Application of Functional Gene Arrays for Analyzing Community Structure at the FRC

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FRC Samples used in this study

	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

Area 3

S-3 Ponds Cap

010

005

015

003   16

275 m

Area 1

Area 2

N

30 m
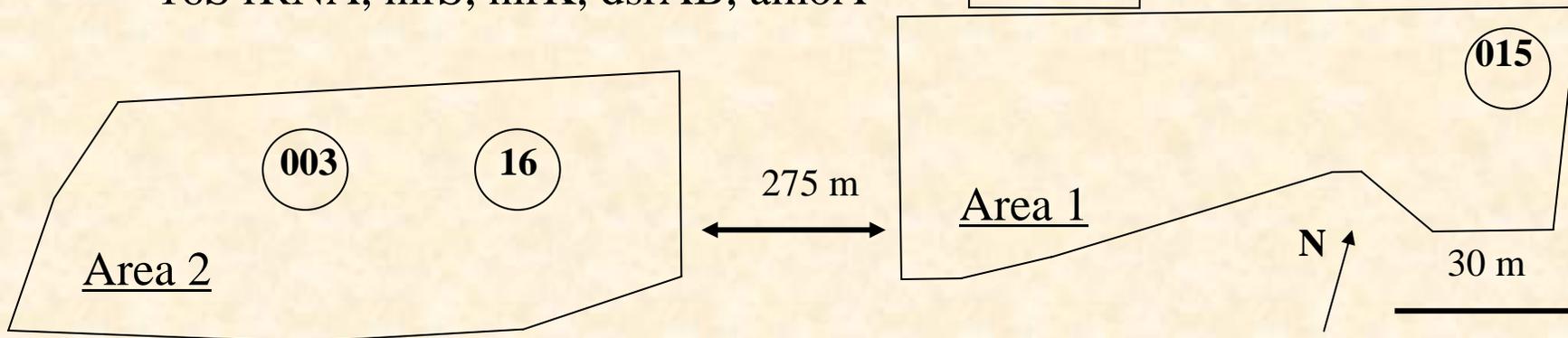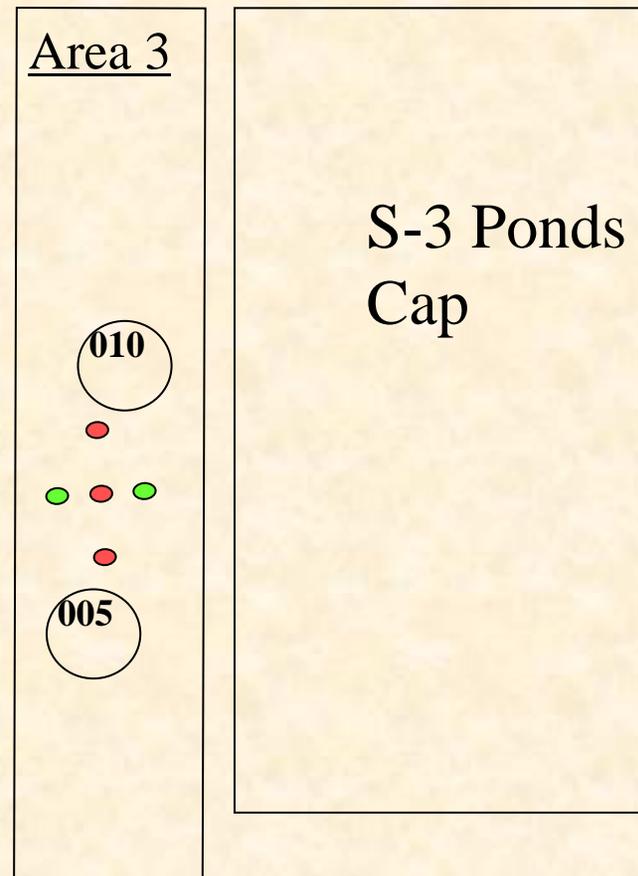
Summary of Diversity in NABIR FRC

Site	SSU rRNA Clones Analyzed (groundwater)	Uranium (mg/l)	pH	Species (OTUs)	H'	1/D
FW-300 (background)	334	-	6.10	158	6.7	66
TPB-16	478	1.10	6.30	244	7.1	73
FW-003	321	0.01	6.00	34	3.0	3.9
FW-005	216	6.40	3.90	47	3.8	6.3
FW-010	**115**	**0.17**	**3.50**	**20**	**3.1**	**4.3**
FW-015	229	7.70	3.40	61	4.3	9.1
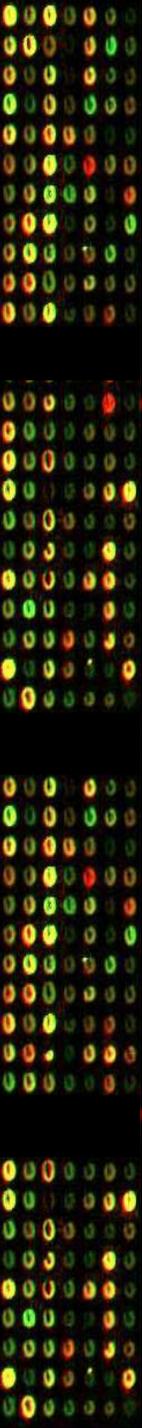
Summary of 50mer-based FGAs for environmental studies

Oligonucleotide probe size: 50 bp

Tiquia et al. 2004. *BioTechniques 36, 664-675*
Rhee et al. 2004, AEM in press

- **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**

Specificity of 50 mer microarrays

>88% sequence similarity can be differentiated.

