
- The proportion of overlapping genes between samples was consistent with the contaminant level and geochemistry.
- A significant portion (5-20%) of all detected genes were unique to each sample, even though they are very close. Thus, important microbial populations appear to be highly heterogeneous in this groundwater system.

CommOligo --- New oligo probe design program for community analysis

Number and specificity of designed probes (50-mer) by different programs

Programs used	Whole-genome sequences of *M. maripaludis* (1766 ORFs)						Group sequences of *nirS* and *nirK* (842 gene sequences)					
	Total ORFs	ORFs rejected	Probes designed	**Specific probe**	Non-specific	**Group-specific**	Total ORFs	ORFs rejected	Probes designed	**Specific probe**	Non-specific	**Group-specific**
ArrayOligoSelector	1766	7	1759	**1415**	344	**0**	842	0	842	**117**	725	**0**
OligoArray	1766	68	1698	**1654**	44	**0**	842	35	807	**70**	737	**0**
OligoArray 2.0	1766	68	1698	**1464**	234	**0**	842	51	791	**35**	756	**0**
OligoPicker	1766	18	1748	**1745**	3	**0**	842	657	185	**141**	44	**0**
CommOligo	1766	9	1757	**1745**	**0**	**12**	842	512	330	**147**	**0**	**183**

- Useful for both whole genome microarrays and community arrays
- Able to design group-specific probes
- Better performance than other programs



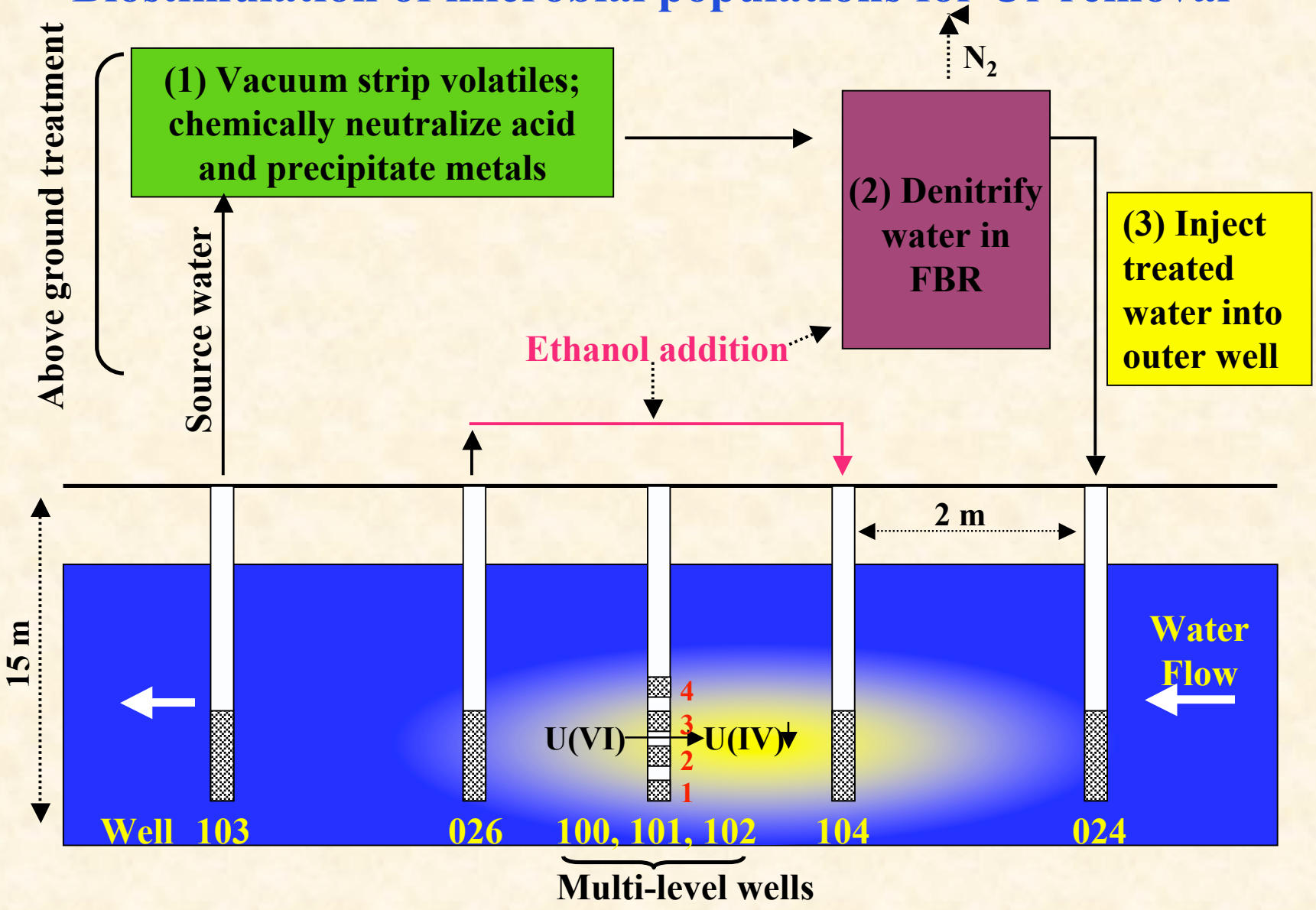
Probes Designed for a Second Generation FGA

- Nitrogen cycling: 5089
- Carbon cycling: 9198
- Sulfate reduction: 1006
- Phosphorus utilization: 438
- Organic contaminant degradation: 5359
- Metal resistance and oxidation: 2303

