

# **Environmental Management Science Program**

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## **Molecular Profiling of Microbial Communities from Contaminated Sources: Use of Subtractive Cloning Methods and rDNA Spacer Sequences**

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## Molecular Profiling of Microbial Communities from Contaminated Sources: Use of Subtractive Cloning Methods and rDNA Spacer Sequences

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### Research Objective

The major objective of the research is to provide appropriate sequences and to assemble a high-density DNA array of oligonucleotides that can be used for rapid profiling of microbial populations from polluted areas. The sequences to be assigned to the DNA array are chosen from from cloned genomic DNA sequences (the ribosomal operon, described below) from groundwater at DOE sites containing organic solvents. The sites, Hanford Nuclear Plant and Lawrence Livermore Site 300, have well characterized pollutant histories, which have been provided by our collaborators.

### Research Progress and Implications

At this mid-point of the project, over 60 unique sequence classes of intergenic spacer region have been identified from the first sample site. The use of these sequences as hybridization probes, and their frequency of occurrence, allow a clear distinction between bacterial communities before and after remediation by acetate/nitrate pumping. We have developed the hybridization conditions for identifying PCR products in a 96 well format, a versatile alignment and visualization program (acronym: MALIGN) developed by Dr. Dennis Maeder, has been used to align the ISRs, which are variable in length and sometimes in position of the tRNAs. Finally, in collaboration with Dr. W. Chen and Dr. J. Zhou at ORNL, we have significant evidence that mass spectrometer analysis can be used to determine the lengths of PCR amplified intergenic spacer DNA.

We are using standard DNA isolation, polymerase chain reaction (PCR), cloning, and sequence analysis methods in 96-well microtiter plate formats in order to reduce the cost and time necessary for sequencing hundreds of bacterial intergenic spacer regions (ISRs). ISRs have been amplified by PCR using generic 16s (5 forward) and 23s (5 reverse) primers using DNA from well samples from the Hanford site, representing upper and lower ground water levels, early, mid., and late in the enrichment/pumping procedure used in bioremediation. Libraries of these ISRs have been sequenced, and the sequences aligned and sorted into bins. Bins are described by type (best blast species) and structure of the ISR. We have 60+ unique sequences (=bins) at present, after sequencing more than 300 ISR sequences. Figure 1 shows the structure of the most common type of ISR found in our samples. Other types are described relative to this model.

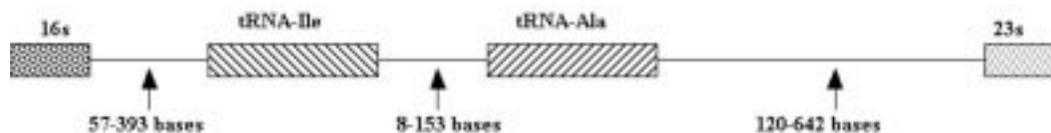
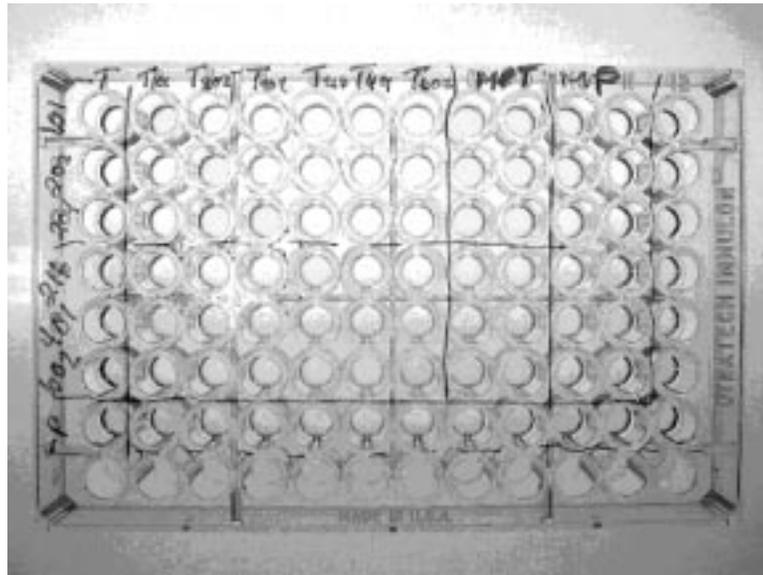