- 5 nirS genes were mixed together
- Only corresponding genes were hybridized
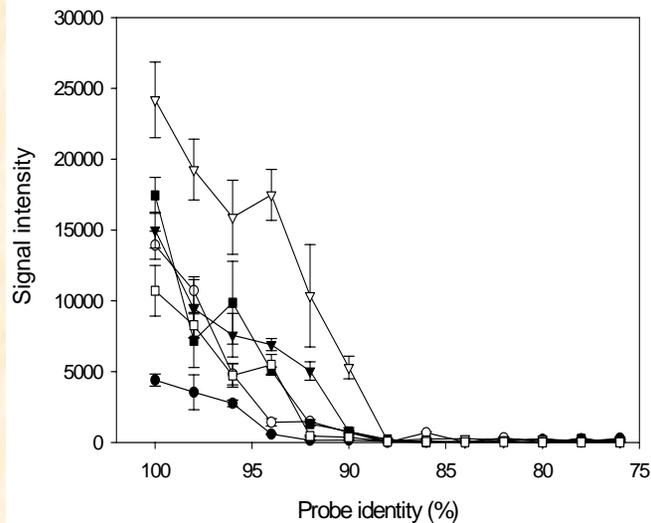
- 6 types of genes were mixed together
- Only corresponding genes were hybridized
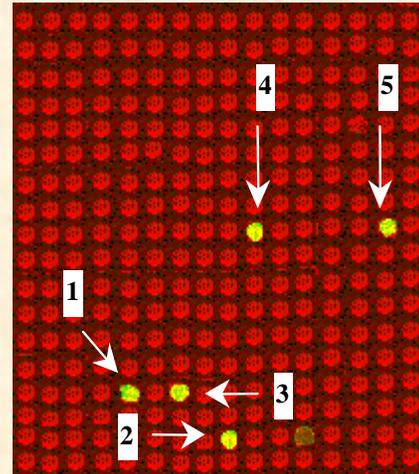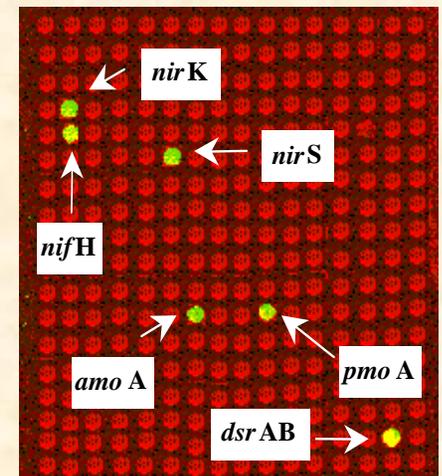
Sensitivity and quantification

Genomic DNA

5
6
7
8
1
2
3
4
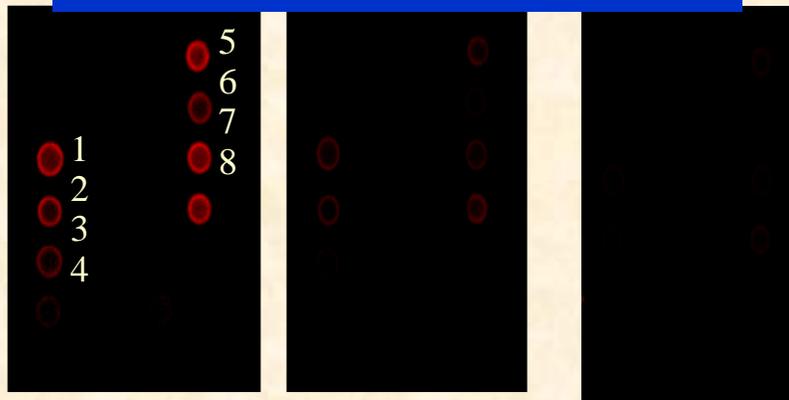
500 ng gDNA   **50 ng**   25 ng

Cells

1.6×10^9 **1.3×10^7** 3.0×10^6

$r^2 = 0.98$

1.6×10^9
8.0×10^8
2.0×10^8 4.0×10^9
1.0×10^7
5.0×10^7
2.5×10^7
3.0×10^6 1.3×10^7
6.0×10^6

Log Signal Ratio (Log R)

Log (Cell Number [N])
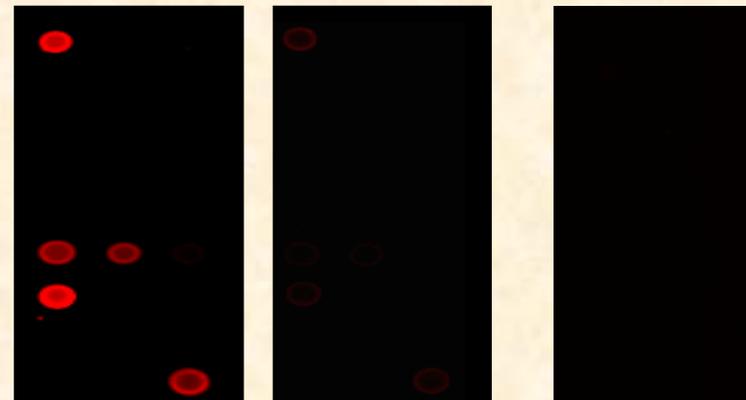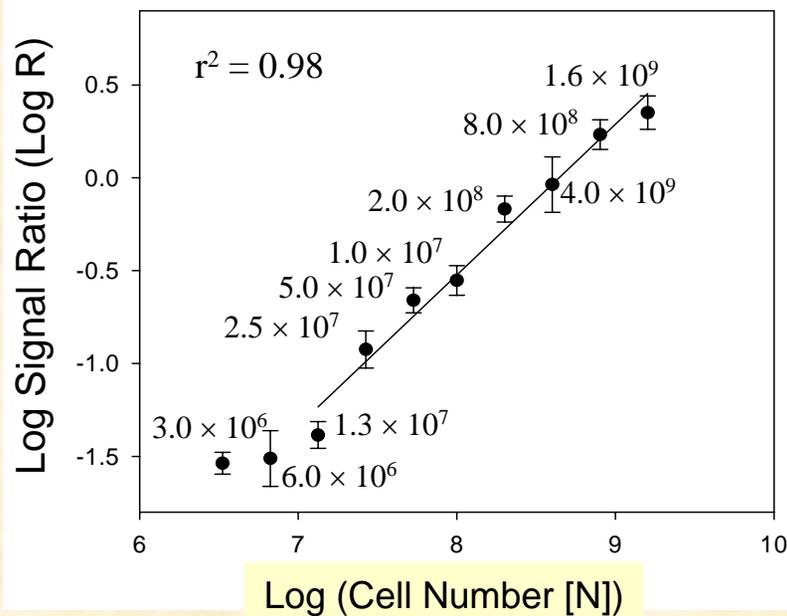
Detection limit