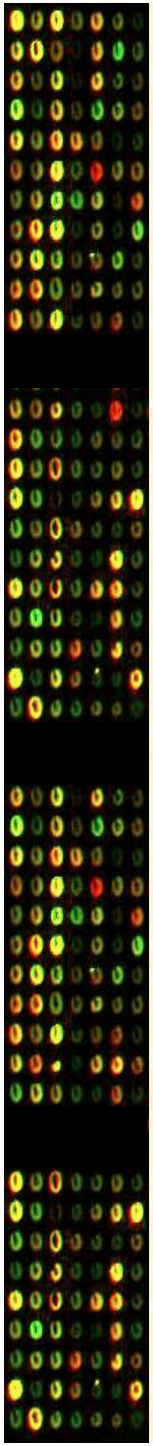
Total: **23,408 genes**

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

Biostimulation of microbial populations for Ur removal



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

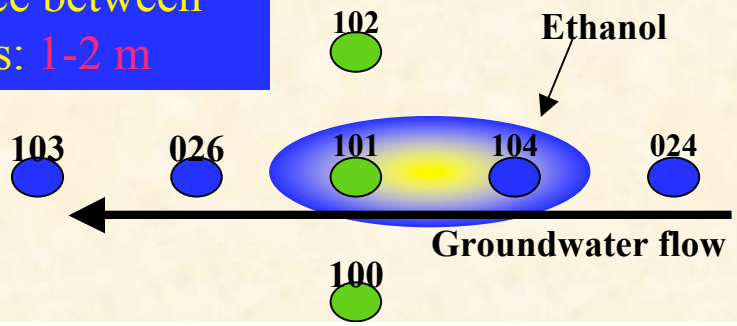


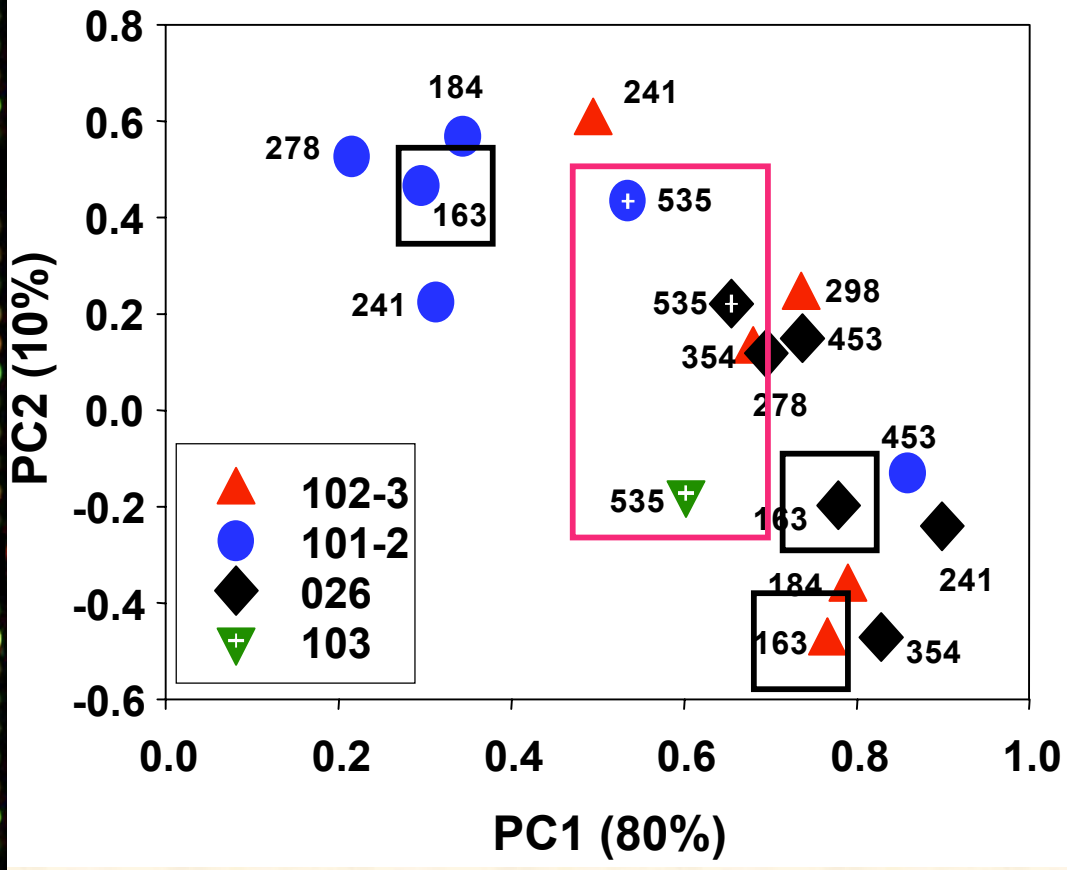
Treatments and sampling

- Ethanol was injected nearly weekly from about Day 137.
- Groundwater was sampled (1-5L) nearly weekly for microarray analysis.
- Geochemical data were measured almost daily.