Figure 1. Structure of the most common (67%) ISRs found in Hanford groundwater samples. Only 9% of our clones have no tRNAs (ISR ~200 bases); the remaining 24% have only an Ile tRNA or Glu tRNA, or else the order of Ala tRNA and Ile tRNA is reversed.

Hypervariable stretches of the ISRs, in the inter-tRNA (itRNA) region, have been synthesized and hybridized against synthetic, labelled templates corresponding to the opposite strand, in collaboration with Molecular Tool, Inc, in a 96-well microtiter plate hybridization format (Figure 2). We are collaborating with Dr. Mary Lowe at Loyola College, MD on a particle hybridization/cell sorting/flow cytometry method for fast, iterative hybridization.

Eleven of the unique sequence classes align with *Pseudomonas* ISRs. Other sequences are related to *Thiobacillus cuprinus*, *T. ferrooxidans*, *Agrobacterium vitis*, *Xylophilus ampelinus*, *Bradyrhizobium japonicum*, *Bartonella bacilliformis*, *Xanthomonas maltophilia*, *Prevotella ruminicola*, *Cryptomonas* sp., and *E. coli*. ISRs that are 95% identical in sequence are considered to be from the same species. ISRs that are 85% similar are tentatively assigned to different strains, and if their distribution is of interest (for example, if the occurrence of the strains is noncongruent), we examine them in more detail.

The development of a large sequence database of ribosomal 16s and ISR sequences will reflect genetic diversity and selection at the contaminated sites. The differences in diversity at Hanford sites before and after remediation echo the effects of environmental pollution on plankton and vertebrates. Sequence information and community dynamics will allow better interpretation of pollution history and the progression of degradation.



**Figure 2. Hybridization of itRNAs vs synthetic templates. Fluoresceinated templates were hybridized to synthetic itRNA primers immobilized on the bottoms of the wells.**

## Planned Activities

Ground water from monitor wells within the LLNL Building 834 Area having varying amounts of VOC contamination are being sampled quarterly for microbiological assessment. Five monitor wells have been selected for this study based on their VOC concentrations. Two of the wells selected (W-834-D3, and W-834-D14) have high VOC concentrations, predominantly TCE, in excess of 100,000  $\mu\text{g/L}$ . A third well (W-834-B3), has lower levels of VOCs, around 10,000  $\mu\text{g/L}$ , which is almost entirely composed of 1,2-DCE. All three of these wells have dedicated extraction pumps used for the treatment facility. The two remaining wells selected (W-834-S6, and W-834-T5) have not historically contained any VOC compounds (clean wells, control populations). All five wells selected are installed within the same hydrologic unit.

We will shortly test the environmental DNA from LLNL (as a PCR amplified sample) in an inter-site hybridization experiment against the sequences from the Hanford sites to find out how much overlap there is in microbial community composition. We will also hybridize against sequences described in the NCBI and Deep Subsurface databases, to probe for sequences without the intermediate cloning step. This new sampling site is an ongoing project for LLNL and DOE and will provide us with progressive sampling to test our arrays, particle detection methods, to follow the time-course of contamination and natural remediation effects on bacterial groundwater communities.

## **Other Access To Information**

Brown, D.C, R.H. Shanks, D. Maeder, M.L. Lowe, F.J. Brockman, and Robb, F.T. Profiling microbial communities in polluted ground water: Effects of bioremediation. In prep. for Applied and Environmental Microbiology.

### **Web Site**

<http://comb5-156.umbi.umd.edu/bags.html> (local ISR database)