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

Quantification

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

Validation by real-time PCR

r=0.86

● Real Time PCR (Log Copy Number)
○ Microarray Hybridization (Log SNR)
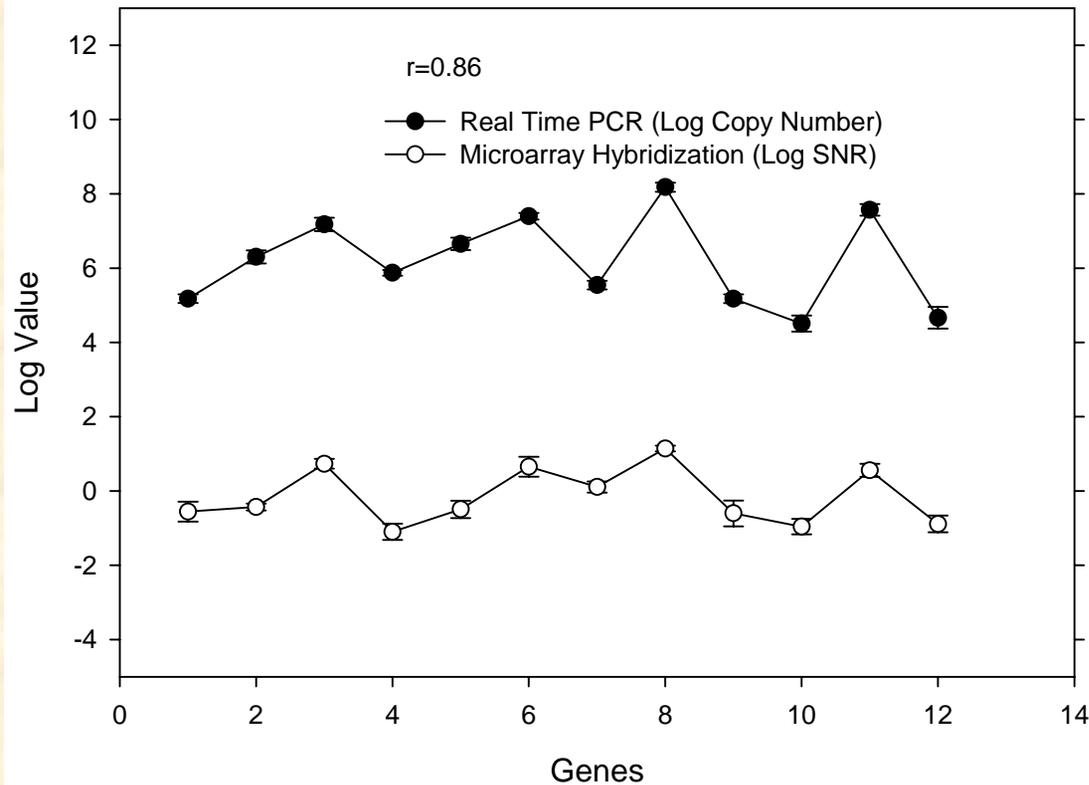
1: gi4704462-TFD
2: gi4704463-TFD-Microcosm
3: gi4704464-TFD-Enrichment
4: gi4704463-TFD
5: gi4704464-TFD-Microcosm
6: gi4704465-TFD-Enrichment
7: gi2828015-TFD
8: gi2828016-TFD-Microcosm
9: gi2828017-TFD-Enrichment
10: gi2828018-TFD
11: gi2828019-TFD-Microcosm
12: gi2828020

Microarray result is consistent with real-time PCR

Hybridization with marine sediment samples
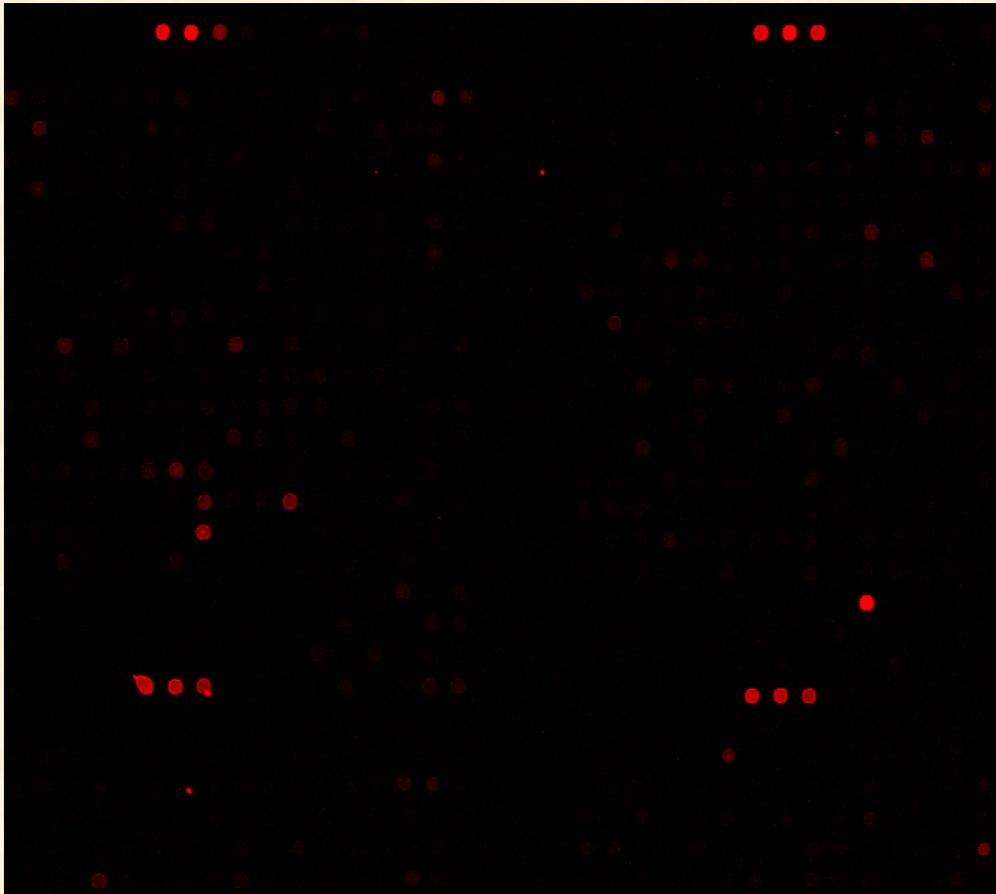
- 5 ug of total DNA from marine sediment was labeled with Cy5 using random primer labeling method.
- Hybridized at 50°C, plus 50% formamide.
- Reasonably good hybridization obtained.
- Dominant populations can be detected.