Overall community similarity

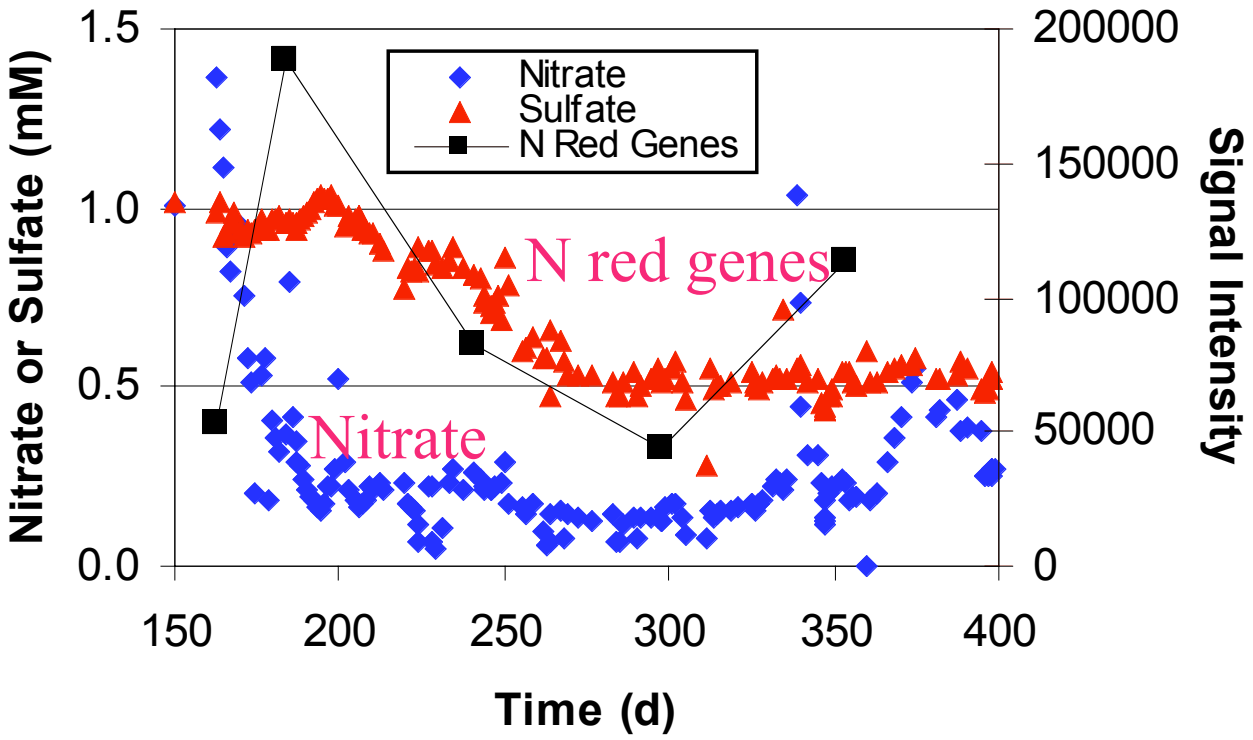
Distance between wells: 1-2 m





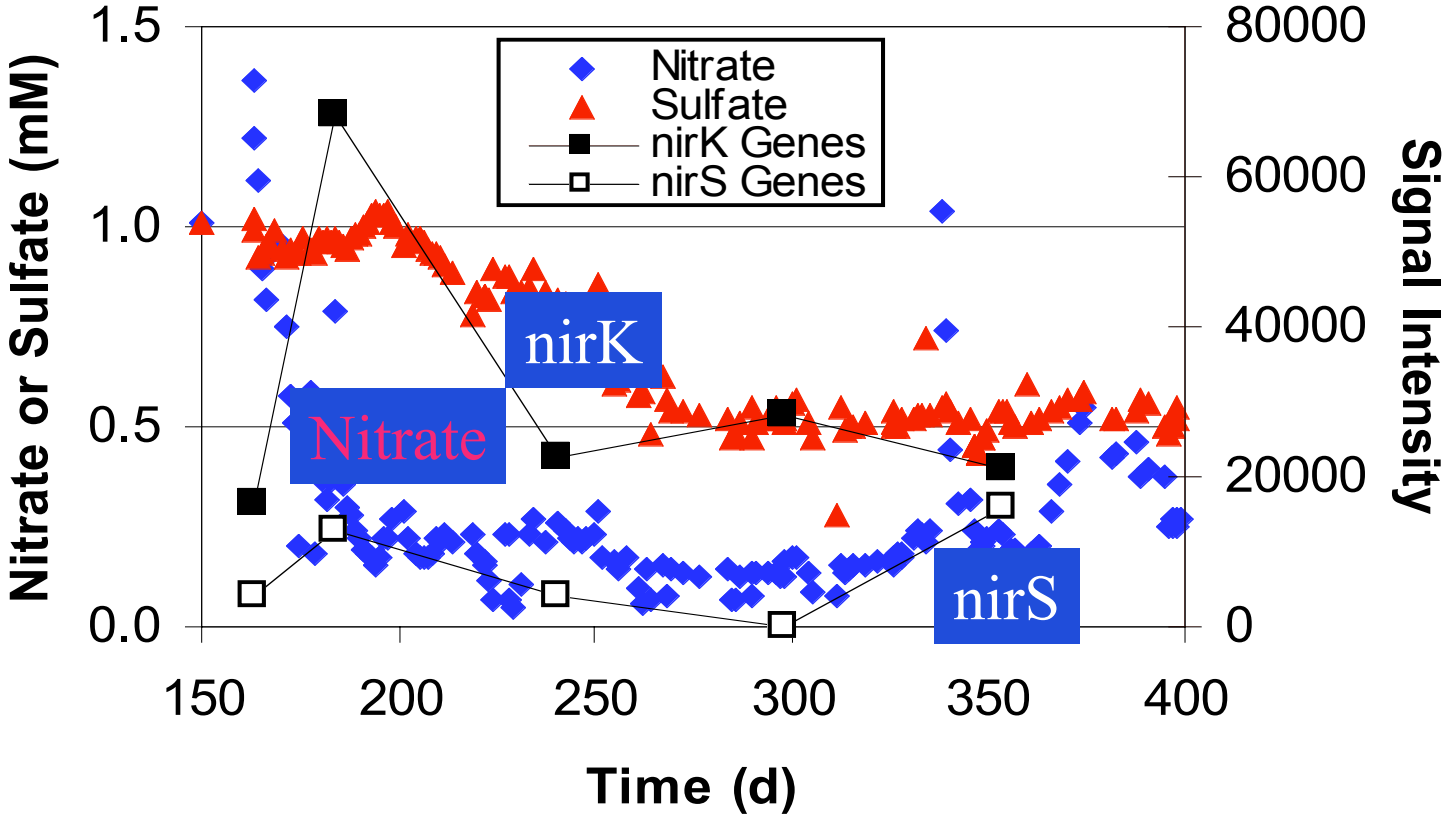
- Initially, 026 & 102-3 were similar but distinct from 101-2 although they are only a few meters away (Black box), indicating heterogeneity in the microbial populations
- Over time, the populations in the different wells became more similar to each other (Red box), possibly due to continual influx of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

