JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

the PRIMER

October 2006 Volume 3 Issue 2

First Tree Genome Is Published:

Poplar Holds Promise as Renewable Bioenergy Resource

Wood from a common tree may one day figure prominently in meeting transportation fuel needs, according to scientists whose research on the fast-growing poplar tree is featured on the cover of the September 15, 2006, edition of the journal *Science* (vol. 313, No. 5793).

The article, highlighting the analysis of the first complete DNA sequence of a tree, the black cottonwood, or *Populus trichocarpa*, lays groundwork that may lead to the development of trees as an ideal "feedstock" for a new generation of biofuels such as cellulosic ethanol. The research is the result of a four-year scientific and technical effort, led by the U.S. Department of Energy's Joint Genome Institute (DOE JGI) and Oak Ridge National Laboratory (ORNL), and including the University of British Columbia; Genome Canada; Umeå University, Sweden; Ghent University, Belgium; and 30 other institutions from around the world,

"Biofuels could

(cont. on page 6)

No Guts, No Worries—

Wonder Worm Enlists Full-Service Microbes for Transportation, Energy, & Waste Management

Researchers have now characterized the unique lifestyle of a gutless worm that commutes through marine sediments powered by a community of symbiotic microbial specialists harbored just under its skin, obviating the need for digestive and excretory systems.

Using DNA sequencing and other diagnostic techniques, scientists from the U.S. Department of Energy's Joint Genome Institute (DOE JGI) have described this complex worm/microbe collaboration in a species of marine oligochaete worm isolated off of the coast of Elba, the Mediterranean island of Napoleon's exile. Their results are published in the September 17 edition of the journal *Nature* (http://www.nature.com/nature/journal/vaop/ncurrent/abs/nature05192.html).

The worm, *Olavius algarvensis*, has no mouth to take in food, but does not go hungry, thanks to the goodwill of its hardworking bacterial tenants. In the transaction, the worm shuttles the bacteria to optimal energy sources it encounters wending its way between the upper oxygen-rich and the lower oxygen-depleted coastal sediments. In exchange, fixed carbon, all required amino acids and vitamins are synthesized by the subcuticular communities of microbial symbionts, providing their host with ample nutrition.

On the other end of the digestive equation, such waste products as ammonium and urea, generated by the worm's metabolism,

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Better Sludge Through Metagenomics—

Researchers Seek to Master Wastewater Treatment Failures

Few stop to consider the consequences of their daily ablutions, the washing of clothes, the watering of lawns, and the flush of a toilet. However, wastewater treatment—one of the cornerstones of modern civilization—is

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Hold the Date

2007 JGI Users Meeting
March 28-30, 2007
Walnut Creek Marriott

For more information,
contact Marsha Fenner
MWFenner@lbl.gov, 925-296-5781

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Finishers Convene in Santa Fe

BY REBECCA E. MCINTOSH

The tedious, sometimes frustrating job of finishing was given well-deserved praise at the recent "Finishing in the Future" workshop, when keynote speaker Julian Parkhill of the Sanger Institute in Cambridge, England, explained exactly why finishing is so important: Even though shotgun sequence can yield about 99.9 percent accuracy, tiny changes such as single base knockouts can have drastic phenotypic effects on an organism. For example, *Y. pestis*, the causative agent of plague, does not have a flagellum, but its genome includes the blueprints for one. A single-point mutation has been found to inactivate the regulator for the entire flagellum biosynthesis mechanism, leaving the organism non-motile.

In the past, finishing has been a topic of discussion at many sequencing meetings and workshops, but Finishing in the Future, held on May 4–5 in Santa Fe, New Mexico, was the first workshop solely dedicated to finishing accomplishments and techniques. It was hosted by the Los Alamos National Laboratory (LANL) and was organized by Chris Detter (LANL), David Bruce (LANL), Patrick Chain (LLNL), Cliff Han (LANL), Alla Lapidus (DOE JGI), and Jeremy Schmutz (Stanford HGC).

Julian Parkhill's keynote address gave an overview of the value of finishing as well as some recent accomplishments in the field. Representatives from many of the major sequencing centers talked about their own finishing protocols and techniques and compared notes on which are more successful and on problems finishers tend to face. Participants also attended a poster session and numerous round table discussions on such topics as technology development, computational finishing methods, and laboratory finishing methods.

