Quick Detection of Pathogens by the Thousands

Medical professionals diagnosing diseases, law-enforcement authorities dealing with an apparent bioterrorism attack, and regulatory agencies testing product safety may have a new detection tool to add to their arsenal—the Lawrence Livermore Microbial Detection Array (LLMDA). Designed by a team of Livermore biologists and informatics specialists, LLMDA can simultaneously identify thousands of known viruses and bacteria within 24 hours.

Current detection systems, such as polymerase chain reaction (PCR) technologies, focus on small, prioritized sets of high-risk biological pathogens. LLMDA, however, can identify a broad range of organisms, including pathogens on a priority screening list, sequenced bacteria or viruses that might not be anticipated, or even emerging pathogens containing DNA sequences previously identified in other pathogens.

Computer scientist Thomas Slezak, who leads the Laboratory’s pathogen bioinformatics team, came up with the idea for LLMDA after working on the biosecurity system deployed by the Department of Homeland Security (DHS) at the 2002 Winter Olympic Games in Salt Lake City, Utah. “The PCR signatures we were building provided diagnostic protection only for the short list of high-risk pathogens that DHS had chosen to focus on,” he says. “I realized that new advances in microarray technology would allow us to design a single assay to detect any sequenced bacterium or pathogen.”

Work on the first-generation LLMDA began in 2007 as a Laboratory Directed Research and Development project. In designing the microarray, the bioinformatics team collaborated with researchers at institutions worldwide, including the University of California at San Francisco; University of Texas Medical Branch at Galveston; National Institute for Public Health and the Environment in Bilthoven, Netherlands; Statens Serum Institut in Copenhagen, Denmark; Imigene in St. Petersburg, Florida; and U.S. Food and Drug Administration. Crystal Jaing, a biologist in Livermore’s Physical and Life Sciences Directorate, leads the LLMDA laboratory research, which includes probe designer Shea Gardner, biostatistician Kevin McLoughlin, and biologist James Thissen.

Pathogens Exposed

The LLMDA process begins by purifying DNA or RNA from a blood or stool sample. The purified DNA or RNA is labeled with a fluorescent dye and then squirted onto the microarray, which sits on top of an incubator heated to 42°C. The microarray contains nearly 400,000 probes arranged in a checkerboard pattern on a 2.5- by 7.5-centimeter glass slide. Scientists examine these probes using a fluorescent scanner and analysis software. “If a DNA sequence from the sample matches a sequence on the microarray, the target DNA will bind to the microarray, and that spot will fluoresce, or light up,” says Jaing. “By looking at the sequences that light up, we can identify the virus or bacterium, sometimes down to the strain level.”
LLMDA is designed to improve on two pathogen identification techniques: PCR analysis and sequencing. PCR analysis is relatively inexpensive and fast. It also has a high sensitivity for known organisms, but it can process no more than 50 DNA signatures at one time. The likelihood of discovering new species is also low with PCR analysis. Sequencing is a highly effective approach—in fact, it may provide the most comprehensive information about biological pathogens, both known and unknown. The process, however, is costly and can take several days to produce results.

LLMDA is less expensive than sequencing and more inclusive than PCR analysis. It can identify any sequenced virus and bacterium. The microarray’s checkerboard has several dozen squares for each of the thousands of organisms sequenced to date, so it can simultaneously examine multiple regions from each organism.

The Livermore team is testing a next-generation LLMDA that contains 2.1 million probes representing about 178,000 sequences from 5,700 viruses and 785,000 sequences from thousands of bacteria. The latest version also includes about 237,000 sequences from hundreds of fungi and about 202,000 sequences from 75 protozoa. “We are designing the next-generation array to be the most comprehensive in the world,” says Jaing. “Our goal is to develop a cost-effective technology that can detect both known and unknown pathogens within 24 hours.”

Wide-Ranging Potential

Jaing foresees many potential applications for the microarray technology. For example, LLMDA could perform routine quality-control checks during a manufacturing process to ensure that no harmful pathogens are present in consumer products. A collaboration with the Blood Systems Research Institute in San Francisco, California, demonstrated how useful the technology can be.

In January 2010, while using a DNA sequencing technology to analyze several vaccines, a scientist at the institute found a contaminant in one of the products being tested. Results showed that a vaccine to prevent rotavirus—a disease that causes severe diarrhea in children—contained a benign pig virus, porcine circovirus-1. Tests of the vaccine with LLMDA confirmed the institute’s results. The manufacturer’s quality-control tests did not detect the pig virus because it was not listed as a known contaminant for the rotavirus vaccine. According to Jaing, technologies such as LLMDA would allow a manufacturer to identify every biological material that is present in quantities large enough to be of concern.
LLMDA could also help law-enforcement officials. The National Biodefense Analysis and Countermeasures Center in Frederick, Maryland, is evaluating the technology as a bioforensic tool. The DHS center analyzes biological samples related to criminal cases under investigation by the Federal Bureau of Investigation. It is considering LLMDA for two scenarios: to perform the initial forensic characterization of a substance, which is typically done by PCR analysis, or to provide an independent identification of the organisms present. Another potential law-enforcement application is characterizing an unknown substance, such as a package of “white powder,” to determine whether it is a dangerous pathogen.

Doctors also might one day use the LLMDA technology to diagnose human disease. Clinical samples could be analyzed to identify the cause of a patient’s symptoms. For example, doctors could easily determine whether flu-like symptoms are caused by influenza or one of dozens of other viruses or bacteria. “One of our collaborators used the microarray to diagnose a range of bacterial and viral organisms in human samples,” says Slezak. “As the cost of the array is reduced, the technology could improve public health diagnostics because it can detect multiple bacteria and viruses in a single test.” He cautions, however, that further work is needed, including approval from the Food and Drug Administration for clinical trials to measure the technology’s efficacy in diagnosing human disease.

A Friend to Animals

Jaing is working with the Marine Mammal Center in Sausalito, California, to diagnose diseases that have struck California sea lions and harbor seals. In June 2009, about 20 harbor seals died in northern California from brain lesions caused by the premature death of living cells. In addition, cancer has killed about 17 percent of the adult sea lions at the center.

The Livermore researchers have used LLMDA to analyze frozen tissue samples from two dead sea lions. One sample contained calcivirus, which does not cause cancer. No virus or bacteria could be positively identified in the other sample. Frozen tissue samples from four sea lions and four harbor seals are being analyzed.

“This research has the potential to be very helpful because the genetic makeup of sea lions and harbor seals has not been well studied,” Jaing says. “LLMDA gives us the potential to identify novel viruses or bacteria that can cause cancer in other marine animals.”

In addition, says Slezak, “The work on marine mammals provides excellent training for emergency response should the nation experience a disease outbreak from an unknown human pathogen. It is only through these sorts of collaborations that the Livermore team can continually improve our microarray probe design and analysis software and our sample preparation techniques to be ready for a day that we hope never comes.”

Looking Ahead

The team will update the array annually to include new viral and bacterial sequences. “We don’t know how many sequences are available worldwide,” says Jaing. “Different outbreaks occur every year, and there could be undiscovered viruses and bacteria that we have yet to access.”

LLMDA has great potential for improving processes used by medical professionals, law-enforcement personnel, and product manufacturers. “Each proposed application will require various levels of validation and approvals before it can be deployed,” says Slezak. “That process could take several years.” Deploying LLMDA in these different applications could better prepare the nation when the next outbreak from an unknown pathogen hits.

—Kristen Light

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