Nitrite Reduction Genes



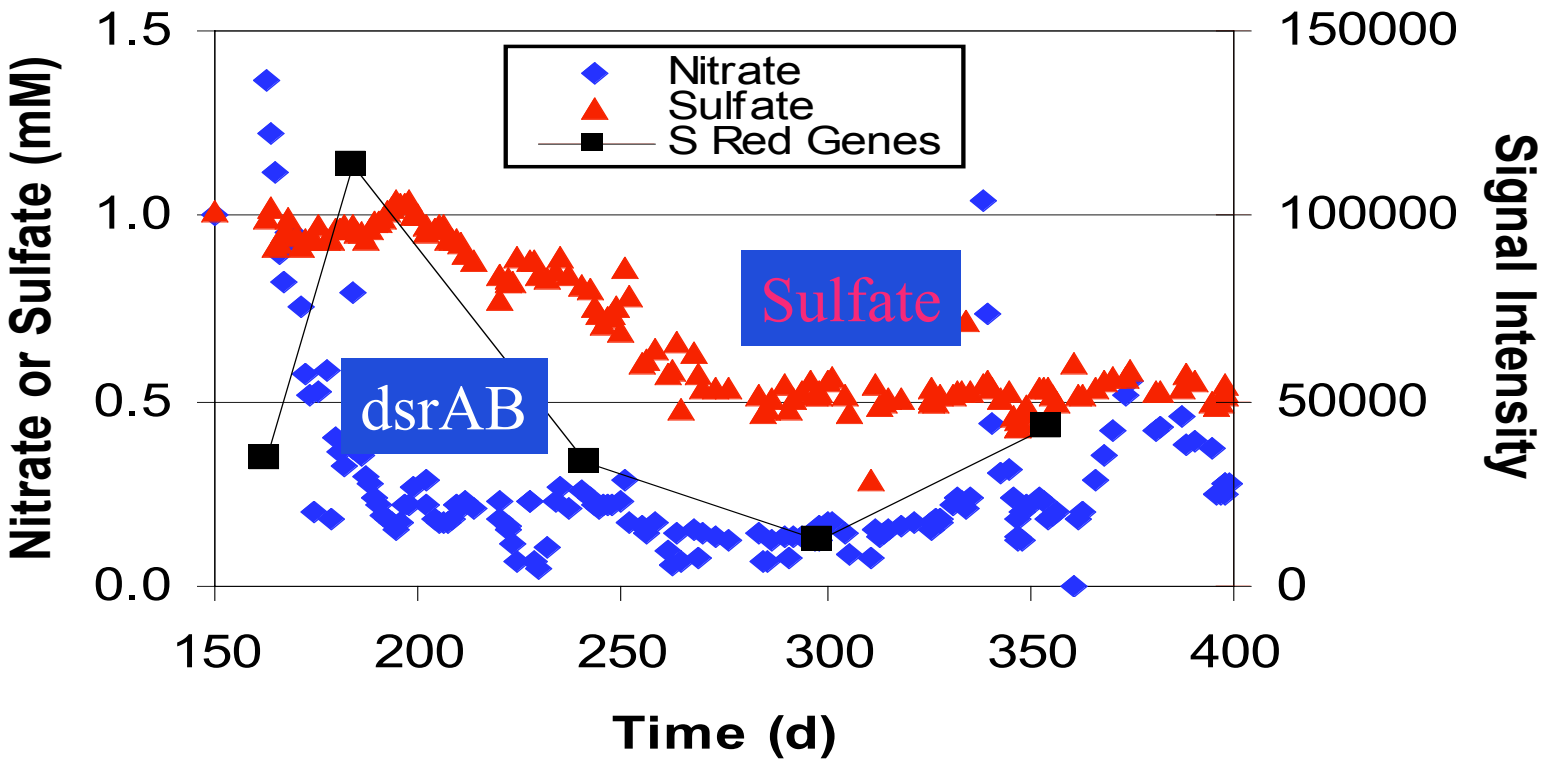
- **Total N reduction gene signals correlated with nitrate levels.**
- **Additional samples after 354 d are being analyzed to see if the trend continued**