As the essence of finishing is to problem-solve or troubleshoot through sections of sequence that were not initially sequenced correctly, many of the talks focused on protocols for specific problems (i.e., hard GC stops and highly repetitive sequences). Many participants found it helpful that the workshops allowed so much time for information sharing. Alla Lapidus noted that participants requested protocols from DOE JGI, and that JGI also requested a few from TIGR and Baylor.

Finishing in the Future also allowed participants to explore broader issues, such as the difficulty of transforming finishing into a high-throughput format.

Members of the Sanger Institute demonstrated their automation of the finishing process using robotics, but showed that completely eliminating the human component and making the process cost-efficient still are major hurdles. However, Cliff Han explained that the future of finishing will need to incorporate automation in process control and data analysis, and in wet lab protocols, to meet the needs of the scientific community. For instance, understanding metagenomes will require finishing enormous amounts of sequence that would stretch current finishing labs to the max. Moreover, future dreams of finishing large genomes and even eukaryotes will depend on finding ways to efficiently take advantage of the skill of technicians as well as the speed of robots.

If you would like to learn more about the Finishing in the Future meeting visit the website *http://finishingfuture.lanl.gov/index.html* or, to get involved with next year's event, contact Chris Detter, cdetter@lanl.gov.

SPOT Award Recipients

Samuel Pitluck - For submitting DOE JGI's DNA sequence data to GenBank, following through on the ever-changing requirements for data submission.

Steve Wilson - For developing the Safety Track system, which has vastly improved JGI's ability to track and learn from safety incidents.

Wendell Hom - For his excellent work in completing the Octopus proposal in record time.

Ron McKeever - For his outstanding performance during the FY 06 JGI property audit.

Duncan Scott - For exceeding expectations in the development of a new inoc list planning and reporting system, yielding a major positive impact on the JGI production line.

Jason Baumohl - For greatly exceeding customer expectations in the development of a new plating process management system.

The Finishing Team - For their efforts in establishing the DOE Joint Genome Institute as a leader in microbial genome finishing—accounting for nearly a quarter of all finished microbial genomes worldwide. Pictured are Alex Copeland, Steve Lowry, Eugene Goltsman, Paul Richardson, Ben Horowitz Vasanth Singan, and Alla Lapidus. Pat Kale and Stephan Trong are not pictured.

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Small Game Hunting in Costa Rica

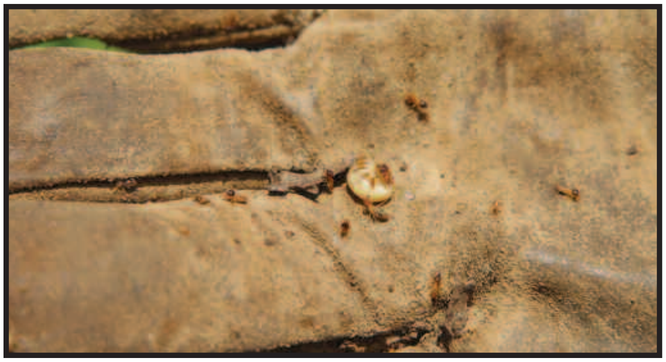
Falk Warnecke (foreground) and Eric Matson, post-doc from collaborator Jared Leadbetter's Caltech lab, secure the termite materials under the watchful eye of Phil Hugenholtz. (Photo by Hector Garcia Martin)

In late May, members of the DOE JGI Microbial Ecology Program headed south for a second metagenomic sampling trip to Costa Rica. As before, the subject of the expedition was the humble termite and the explorers were not disappointed. The rainy season was just beginning in Costa Rica and there was a good chance that the field trips would be rained out. However, the weather gods smiled upon the expedition and thousands of termites were successfully collected and brought back to the remote lab for processing.

A key target for the sampling trip was the same genus collected last year, *Nasutitermes*, in which hundreds of wood-hydrolyzing enzymes were identified. This time around, a different part of the hindgut was targeted—the alkaline P1 section—in the hope that a similar number of enzymes would be discovered that were optimized for high pH. These enzymes would be very useful in industrial cellulosic ethanol production where an alkaline pretreatment is used. In addition, samples of the termite hindguts were taken for investigating viruses and gene expression (mRNA), and for microscopy.