Amplification for increasing hybridization sensitivity
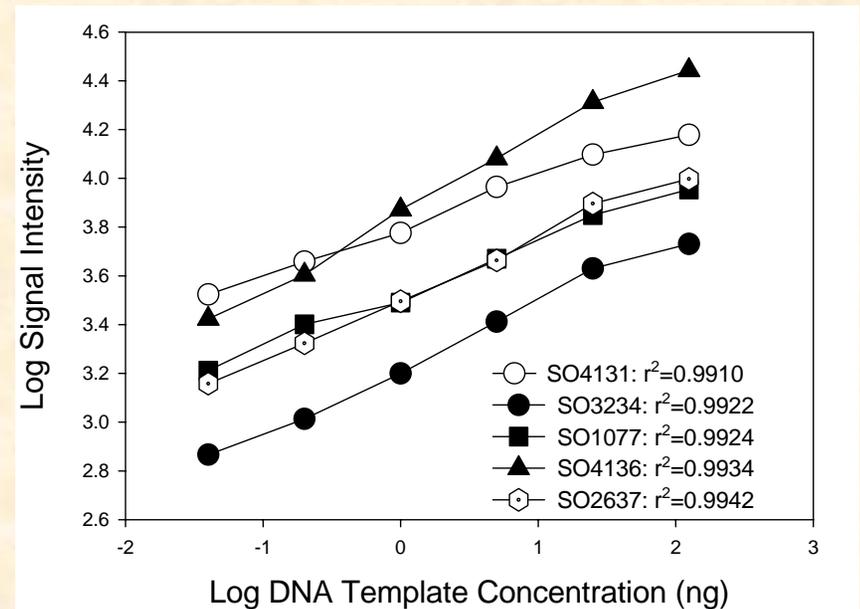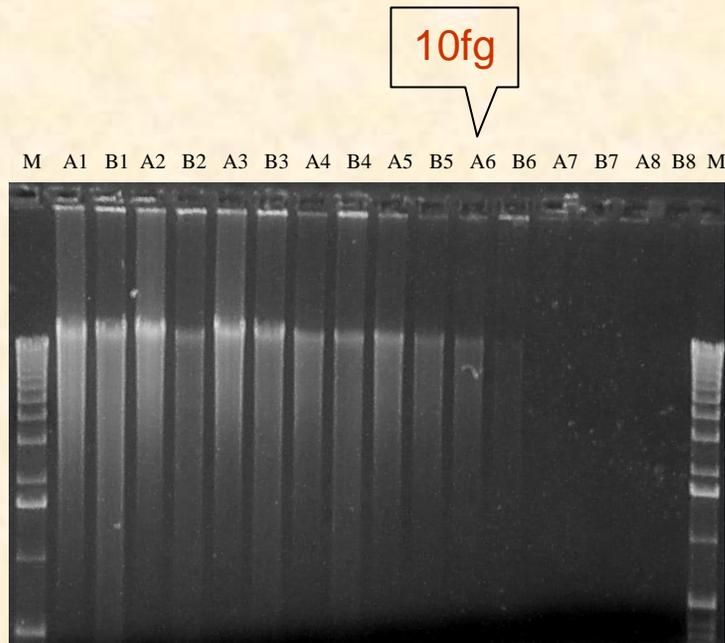
10fg