Nitrite Reductase Genes



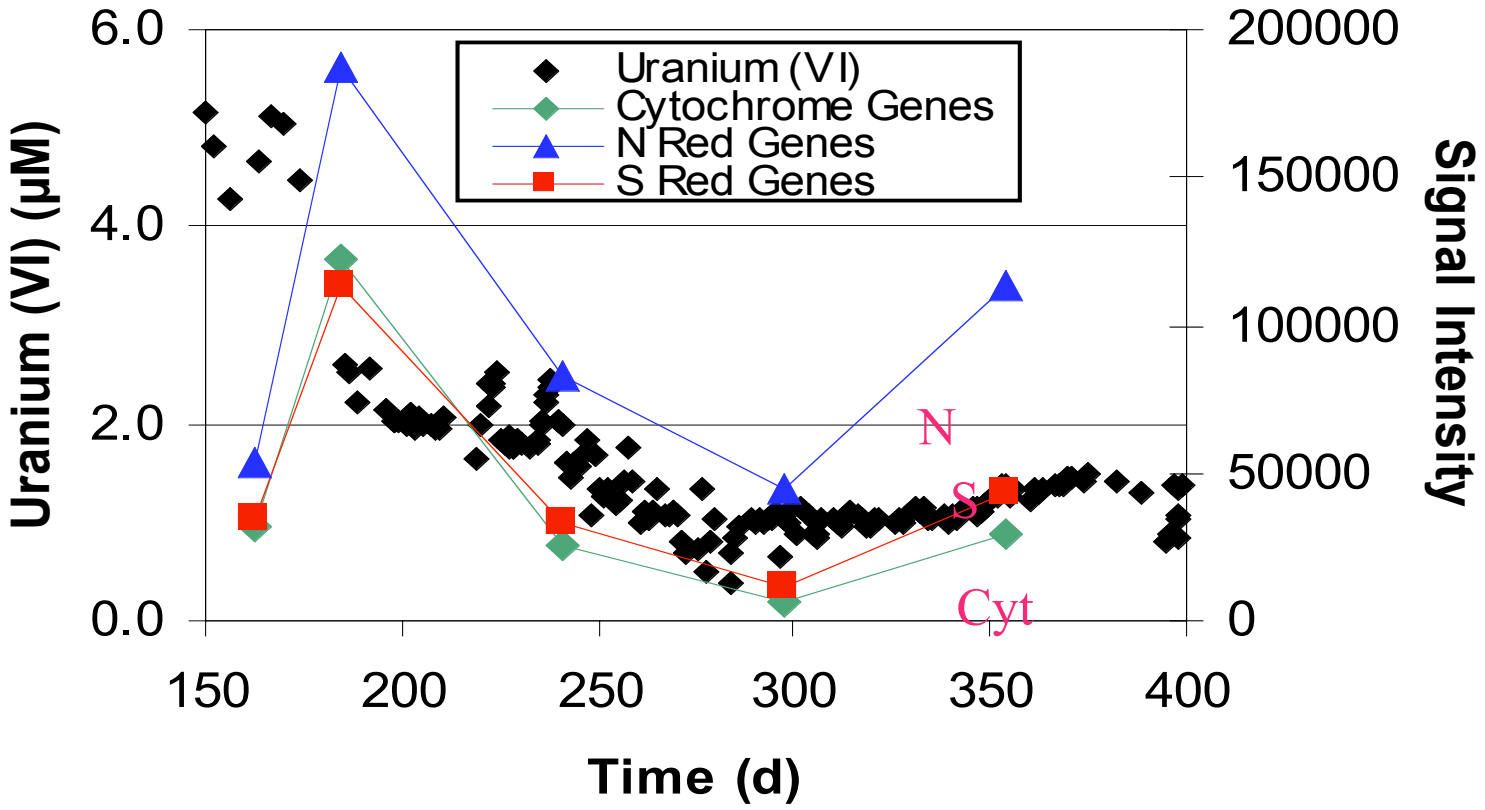
- Total *nirS* and *nirK* signals correlated with nitrate levels.
- *nirK* genes were dominant but *nirS* increased in 354 d sample.
- Total numbers of *nirS* and *nirK* genes detected had same trends.
- Bacteria containing nirK genes respond to biostimulation more rapidly.

Sulfite reduction Genes

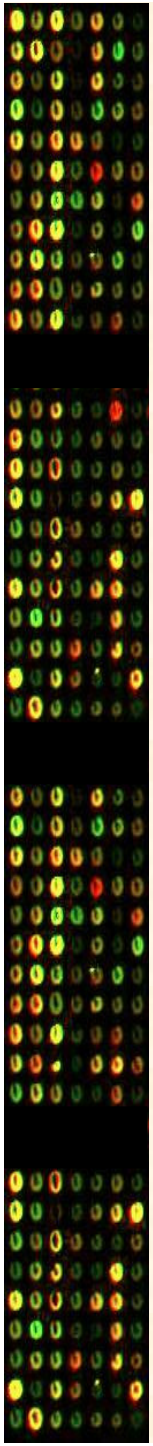


- **Total S reduction gene signals correlated with sulfate levels.**
- **Total numbers of S reduction genes detected had same trends.**
- **Several FRC *dsrA/B* clones were detected.**

