Microarrays

EMD Team Fact Sheet—November 2011

This fact sheet, developed by the ITRC Environmental Molecular Diagnostics (EMD) Team, is one of 10 designed to provide introductory information about and promote awareness of EMDs. Please review the Introduction to EMDs Fact Sheet along with this one. A glossary is included at the end of this fact sheet.

Why are microarrays relevant?

Microarrays offer the ability to simultaneously detect and semiquantitatively assess the relative abundance of thousands of different microbial biomarker genes as a comprehensive evaluation of the microbial community composition and its potential activity within an environmental sample. Microarray analysis offers advantages at sites that require a comprehensive view of the microbial community and where a larger number of biomarker gene targets need to be monitored to assess biodegradation. Microarray analysis may provide valuable insight into biodegradation of emerging contaminants, for which little is known regarding the microorganisms and degradation pathways involved. Microarrays have been used in research settings since 1996 but have only recently become commercially available for environmental applications. Microarrays have been used to document microbial diversity in a number of environments, including the petroleum release in the Gulf of Mexico (Hazen et al. 2010) and sites impacted by radionuclides like uranium (Chandler et al. 2010, Rastogi et al. 2010).

What do microarrays do?

Environmental samples can contain thousands of different microorganisms and many different functional genes, some of which can serve as process-specific biomarkers. Phylogenetic microarrays evaluate community composition based on the presence/absence of microbial 16S rRNA genes present in a sample and answer the question, “Who is there?” A functional gene microarray targets genes involved in specific processes, for example a gene encoding a key enzyme involved in a degradation pathway, and can help answer questions about “potential activity.” For example, functional gene microarray analysis can provide information on the capabilities of the microbial population to transform contaminants (e.g., degrade organic compounds, reduce metals such as Cr[VI]). Phylogenetic and functional microarrays can also be interrogated with RNA extracted from environmental samples and provide information about general activity (phylogenetic arrays) or about the activity of specific functional genes and pathways (functional array). Thus, microarrays can provide information about activity and determine, “Who is active?” and, “What pathway is active?”

How are the data used?

The strength of the microarray approach is that many species or genes can be monitored simultaneously, and the overall responses of a microbial community to perturbations such as implementation of a remedy can be monitored over time or compared within impacted and background zones. A gram of soil or a liter of groundwater can contain billions of microorganisms, representing thousands of unique species that carry out different processes. Biodegradation of a contaminant of interest may require a single microbial population, a group of microorganisms, or a diverse community. In other words, a process of interest may be sufficiently monitored by looking at the dynamics of a couple of genes (e.g., genes encoding oxygenases involved in aerobic benzene biodegradation) in a couple of candidate species, while monitoring of more complex processes (e.g., nitrogen cycle, sulfur cycle, heavy-metals reduction) may be substantially improved by the analysis of hundreds or thousands of genes or assessment of flux of species present in a diverse community. Thus, microarrays can provide valuable insights for environmental remediation and monitoring.

Example Environmental Remediation Questions Microarrays Can Help Answer

- **Site Characterization**
  - Assess current conditions and potential for biodegradation
    - What known microorganisms or functional genes are present?
    - How diverse is the microbial community?
  - Emerging contaminants
    - What types of microorganisms are present in impacted wells versus background wells?
- **Remediation**
  - Is monitored natural attenuation feasible?
    - Are supporting and contaminant-degrading microorganisms present in impacted zones?
    - Are competing microorganisms present?
  - Is biostimulation necessary? Should an amendment be added?
    - What kind of microorganisms will respond to the amendment?
- **Monitoring**
  - Monitored natural attenuation
    - What known microorganisms or functional genes are present?
    - Are competing microorganisms present?
    - How diverse is the microbial community?
    - Does microbial community structure (e.g., diversity) change over time?
    - What microorganisms are detected in impacted versus nonimpacted wells, and do these microorganisms support contaminant biodegradation?
  - Biostimulation
    - What changes were evident in the overall microbial community composition following amendment?
    - Did amendment addition promote growth of a specific group of microorganisms or increase of functional genes?
    - Is the shift in the microbial community consistent with the biostimulation strategy?
- **Closure**
  - How does the diversity of the microbial community in formerly impacted wells compare to the diversity of the microbial community in background wells?