In addition to the target species, a number of other fascinating termite species were discovered, usually by Jared Leadbetter, a lead investigator from Caltech, who seems to have a sixth sense when it comes to finding termites. A dark-purple ground-dwelling termite was uncovered at the base of a plant and appears to feed on very decayed and blackened leaf litter. This could be of interest for bioenergy production, since the hindgut community may be adapted to degrading lignin, tannins, and humic acids not degraded by other termite genera such as *Nasutitermes*. The purple termite proved to be a very aggressive species, and the soldiers have large, powerful jaws and a long unicorn-like “nasute” from which terpenes are fired at enemies. A second *Nasutitermes* species with a jet black head was found to have built a covered pathway up the trunk of a tree that extended for over 50 feet, presumably leading to their nest (the tree was too tall to spot the nest). The pathway was so established that moss had grown over it, providing the termites with a secure and discrete channel for foraging in the undergrowth below.

The termite project is a collaboration between DOE JGI, Caltech, the San Diego-based biotechnology company Diversa, and INBIO, the National Biodiversity Institute of Costa Rica.



Termites in hand.

A termite nest.

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DOE JGI FACES

Erika Lindquist—Point Person

Sometimes, a career path isn't a straight line. Sometimes it requires searching and trying many things until you discover what's right for you. In the case of Erika Lindquist, her career as a scientist was just that—a journey. Erika's journey included jumping out of airplanes, creating and testing detonators, fighting in a war, and spending endless hours in a scientific laboratory before she decided to focus and become a scientist. At present, Erica is the point person for DOE JGI's EST and cDNA projects. All of her adventures and experiences made her a better scientist by providing her with patience, persistence, and a different perspective.

Erika Lindquist was destined to be an engineer. Her father was a fusion engineer and project manager at LLNL who exposed her to math and science at an early age. He taught her the basic methods for problem solving and encouraged her to learn from her mistakes. It was easy for a child who had a naturally inquisitive mind and loved to solve problems. In fact, Erika excelled in math and science, and her favorite subject at San Ramon High School was physics. It was no surprise to her family when Erika decided to study Electrical Engineering at Santa Clara University.

Away from the watchful eye of her family and the sheltered life in Alamo, California, she found that the University had many interesting distractions. The first was ROTC. She was excited to take an elective class in rappelling. The class was sponsored by ROTC and she was invited to an advanced ROTC training course held at Fort Knox, Kentucky, where she learned marksmanship with an M16 and M60, survival techniques, grenade launching, orienteering, and leadership skills. Prepared for anything when she returned to school in the fall, she was offered and accepted an ROTC scholarship for the remaining two years of school.

After graduating from Santa Clara, Erika completed her ROTC training by attending jump school and officer training school. She was commissioned as a Lieutenant in the Army National Guard and assigned to a Military Police unit in Pittsburg. She also accepted a position as an electrical explosive engineer at Reynolds Industry Systems. Reynolds created precision secondary explosives for highly specific and reliable uses such as airbag release cartridges and mining. Her primary task was to design firing systems and test explosive detonators. It was an interesting and challenging job in the beginning, but the work was in a well-developed field that required little problem solving.

Motivated to discover a new career, Erika decided to go back to school to study environmental engineering at Humboldt State University. Part of the preparatory coursework required her to take biology, bacteriology, and genetics. "I found these courses fascinating and loved the hands-on lab work." She then decided to pursue a graduate degree in Microbiology at Berkeley. However, before she was able to attend, Desert Storm began and Erika's unit was deployed to Saudi Arabia.

She didn't think about the seriousness of the situation until her unit took off from Travis AFB in a C-130 filled with Humvees. "I'll always remember the scene, sitting on the plane looking at the somber faces of my colleagues—some of whom were crying." Until that moment, her career in the military was more like a hobby. One weekend a month she was allowed to play with guns and drive Humvees. As an officer, Erika was required to play a leadership role in her unit. "I learned that there were many types of leaders: the loud, fearless Patton type and the not so in-your-face, quiet and patient type."