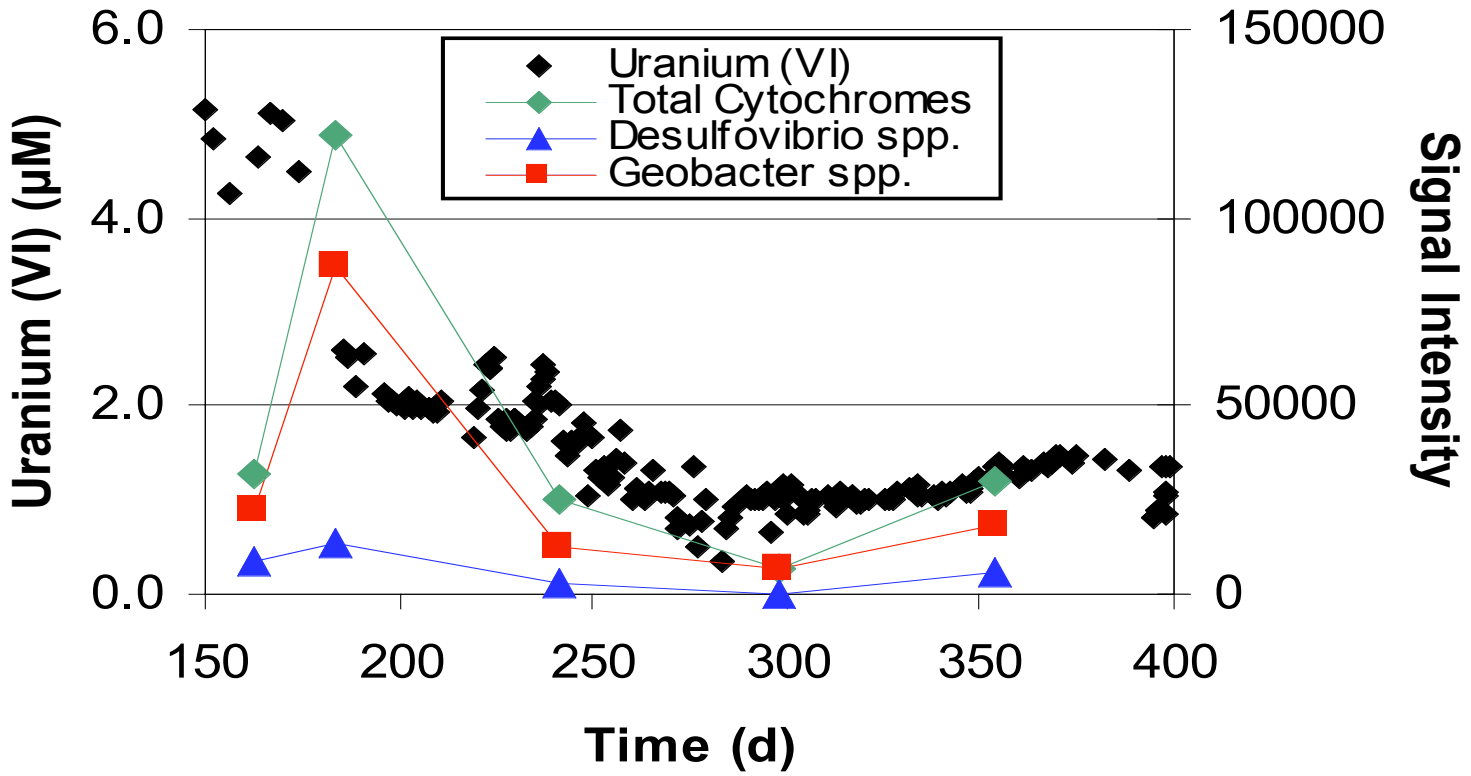
N & S Reduction and Cytochrome Genes



- N and S reduction and cytochrome C genes followed trends in U(VI) levels.