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

Amplification is quantitative for majority of the genes

Groundwater samples with low biomass
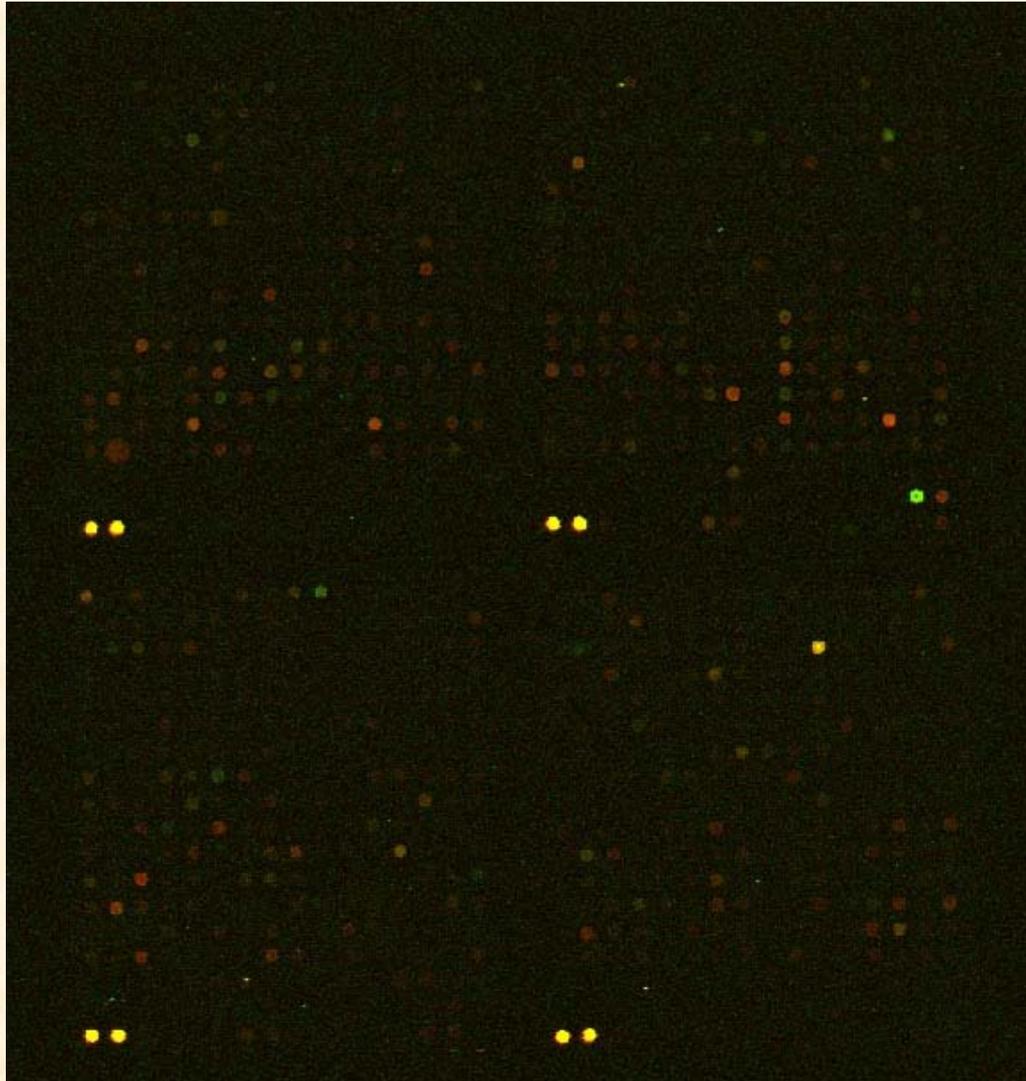
- 2L groundwater from six different sites.
- Cell counts: $1\text{-}5\text{x}10^5$/ml
- DNA was isolated, 1/20 of the DNA was manipulated and used for hybridization.
- Nice hybridization was obtained with the DNA manipulated with the new method.
- No hybridization were obtained if the DNA is not manipulated.

Difference of functional genes in samples from NABIR Field Research Center
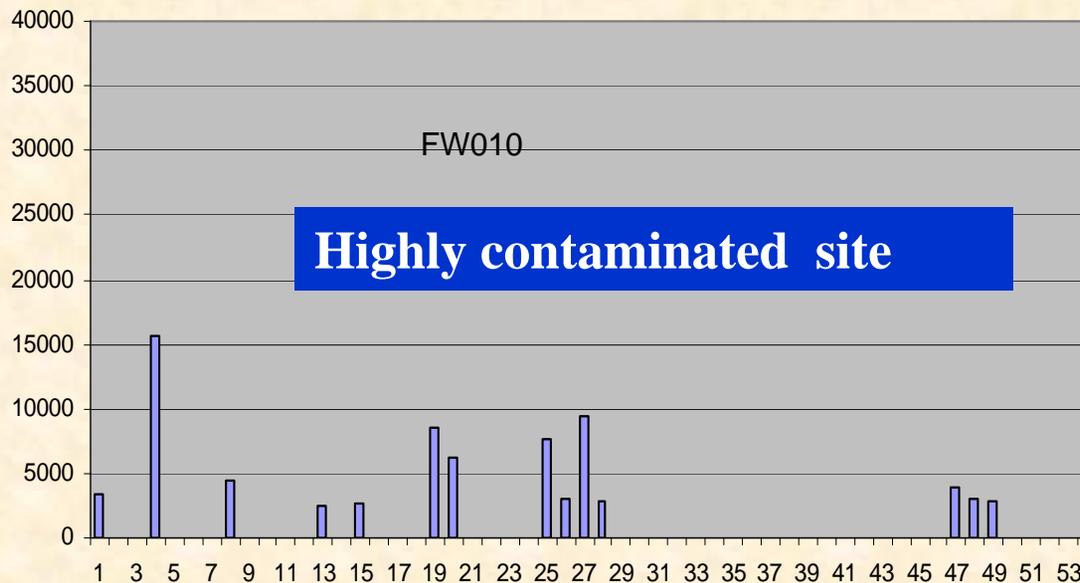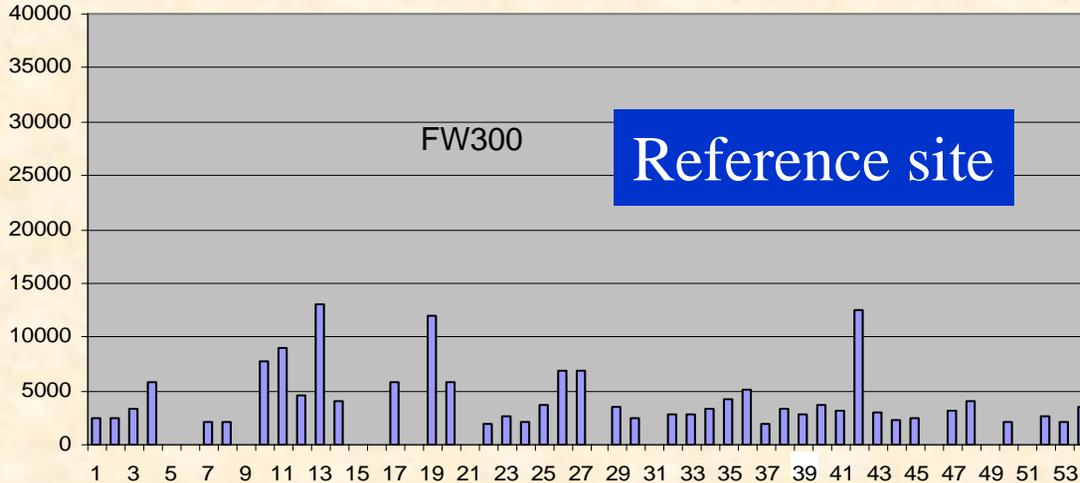
- Clear difference was observed among contaminated and noncontaminated sites.
- E.g., some genes are present in noncontaminated site but not in contaminated sites