Erika's MP unit was responsible for managing POWs, and it followed the troops advancing into Iraq. "Outside of the Republican Guard units, the Iraqi infantry were not well-trained, nor cared for, therefore many soldiers gave up hoping that the Americans would clothe and feed them." Her unit spent six months caring for the Iraqi soldiers in temporary POW camps in the desert.

Erika returned to school with a renewed focus. She

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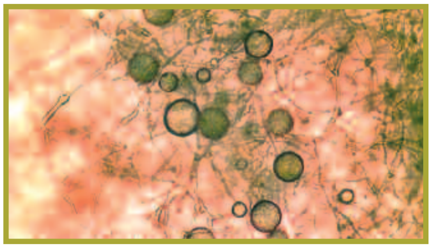
JGI New Employees

Bill Cannan
Senior Recruiter, HR
Cindy Choi
Research Associate, Genome Technologies
Mansi Chovatia
Senior Research Technician, Production
Julianna Chow
Research Associate, Genomic Tech.
Alicia Clum
Research Associate, Genome Tech.
Nicholas Eattock
Biosciences Tech 1, Production
Rob Egan
Software Developer 3, Informatics
Gerald Ilog
Research Tech, Production
Megan Kennedy
Biosciences Tech 1, Production
Duane Kubischta
Systems Analyst 3, Production
Kathleen Lail
Sequencing Supervisor, Production
Michael Lee
Principal Safety Technician, EH&S
Aiyesha Ma
Student Assistant, Quality Assurance Group
Gladys McCay
Student Assistant BBEI, Genome Tech.
Yanan Meng
Research Tech, Production
Bonnie Millenbaugh
Administrative Specialist IV, Operations
Mary Ann Pedraza
Senior Research Associate, Genome Tech.
Michael Pintor
Research Tech, Production
Nicole Shapiro
Senior Research Technician, Production
Gurjot "Jay" Singh
Research Technician, Production
Hui Sun
Senior Research Associate, Genome Tech.
Zalak Trivedi
Senior Research Technician, Production
Mario Velazquez
Research Technician, Production
Chris Wong
Porduction Manager
Andy Yuen
Biosciences Technician, Production
Michael Zhang
CSE Trainee, Informatics
Tao Zhang
Senior Research Associate,
Genetic Analysis Programs

DOE JGI and VBI Decode Plant-Killing Pathogens

By comparing the complete genome sequences of two plant-killing pathogens, *Phytophthora ramorum* and *Phytophthora sojae*, and related organisms, researchers from DOE JGI, with the Virginia Bioinformatics Institute (VBI) and others, have uncovered crucial aspects of the disease-causing mechanisms of sudden oak death (SOD) and soybean root rot disease. Results of a four-year, $4 million multi-agency project supported by DOE, the US Department of Agriculture (USDA), and the National Science Foundation (NSF), appear in the Sept 1, 2006, edition of Science (vol. 313, No. 5791).

"What is extraordinary about the *Phytophthora* genomes is that almost half of the genes contained in them show signs of rapid adaptation. We speculate that the rapidly changing genes are being driven to evolve by pressure from the defense systems of the pathogens' host plants," said VBI Professor Brett Tyler, the project's coordinator and lead author of the *Science* paper. "The unprecedented level of genetic flexibility in these organisms gives us insights into how these pathogens have become successful. At the same time, it has helped us identify weak points in the organisms that can be targeted to control them."



Typical spores formed by the vegetative body, or hyphae, of *P. ramorum*. Matteo Garbelotto Lab - UC Berkeley.

OPA Recipients

Harris Shapiro - This OPA recognizes your outstanding leadership and commitment in ensuring the JGI sequencing pipeline continues assembling genomes.

Simon Minovitsky - In recognition of your successful development of the VISTA Enhancer database.

Genome Assembly Team: Serge Dusheyko and Jasmyn Pangilinan - This OPA recognizes the extraordinary efforts of Serge Dusheko and Jasmyn Pangilian in keeping the Sequencing Pipeline operational and ensuring critical JGI sequencing goals were met for 2005.

Venonat LIMS Team: Dana Alcivare, Patrick Hajek, Aaron Porter, Jason Baumohl, Arkady Voloshin - This OPA recognizes the team's innovation and dedication for migrating the Venonat LIMS to a modern platform, defying all conventional wisdom that it would be impossible. Their work ensures a stable future for this critical information system.