How do they work?

Microarrays are a collection of many short DNA strands, called “probes,” that are attached to a solid surface (e.g., a glass slide). The probes are selected for their specific, known DNA sequence, to which only complementary pieces of DNA (target) will bind (hybridize). After DNA is extracted from an environmental sample, it is fragmented and labeled with fluorescent chemicals and applied to the microarray. When hybridization (i.e., specific binding) occurs, the labeled DNA that complements its respective microarray probe is bound in place, producing characteristic fluorescent signals. DNA that does not have a complementary probe on the microarray slide is removed in a washing step. Detection and relative quantification are based on the fluorescent signal remaining after the washing step. This approach can also be applied to RNA obtained from the environmental sample. In this case, the RNA is transformed to complementary DNA (cDNA) in a step called “reverse transcription.” Hybridization of the cDNA to the array can provide information about activity. The strengths of the microarray approach are that many genes or species can be monitored simultaneously and the overall responses of a microbial community in response to remedial action can be monitored over temporal and spatial scales.

Figure 1 illustrates the results from a microarray analysis of DNA extracted from two individual environmental samples. For example, Sample A could be a groundwater sample obtained from a monitoring well in the contaminant source area, whereas Sample B could have been collected from a background well located upgradient of the site. The DNA from Sample A is labeled with a green fluorescent dye, and DNA from Sample B is labeled with a fluorescent red dye. Each position or “spot” in the microarray grid contains a specific gene probe. If Sample A contains complementary DNA target

sequences, the labeled DNA fragments will bind to the corresponding gene probes, producing a green signal at each of these positions. If Sample B DNA binds to the gene probes, the signals will be red. If DNA from both samples binds to the gene probes, a combination of both colors will appear (i.e., yellow). In the end, genes detected in Sample A only appear green, genes detected in Sample B only appear red, and genes detected in both samples appear yellow (a mix of green and red). Thus, in this example, the microarray results illustrate which microorganisms are unique to the contaminant source area (green), which are detected only in the background area (red), and which are present in both areas (yellow).

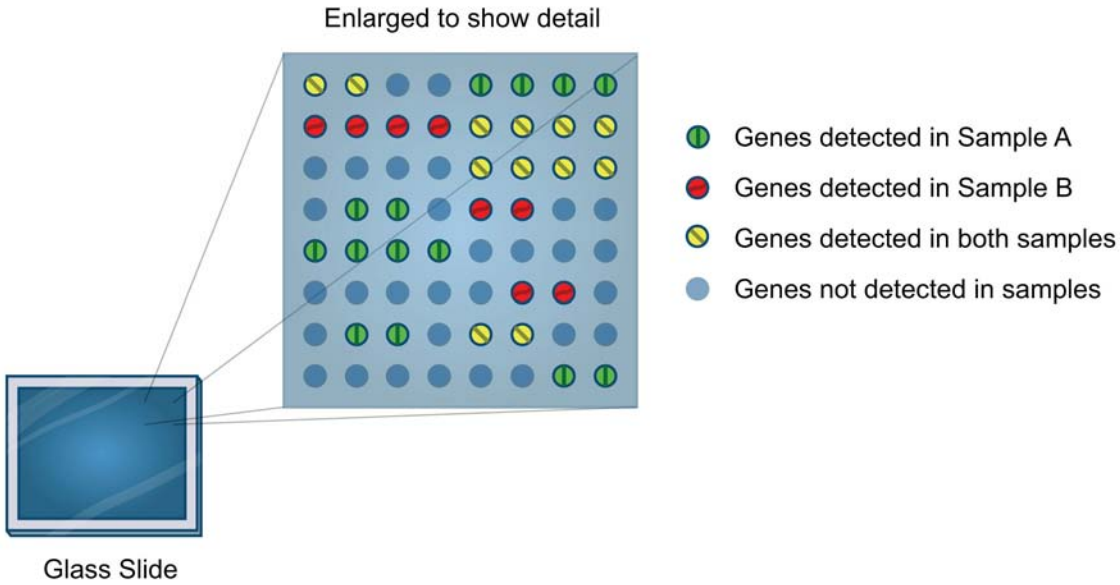

Figure 1. Example of results from a two-dye microarray.

