DNA Microarray Rapidly Profiles Microbial Populations

Lawrence Berkeley Natl Lab

Microbial threats, it seems, might be anywhere — bioweapons in the air and water, pathogens in the food supply, diseases in the human population, and changes in microbial populations in the environment, whether the result of natural or human activity.

A number of government agencies and health and medical researchers would like to monitor microbial populations to help keep us safe, cure our diseases and protect the environment. But it's not that easy. In fact, until recently it's been hard — exceedingly hard — to monitor microbial populations with any kind of depth and accuracy.

Oh, sure, a researcher could grab a sample, try to culture it in a petri dish and see what's there. But when you culture bacteria, you're creating an artificial, unnatural environment for the bacteria, and only a small proportion of the bacteria will actually grow under those conditions. If you want to do a more rigorous DNA analysis on the sample, the conventional techniques for preparing samples are tedious, finicky, expensive, error-prone and grindingly slow. Some labs have tried to use robots to automate the sample preparation process. It's rumored that the robots quit because the work was too boring.

> *Now, thanks to research at Lawrence Berkeley National Laboratory, efficient technology transfer and a startup company, there is a much faster, efficient and accurate way to identify and monitor microbial populations. It's called PhyloChip technology.*

The Evolution of a Breakthrough

Gary Andersen, Ph.D., Molecular Microbial Ecology group leader at Lawrence Berkeley National Laboratory (LBNL), is not a man given to puffing himself up. He says, "To start, you have to realize that we have made our accomplishments only by standing on the shoulders of giants. The mid-1990s were a revolutionary time in biology, and my colleagues and I owe a lot to the achievements of those researchers.

"There are two keys that make PhyloChip technology possible," Andersen says. "The first is the discovery that the 16S ribosomal gene — a 1,500-base DNA sequence that all bacteria have — is like a barcode for a bacterium. The 16S gene is used in the assembly of protein, and it's different for every species of bacteria. Researchers have built up a large database of 16S gene sequences from different bacteria, so that now if you have a new community of bacteria, you could identify it on the basis of the previously known 16S sequences."

The second key development, Andersen relates, was the use of DNA microarrays to identify DNA sequences. A DNA microarray is a pattern of microscopic DNA "probes" arranged on a chip. Each probe is a precise sequence of DNA, and it has the capability to detect a specific DNA target. When a samplethat has been treated with fluorescent dye is passed over a microarray,researchers can then tell, by looking at the glowingdots on the microarray, which specific DNA targets have beendetected. Initially researchers were using microarrays to detectthe expression of genes. At first it was possible to test only for100 different DNA targets, but soon it was possible to test forthousands using more advanced microarrays.

"We were the first research team to figure out that this technology could be used to identify bacteria by looking for specific pieces of the 16S gene," Andersen says. "Originally, I experimented with 98-well microtiter plates, and it worked. The first array in its present form that we used had 16,000 probes. That was a huge jump, and it paved the way for making much larger arrays and eventually analyzing them by computer."

Andersen adds, "The other key things we did were to figure out a way to group the probes together to rapidly distinguish one set of targets from another, to make the probes smaller to increase specificity of identification and to use multiple probes to identify each bacteria, which greatly increases our confidence of correct identification."

Going Big Time

With funding from the Department of Homeland Security, Andersen continued to develop and refine PhyloChip technology. And he wasn't alone.

Todd DeSantis, software developer in the Molecular Microbial Ecology group at LBNL, says, "We found out three important things. First, the technology is highly scalable. We started with 16,000 probes in an array, and now we're up to over a million.

"Second, our results are not just qualitative — that is, we can see which species of bacteria are present — but also quantitative: From sample to sample we can see which bacteria are growing in a population. It's tremendously important in ecological and clinical studies to see what the trend is over time."

Third, DeSantis states, the results are highly reproducible in tracking even the low abundance organisms. "That's

critical to making sure the changes you are seeing are real and not some artifact of testing error," he says.

He also notes that as the number of probes on a PhyloChip array has exploded, the ability to analyze the results by computer has become an absolute necessity. "If you were to try to analyze a million-probe chip by hand, it would be just as tedious, time consuming and error-prone as the old DNA analysis techniques that PhyloChip technology replaces. The computer speeds and refines the process."

Licensing the Technology

Virginia de la Puente, senior licensing associate in Technology Transfer and Intellectual Property Management at LBNL, is the first to admit that the licensing history of PhyloChip technology is unusual. "This technology was the overall third-place winner for The Wall Street Journal's 2008 Technology Innovation Awards. You might think that would pretty much guarantee a licensing deal, but it was not to be. We had three or four companies interested, but none of them came back with a proposal." She adds, "There is a certain amount of tension in tech transfer. Big companies want a certain level of development, and small companies generally don't have a lot of money. You have to find the company that's the right fit for the technology."

It took, instead, a chance conversation to get PhyloChip technology licensed. One day Corey Goodman, who was between executive assignments and would ultimately become one of the founders of the company that licensed the technology, was chatting with a neighbor. The neighbor was involved in a water quality experiment involving PhyloChip technology and was raving about it. "You have to check this out!" he said to Goodman.

De la Puente says, "Goodman did check it out and soon entered into a six-month option agreement for the PhyloChip

technology. Then this agreement was extended for another six months. The team Goodman put together hired a pretty aggressive law firm and negotiated hard. Finally a license agreement was signed."

The Realities of Bringing PhyloChip Technology to the Marketplace

In April 2009, a company called PhyloTech was set up specifically to commercialize the PhyloChip technology. "In a very short time, we got the technology transferred and up and running," says Rachel Steger, marketing director for the company. By July 2010, we started selling to customers. That speaks very well to the tech transfer process, which allowed us to commercialize very quickly. In 2011, we're making money and paying royalties."

Steger notes that there is a huge effort in the research community to understand the human microbiome — that is, all the bacteria that reside in and on the human body. She says, "It turns out that any individual has far more bacterial cells than human cells, so many in fact that it amounts to a second genome. This second genome is not well-understood, but our technology is uniquely useful in helping researchers to get a handle on it. Because of that, our company was recently renamed Second Genome."

She adds, "We are selling a service. Customers send us raw or processed biological samples. We do laboratory processing using the PhyloChip technology and perform data analysis on the results. Most of our customers are doing research in academic labs, but we also have customers in industry and in specific applied markets involving waterborne detection. Using conventional techniques, it could take years to get the detailed data that we can provide in just weeks."

PhyloChip technology has already been put to good use in detecting oil-digesting bacteria in the plume from the BP

Deepwater Horizon oil spill, in profiling microbial populations in floodwaters from levee breaks following Hurricane

Katrina and in assessing the health of coral reefs. When the next microbial threat or research question arises, it seems likely Second Genome and PhyloChip technology will play a significant role in keeping us safe, curing our diseases and protecting the environment.

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