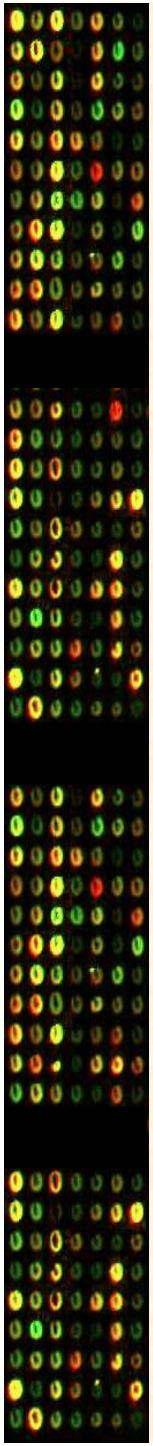
Cytochrome Genes

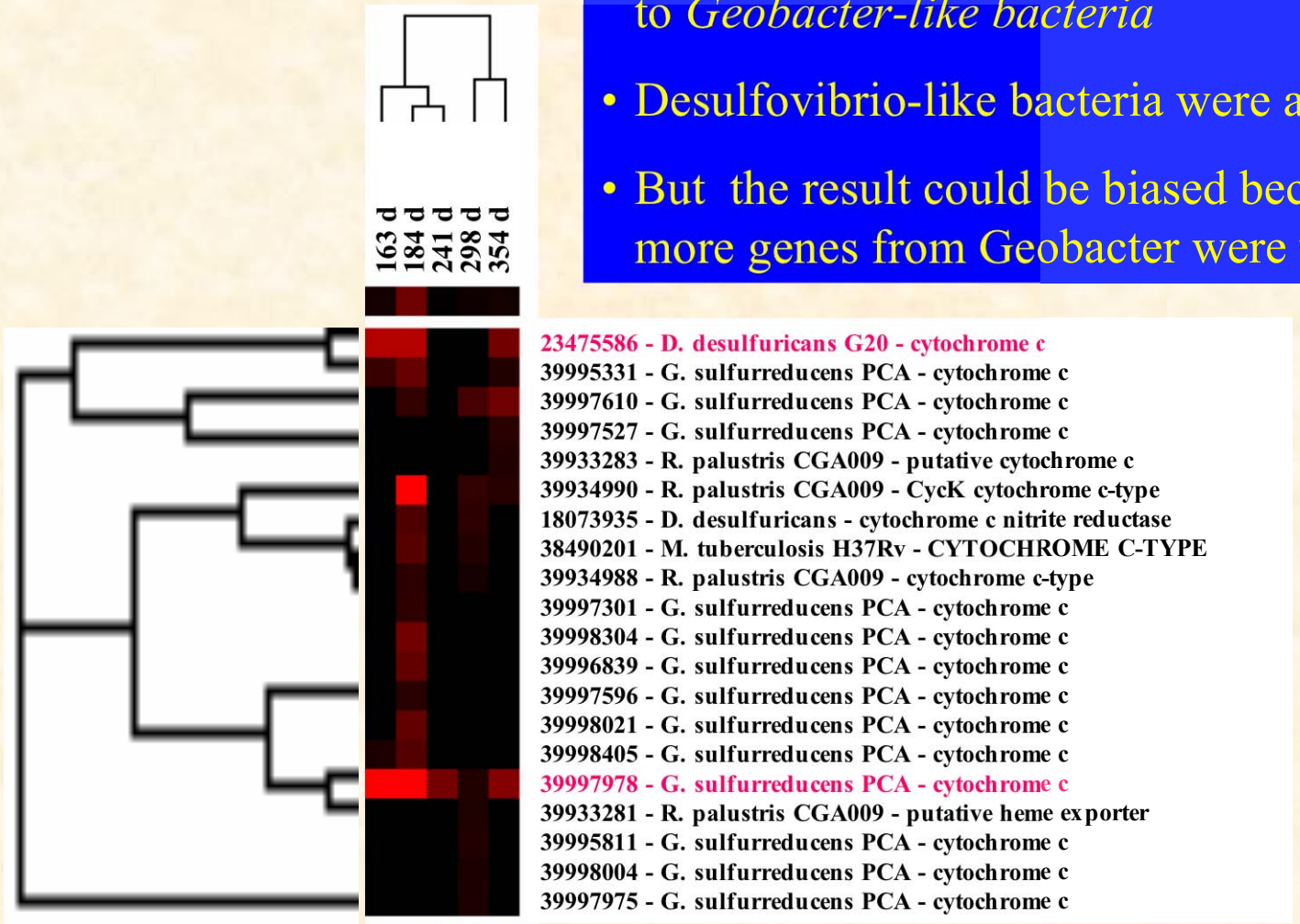


- **Both Geobacter- and Desulfovibrio-like species were detected.**
- ***Geobacter* sp.- like cytochrome C genes followed trends in U(VI) levels.**
- **Most prominent during initial denitrification phase.**