How are the data reported?

Phylogenetic microarray results are usually reported as a list of probable microorganisms (genus and species) detected in the sample. Similarly, functional gene microarray results include a list of the specific genes detected (e.g., a gene encoding nitrite reductase) and the gene type based on the biological process involved (e.g., denitrification). Statistical procedures have been developed that can aid in the interpretation of the results; however, microarray data interpretation requires expertise.

Advantages

- Detection and relative quantification of thousands of organisms or functional genes in a single analysis.
- Information about gene expression (i.e., activity) can be obtained.
- Databases of known microorganisms and functional genes are becoming more comprehensive, making interpretation of results more meaningful and thus microarray analysis more applicable to environmental remediation. Microarrays provide a large quantity of information, which can be used to develop an understanding of the site that may not be possible using conventional environmental sampling and analytical testing. The microarray results may provide project managers with better information to use in the selection of remedial action alternatives or guide the selection of specific EMDs for efficient site monitoring. For example, microarrays can identify site-specific biomarker genes that provide meaningful information, and qPCR can then be applied to specifically monitor these genes.
- Gives an indication of the microbial diversity and possibly identifies the presence of microbes implicated in the biodegradation of the target contaminants. Microarrays can be based on both DNA and RNA, providing information on microbial community structure and metabolic activities, respectively.

Limitations

- At the present time, few microarrays are commercially available that are relevant to environmental remediation.
- Careful design and thorough optimization and testing are needed to eliminate false positive signals (unspecific hybridization). Users should be sure to request documentation from the laboratory about the testing and validation of the microarrays.
- Quantification of the results can be difficult. Although recent studies have demonstrated relationships between signal intensity and target gene abundance, the dynamic range of the signal (i.e., the difference between the maximum and minimum signal) is limited and can hinder accurate quantification.
- Standardization of performance testing across different microarray platforms and guidelines for application and data interpretation are not readily available.
- The interpretation of data typically requires significant expertise, including knowledge of advanced statistical analyses.
- Microarray probes are based on genetic sequences of known microorganisms and biodegradation pathways cataloged in public databases—novel or as-yet undiscovered genes cannot be detected with microarrays. However, as new microorganisms and biodegradation pathways are identified, corresponding probes can be readily added to existing microarrays to expand the applicability of the technique to other contaminants and newly identified biodegradation pathways.

Sampling Protocols

Almost any type of sample matrix (e.g., soil, sediment, groundwater) can be submitted for microarray analysis. Sampling usually involves collecting 10–20 g of soil or 1–2 L of groundwater and placement in sealed containers. Microarrays need a minimum of 2–5 µg of DNA; otherwise, it is necessary to amplify the sample prior to microarray analysis. The following items are typical requirements for microbiological sampling: (a) use of aseptic sample collection techniques and sterile containers, (b) shipment of the samples to the laboratory within 24 hours of sample collection, and (c) maintenance of the samples at 4°C during handling and transport to the laboratory. Sample collection techniques and containers may vary depending on the matrix sampled and the laboratory analyzing the samples. Users should work with the analytical laboratory to ensure sampling protocols for collecting, handling, and transporting the samples are in place and understood.

Quality Assurance/Quality Control

To date, most EMDs do not have standardized protocols accepted by the U.S. Environmental Protection Agency (EPA) or other government agencies. However, EPA (2007) has an interim guidance for microarray analysis. In addition, most laboratories work under standard operating procedures (SOPs) and good laboratory practices, which can be provided to the user (e.g., consultant, state regulator) on request.