Principle component analysis of microarray data

A: PCA-array

B: PCA-chemistry

A
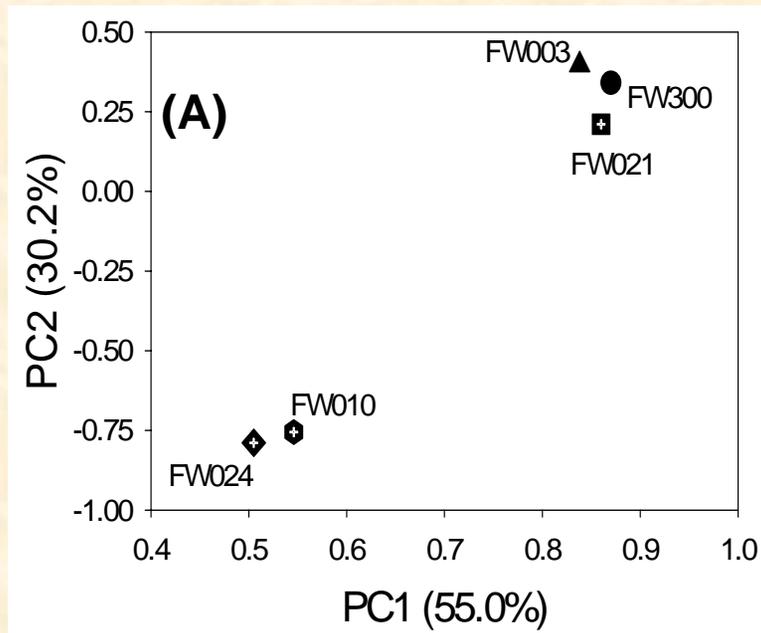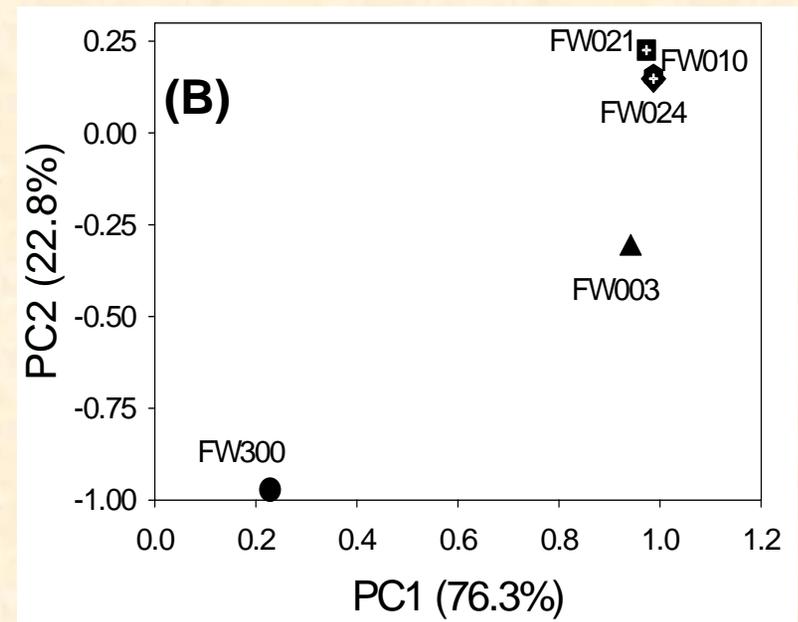
18369658:benzoate, benzoyl CoA reductase subunit , Azoarcus evansii
2429345:Metal, tellurite resistance protein , Rhodobacter sphaeroides
PB20:nirK, lab clone
NKTT70:nirK, lab clone
B9-12S:nirK, lab clone
14523811:nor, NorB nitric oxide reductase [Sinorhizobium meliloti],
NIRND84475:nirS, lab clone,
3641341:catechol, lactone-specific esterase , Pseudomonas fluorescens
21322691:metal, putative mercuric reductase , Pseudomonas aeruginosa
7160897:nirS, nitrite reductase, uncultured bacterium vD4
294671:ammeline, N-ethylammeline chlorohydrolase , Gordonia rubripertinctus
TPB16070B:dsr, lab clone
NKTT43:nirK, lab clone
15077492:dsr, dissimilatory sulfite reductase alpha subunit, Thermodesulfobacteriumthermophilum
3059191:biphenyl, ferredoxin reductase , Rhodococcus erythropolis
Y32S:nirK, lab clone
17549724:nor, PROBABLE NITRIC OXIDE REDUCTASE (SUBUNIT B) TRANSMEMBRANE PROTEIN, Ralstonia solanacearum
FW005271B:dsr, lab clone
FW010274B:dsr, lab clone
M300308B:dsr, lab clone
3021352:dsr, dissimilatory sulfite reductase subunit alpha, Desulfobacterium autotrophicum
11496137:chitinase, chitinase, Ustilago maydis

B

1655815:xylanse, xylanase, Orpinomyces sp. PC-2
18495817:cyclohexane, 6-hexanolactone hydrolase , Xanthobacter flavus
11323312:dibenzothiophene, DszD , Rhodococcus erythropolis
2935034:catechol, dienelactone hydrolase , Rhodococcus opacus
TPB16340A:dsr, lab clone
7363464:endoglucan, endoglucanase M, Clostridium cellulovorans
4239914:phenol, phenol hydroxylase alpha subunit , Burkholderia cepacia
295708:phthalate, PHT1 , Pseudomonas putida
22252786:nirS, nitrite reductase, uncultured bacterium
22252786:nirS, nitrite reductase, uncultured bacterium
22276202:nirS, nitrite reductase, gamma proteobacterium C10-1
21242882:metal, cation efflux system protein , Xanthomonas axonopodis pv. citri str. 306
3057083:nosZ, nitrous oxide reductase, Paracoccus pantotrophus
18034349:dsr, dissimilatory sulfite reductase alpha subunit , Desulfobacterium vacuolatum
13249545:dsr, dissimilatory sulfite reductase subunit B, uncultured sulfate-reducingbacterium
14715456:catechol, 4-oxalocrotonate decarboxylase , Pseudomonas putida
45966:endoglucan, endoglucanse B, Ruminococcus albus
580995:nifD, nitrogenase MoFe protein alpha-subunit (AA 1-534), Clostridium pasteurianum,
M305054:nirK, lab clone
14719374:cyclohexanol, Fe-dependent alcohol dehydrogenase , Brevibacterium sp. HCU
FW010010B:dsr, lab clone
16304107:ppkase, polyphosphate kinase, Nodularia spumigena
FW003218B:dsr, lab clone