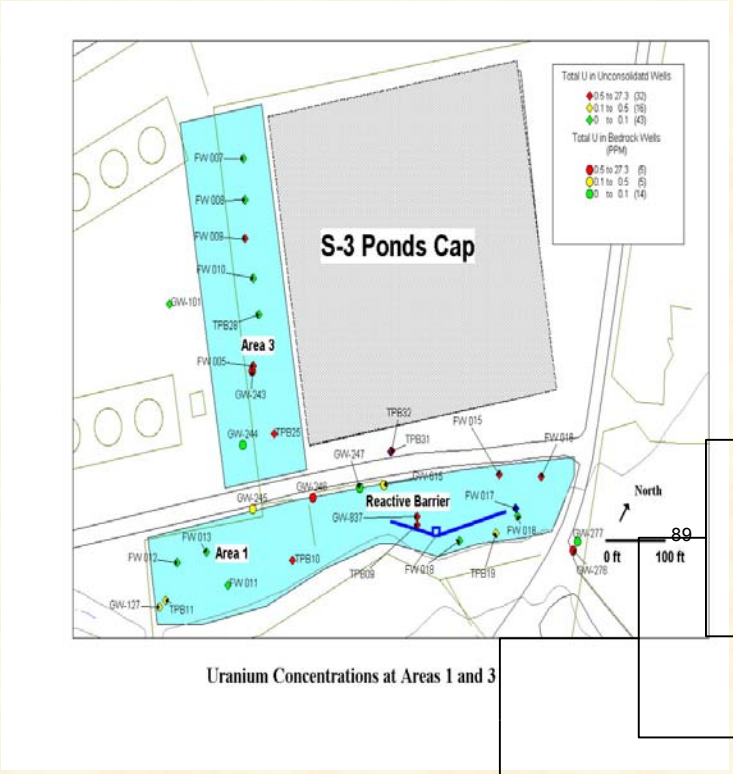
Cytochrome Genes

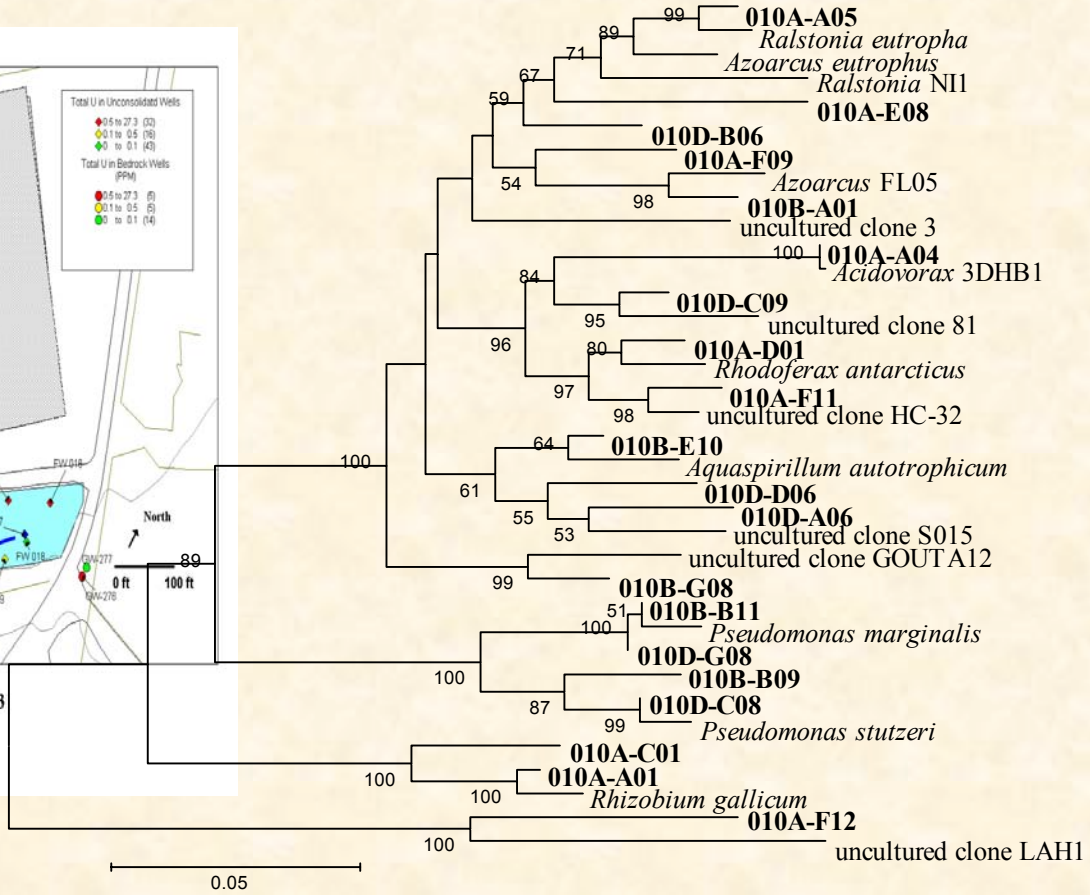
- Most detected cytochrome genes were similar to *Geobacter-like bacteria*
- Desulfovibrio-like bacteria were also detected.
- But the result could be biased because many more genes from Geobacter were used as probes



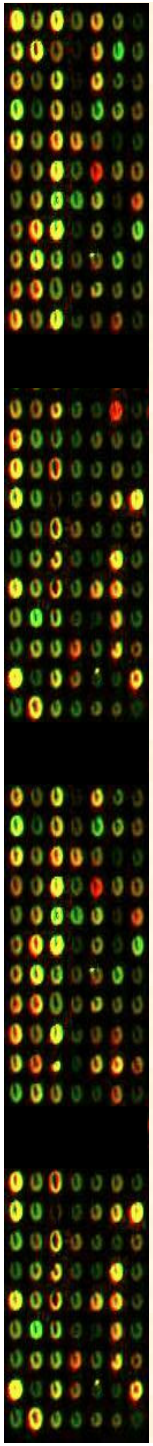


Whole community sequencing



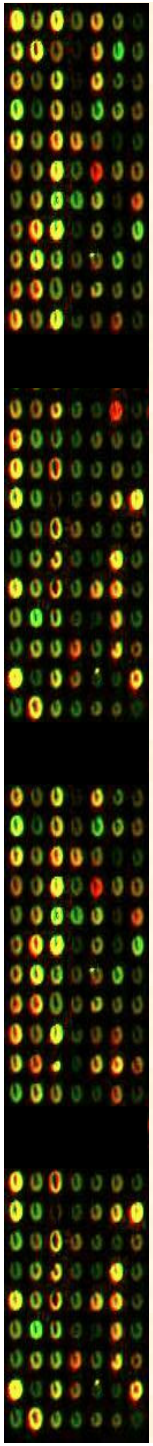


- Sample from NABIR Field Research Center at ORNL
- Sequenced by DOE Joint Genome Institute
- 20 species based on 16S rRNA



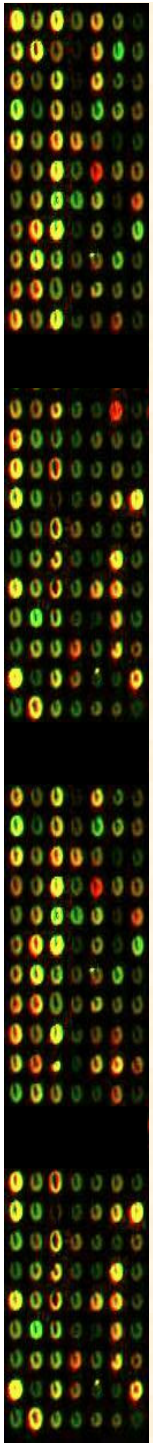
Current status of sequencing

- Collected 2,000 L groundwater
- Took about 6 months to optimize the protocols.
- 300 ug DNA was isolated.
- Sent DNA twice to JGI for library construction.
- Libraries
  - 40 kb fosmid library
  - 8 kb library
  - 3 kb library
- Very good 40kb library was obtained at the first but not 8 and 3 kb library.
- Sequencing is in process



Conclusions

- Development
  - Very comprehensive oligonucleotide arrays for environmental studies were developed. This is the most comprehensive arrays available today.
  - The arrays are specific and quantitative.
  - Novel approach for increasing sensitivity is developed. This made it possible to use microarrays for analyzing environmental samples.
  - New computer program was developed for probe designing.
- Applications
  - Microbial populations are highly heterogeneous in NABIR FRC.
  - Contaminants have significant effects on microbial community structure and dynamics.
  - Microbial populations at the FRC sites can be stimulated for removing uranium.
  - Geobacter and Desulfovibrio-like species could be responsible for uranium reduction after stimulation.



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  - Zhijian Huang
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- **JGI**
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  - Chris Detter
- **Miami University**
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