GeneLib Team : Erika Lindquist and Ed Kirton - This OPA recognizes the outstanding accomplishments of Dr. Erika Lindquist and Ed Kirton for developing and implementing the "GeneLib" program, an essential tool for sequencing and the R&D programs.

To nominate your colleagues for such honors, see:

- LBNL SPOTs/OPAs - http://www.jgi-psf.org/HR/awards.html
- LLNL Awards - http://www.jgi-psf.org/HR/forms_templates/llnl/BIOAwardsGuidelines2006.pdf

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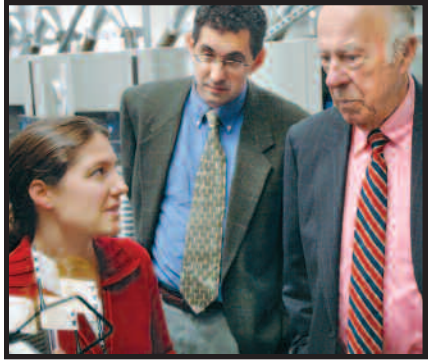
George Shultz Visits PGF

Former Secretary of State George Shultz with DOE JGI Sequencing Department Head Susan Lucas (left) and Computational Genomics Program Head Dan Rokhsar (middle). Now Thomas W. and Susan B. Ford Distinguished Fellow at the Hoover Institution, Mr. Shultz visited DOE JGI in April, along with Sidney Drell, senior fellow and professor of theoretical physics (emeritus) at the Stanford Linear Accelerator Center (SLAC), and Lucy Shapiro and Harley McAdams from the Stanford University Medical Center.



Poplar Holds Promise as Renewable Bioenergy Resource

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DOE JGI staff and guests gathered on September 14 to celebrate the publication of the first tree genome in the journal *Science* by planting a black cottonwood, or poplar, of the same type sequenced, *Populus trichocarpa*. At planting, the sapling measured 50.5 inches. *The Primer* will continue to monitor its growth.

provide a major answer to our energy needs by giving the United States a home-grown, environmentally friendlier alternative to imported oil," said DOE's Under Secretary for *Science*, Dr. Raymond L. Orbach. "Fine-tuning plants for biofuels production is one of the keys to making biofuels economically viable and cost-effective. This research, employing the latest genomic technologies, is an important step on the road to developing practical, biologically-based substitutes for gasoline and other fossil fuels."

"Biofuels are not only attractive for their potential to cut reliance on oil imports but also their reduced environmental impact," said Dr. Gerald A. Tuskan, ORNL and DOE JGI researcher and lead author of the Science study. "Biofuels emit fewer pollutants than fossil fuels such as gasoline. In addition, poplar and

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Phil Hugenholtz—ISME Young Investigator Award Winner

At the 11th International Symposium on Microbial Ecology in Vienna, August 20-25, the International Society for Microbial Ecology (ISME) presented The Young Investigator Award to DOE JGI's own Phil Hugenholtz. The award (a glass plaque with an embedded solid gold coin) recognizes scientists who have made significant contributions to microbial ecology. In his acceptance remarks, Phil thanked the Society for its timely selection because "…next year, I'm officially a middle-aged investigator." He went on to say that he was indebted to his past and present collaborators "…because research, much like a microbial community, is all about teamwork."

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related plants are vital managers of atmospheric carbon. Trees store captured carbon dioxide in their leaves, branches, stems, and roots. This natural process provides opportunities to improve carbon removal from the air by producing trees that effectively shuttle and store more carbon below ground in their roots and the soil. Moreover, bioenergy crops re-absorb carbon dioxide emitted when biofuels are consumed, creating a cycle that is essentially carbon neutral."

Poplar's extraordinarily rapid growth—it can grow a dozen feet a year—and its relatively compact genome size of 480 million nucleotide units—40 times smaller than the genome of pine—are among the many features that led researchers to target poplar as a model crop for biofuels production.