C

FW300347A:dsr, lab clone
M300012B:dsr, lab clone
M306059B:dsr, lab clone
FW005196A:dsr, lab clone
15127807:catechol, protocatechuate 3,4-dioxygenase type II alpha subunit PcaG-II , Hydrogenophaga
7160923:nirS, nitrite reductase,uncultured bacterium Y32K
20502013:dsr, dissimilatory sulfite reductase subunit A, uncultured sulfate-reducingbacterium
13249569:dsr, dissimilatory sulfite reductase subunit B, uncultured sulfate-reducingbacterium
15055587:dsr, dissimilatory sulfite reductase alpha subunit, Desulfococcus multivorans
22324383:mmo, particulate methane monooxygenase subunit A, uncultured bacterium
2463460:hydroxybenzoate, 4-Hydroxybenzoyl-CoA reductase beta-subunit , Thauera aromatica
12667602:dsr, dissimilatory sulfite reductase subunit A, uncultured sulfate-reducing bacteriumUMTRAdsr617-4
13249563:dsr, dissimilatory sulfite reductase subunit B, uncultured sulfate-reducingbacterium
3892198:dsr, dissimilatory sulfite reductase beta, Archaeoglobus profundus
14588936:Metal, H+ATPase subunit, plasma membrane , Saccharomyces cerevisiae

D

FW003205B:dsr, lab clone
W306762A:dsr, lab clone
M300002B:dsr, lab clone
13249547:dsr, dissimilatory sulfite reductase subunit B, uncultured sulfate-reducingbacterium
3046914:phenol, phenol hydroxylase , Geobacillus thermoleovorans
W307294A:dsr, lab clone
NKTT09:nirK, lab clone
NIRFD50473:nirS, lab clone,
S86:nirS, lab clone
W306517A:dsr, lab clone
20142110:dsr, dissimilatory sulfite reductase alpha subunit, uncultured bacterium
W301328A:dsr, lab clone
WC1:nirK, lab clone
22776523:nor, nitric-oxide reductase, Oceanobacillus ihevensis
10279673:dsr, sulfite reductase, Desulfovibrio desulfuricans
15080890:dsr, dissimilatory sulfite reductase beta subunit, Desulfotomaculum thermosapovorans
W306570B:dsr, lab clone

	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

•The proportion of unique genes (grey shading in diagonal) in individual wells and a matrix representation of the overlapping number of detected genes between wells.

•In addition the total number of genes detected in each sample and the diversity of those genes is reported.
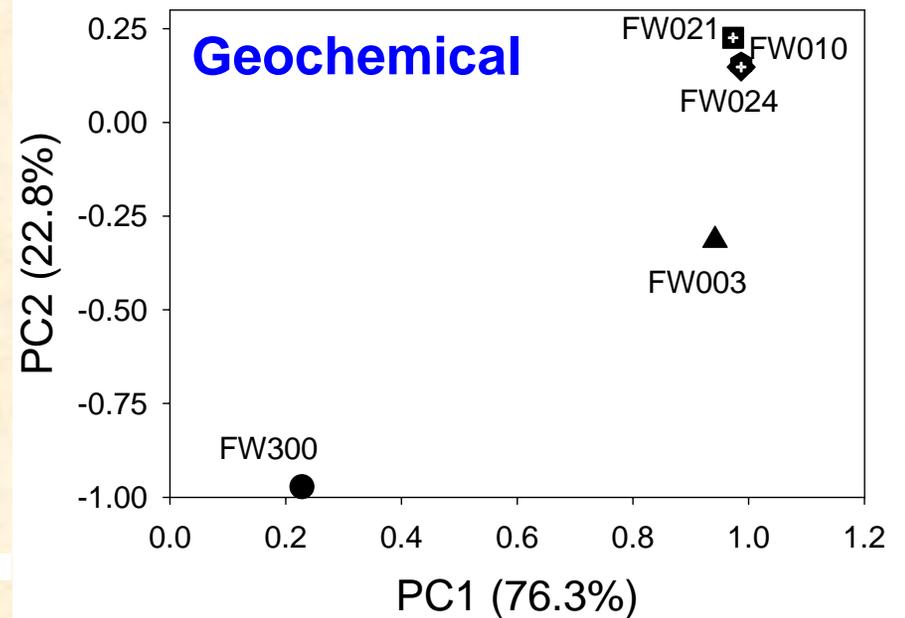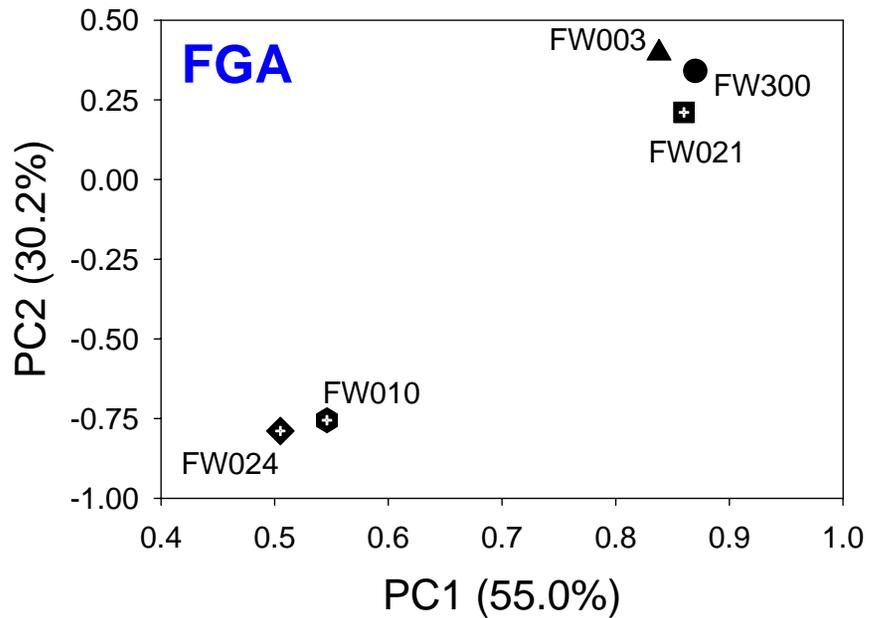
- **Principal components analysis of the FGA data grouped two of the highly contaminated wells (FW010 & FW024) together, thus suggesting that the contaminants impacted the microbial community structure.**