Currently, users can best ensure data quality by detailing the laboratory requirements in a site-specific quality assurance project plan (QAPP). This plan should include identification of the EMDs being used; the field sampling procedures, including preservation requirements; the SOPs of the laboratory performing the analysis; and any internal quality assurance/quality control information available (such as results for positive and negative controls). Specifically for microarrays, the arrays typically contain control probes and internal controls for analytical and technical performance of the system, as well as controls for normalization of signal. Standards currently exist for reporting data from microarray analysis (Brazma et al. 2001).

Additional Information

He, Z., Y. Deng, J. D. Van Nostrand, Q. Tu, M. Xu, C. L. Hemme, X. Li, L. Wu, T. J. Gentry, Y. Yin, J. Liebich, T. C. Hazen, and J. Zho. 2010. “GeoChip 3.0 as a High-Throughput Tool for Analyzing

Microbial Community Composition, Structure and Functional Activity," *The ISME Journal* **4**: 1167–79.

He, Z., T. J. Gentry, C. W. Schadt, L. Wu, J. Liebich, S. C. Chong, Z. Huang, W. Wu, B. Gu, P. Jardine, C. Criddle, and J. Zhou. 2007. "GeoChip: A Comprehensive Microarray for Investigating Biogeochemical, Ecological and Environmental Processes," *The ISME Journal* **1**: 67–77. PMID 18043615. www.ncbi.nlm.nih.gov/pubmed/18043615.

U.S. Navy. 2009. "Molecular Biological Tools" website, vers. 1.1. www.ert2.org/MBT/tool.aspx.

Wagner, M., H. Smidt, A. Loy, and J. Zhou. 2007. "Unraveling Microbial Communities with DNA-Microarrays: Challenges and Future Directions," *Microbial Ecology* **53**: 498–506. DOI 10.1007/s00248-006-9197-7.

Wilson, K. H., W. J. Wilson, J. L. Radosevich, T. Z. DeSantis, V. S. Viswanathan, T. A. Kuczmarski, and G. L. Andersen, 2002. "High-Density Microarray of Small-Subunit Ribosomal DNA Probes," *Applied and Environmental Microbiology* **68**(5): 2535–41. PMID 11976131. www.ncbi.nlm.nih.gov/pubmed/11976131.

Wu, C. H., B. Sercu, L. C. Van De Werfhorst, J. Wong, T. Z. DeSantis, E. L. Brodie, T. C. Hazen, P. A. Holden, and G. L. Andersen. 2010. "Characterization of Coastal Urban Watershed Bacterial Communities Leads to Alternative Community-Based Indicators," *PLoS One* **5**(6): 11285. www.plosone.org**.**

Zhou, J. 2003. "Microarrays for Bacterial Detection and Community Analysis," *Current Opinion in Microbiology* **6**: 288–94.

Zhou, J., Z. He, J. D. Van Nostrand, L. Wu, and Y. Deng. 2010. "Applying GeoChip Analysis to Disparate Microbial Communities," *Microbe* **5**(2): 60–65.

References

Biology Online. n.d. "Biology-Online Dictionary." www.biology-online.org.

Brazma, A., P. Hingamp, J. Quackenbush, G. Sherlock, P. Spellman, C. Stoeckert, J. Aach, W. Ansorge, C. A. Ball, H. C. Causton, T. Gaasterland, P. Glenisson, F. C. P. Holstege, I. F. Kim, V. J. Makowitz, J. C. Matese, H. Parkinson, A. Robinson, U. Sarkans, S. Schulze-Kremer, J. Stewart, R. Taylor, J. Vilo, and M. Vingron. 2001. "Minimum Information about a Microarray Experiment (MIAME): Toward Standards for Microarray Data," *Nature Genetics* **29**: 365–71.

Chandler, D. P., A. Kukhtin, R. Mokhiber, C. Knickerbocker, D. Ogles, G. Rudy, J. Golova, P. Long, and A. Peacock. 2010. "Monitoring Microbial Community Structure and Dynamics during In Situ U(VI) Bioremediation with a Field-Portable Microarray Analysis System," *Environmental Science and Technology* **44**: 5516–22. PMID 20560650. www.ncbi.nlm.nih.gov/pubmed/20560650.

EPA (U.S. Environmental Protection Agency). 2007. *Interim Guidance for Microarray-Based Assays*. External review draft. www.epa.gov/spc/pdfs/epa_interim_guidance_for_microarray-based_assays-external-review_draft.

EPA. 2011. "Glossary of Technical Terms." www.epa.gov/oust/cat/tumgloss.htm#a.

Hazen, T. C., E. A. Dubinsky, T. Z. DeSantis, G. L. Andersen, Y. M. Piceno, N. Singh, J. K. Jansson, A. Probst, S. E. Borglin, J. L. Fortney, W. T. Stringfellow, M. Bill, M. E. Conrad, L. M. Tom, K. L. Chavarria, T. R. Alusi, R. Lamendella, D. C. Joyner, C. Spier, J. Baelum, M. Auer, M. L. Zemla, R. Chakraborty, E. L. Sonnenthal, P. D'haeseleer, H. N. Holman, S. Osman, Z. Lu, J. D. Van Nostrand, Y. Deng, J. Zhou, and O. U. Mason. 2010. "Deep-Seas Oil Plume Enriches Indigenous Oil-Degrading Bacteria," *Science* **330**(6001): 204–08.

Nannipieri, P., J. Ascher, M. T. Ceccherini, L. Landi, G. Pietramellara, and G. Renella. 2003. "Microbial Diversity and Soil Functions," *European Journal of Soil Science* **54**: 655–70.

Rastogi G, S. Osman, P. A. Vaishampayan, G. L. Andersen, L. D. Stetler, and R. K. Sani. 2010. "Microbial Diversity in Uranium Mining-Impacted Soils as Revealed by High-Density 16S Microarray and Clone Library," *Microbial Ecology* **59**(1): 94–108. PMID 19888627. www.ncbi.nlm.nih.gov/pubmed/19888627.

Glossary

16S rRNA—A subunit of the ribosome composed of ribonucleic acid (RNA). The RNA sequence is used to classify and identify microorganisms (e.g., genus and species).

biodegradation—A process by which microorganisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment (EPA 2011).

biomarker—A distinctive (unique) characteristic of a biomolecule that can be measured and used as an indicator of a target microorganism or biological process. For example, a specific DNA sequence (used as a probe on a microarray) could be a biomarker for a particular microorganism (e.g., *Desulfotomaculum*).

functional gene—A segment of DNA that encodes an enzyme or other protein that performs a known biochemical reaction. For example, the functional gene *tceA* encodes the reductive dehalogenase enzyme that initiates reductive dechlorination of trichloroethene. Other genes can code for RNA entities which can regulate the activity of other DNA target sequences.

genus—A category of organism classification (taxonomy). A particular genus is a group of related species. For example, *Pseudomonas* is a genus of bacteria.

microarray probe—A short, defined segment of DNA designed to bind with the target gene if found in the environmental sample. The probe is attached to the solid surface of the microarray.

microbial community—The microorganisms present in a particular sample.

microbial diversity—Microbial diversity can have many definitions but in this context generally refers to the number of different microbial species and their relative abundance in an environmental sample (Nannipieri et al. 2003).

nitrite reductase gene—Functional genes encoding the enzymes that catalyze nitrite reduction. Nitrite reductase genes are commonly used as the target gene to detect microorganisms capable of denitrification.

phylogeny (phylogenetic analysis)—Classification of microorganisms into groups (e.g., genus and species) based in part on the rRNA sequences.

ribosome—A multicomponent biological molecule which is part of the protein-synthesizing machinery of the cell.

species—The lowest taxonomic rank and the most basic unit or category of biological classification (Biology Online n.d.).

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ABOUT ITRC

The Interstate Technology & Regulatory Council (ITRC) is a public-private coalition working to reduce barriers to the use of innovative environmental technologies and approaches so that compliance costs are reduced and cleanup efficacy is maximized. ITRC produces documents and training that broaden and deepen technical knowledge and expedite quality regulatory decision making while protecting human health and the environment. With private- and public-sector members from all 50 states and the District of Columbia, ITRC truly provides a national perspective. More information on ITRC is available at www.itrcweb.org.

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