- **The presence of sulfate-reducing bacteria at all sites implies the potential for *in situ* metal (uranium) reduction/remediation.**

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**

- **23,864 probes designed**
- **Will be very useful for community and ecological studies**

Overall Summary of Probes on FGA

Gene Category	Number of Probes		
	Unique	Group	Total
Carbon Degradation	2,532	276	2,808
Carbon Fixation	584	215	799
Metal Resistance/Reduction	4,039	507	4,546
Methane/Methanogenesis	437	333	770
Nitrogen Fixation	1,225	0	1,225
Nitrogen Metabolism	865	902	1,767
Nitrogen Reduction	1,805	501	2,306
Organic Contaminant	6,920	1,087	8,007
Perchlorate Remediation	21	0	21
Sulfur Reduction	1,286	329	1,615
Total	19,714	4,150	23,864

Whole community sequencing – NABIR FRC by JGI
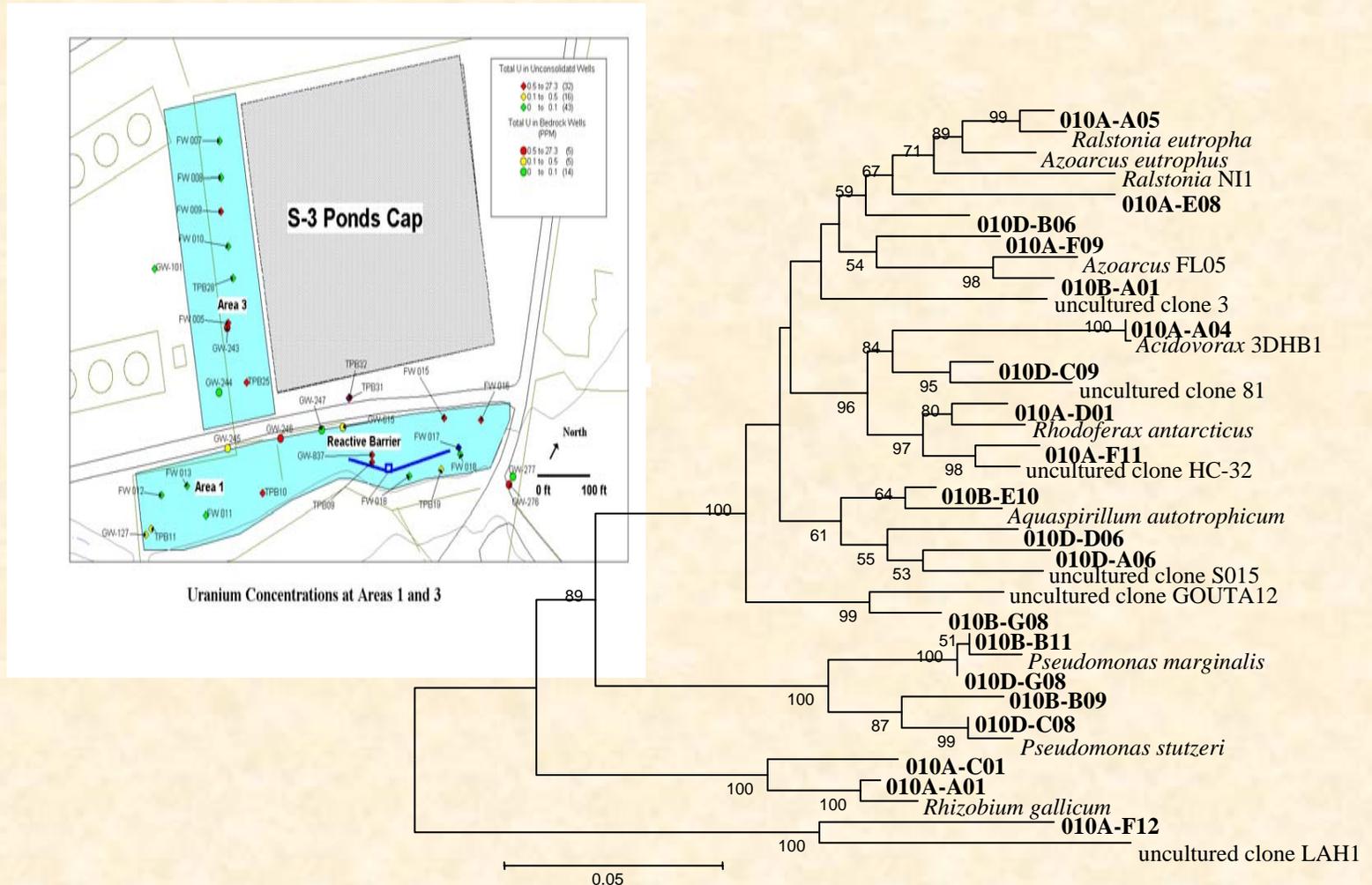
20 species based on 16S rRNA