Among its major discoveries, the poplar project identified over 45,000 protein-coding genes—more than any other organism sequenced to date and about double those in the human genome (which is six times larger than the poplar's). The research team identified 93 genes associated with the production of cellulose, hemicellulose and lignin, the building blocks of plant cell walls. The biopolymers cellulose and hemicellulose are the most abundant organic materials on earth. Once broken down into sugars by enzymatic action, they can be fermented and distilled as fuel-quality ethanol and other liquid fuels.

Poplar is the most complex genome to be sequenced and assembled by a single public sequencing facility, and only the third plant to have its genome completely sequenced and published. The first two were a tiny weed, *Arabidopsis thaliana*, and rice. Native to the Pacific coast from San Diego to Alaska, *Populus trichocarpa* is one of the tallest broadleaf hardwood trees in the western US. The sequenced DNA was isolated from a specimen collected along the banks of the Nisqually River in Washington State.

The poplar project supports a broader DOE drive, under the Bush Administration's Advanced Energy Initiative, to accelerate research into biofuels production. In August, DOE announced it would spend $250 million over five years to establish and operate two new bioenergy research centers. The DOE-supported research focuses on both plants and microbes in seeking new biotechnology-based, cost-effective methods of producing fuels from plant matter (biomass).

DOE scientists see a future in which vast poplar farms in the Pacific Northwest, upper Midwest, and portions of the southeastern U.S. provide a steady supply of cellulose-rich tree biomass which specialized biorefineries would transform into fuels like ethanol. Other regions might specialize in different "energy crops," such as switchgrass and willow, suited to their climates and soil conditions. A large quantity of biofuel might also be produced from agricultural and forestry waste.

Chris Wong—DOE JGI Production Manager

Chris Wong joined the PGF staff in July. He got his start in science in an unenviable position in Genentech's Process Development Group where he conducted media screening for fermentation. Genentech farmed out Chris as a resource to IDEC and Immunex before he moved on to Cell Genesys, where he helped build out a facility from 5,000 to 100,000 square feet, and a staff of four to 60. His wife Deanna, a tax attorney, does forecast flow planning for The Gap. They have two kids, Brian (four and a half) and Abigail (10 months).

SPOTLIGHT ON SAFETY—ERGONOMICS

Ergonomics is about getting a good match between the work we do and what our bodies can handle. When there's a mismatch, it can lead to aches and pains, inefficiencies, and even to serious injuries if not promptly addressed. Unfortunately, ergonomics-related problems are now the most common type of injury at DOE JGI, and for this reason, improving ergonomics has become a major focus here.

A major step that DOE JGI has taken to address ergonomic injuries was to obtain the services of UCSF senior ergonomics consultant Ira Janowitz, who now holds regular office hours at the PGF to provide advice and assistance on ergonomics issues. Ira's role is to support the existing program at DOE JGI and add analysis, training, and advice as needed. According to Janowitz, "Good ergonomics is not based on forcing employees to follow rules, but is a problem-solving process best carried out with the involvement of those who actually do the work." Everyone's involvement is critical in addressing ergonomic issues.

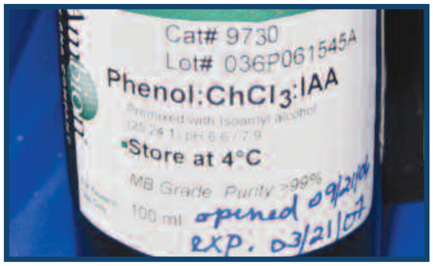
In addition, Ira is working with the DOE JGI EH&S staff to arrange for the creation of an ergonomic equipment demonstration room, which will be supplied with several different computer mice, keyboards, and other equipment to make the time employees spend at the computer a little bit more comfortable. The selection of these devices and chairs will combine existing LBNL and LLNL standards with recommendations by Janowitz, and will give employees the opportunity to test these out in one centralized location. EH&S hopes to have several different types of chairs that employees will be able to bring to their work area and evaluate on site, thereby reducing the lag time currently experienced through the LBNL ergonomic chair loaner program. This demonstration room will be located in Building 400, room 405. Some examples of ergonomic equipment that are currently available for trial are:

- Goldtouch Key Ovation keyboard
- Microsoft Natural Ergonomic Keyboard 4000
- Logitech Marble mouse
- Evoluent Vertical mouse (left and right handed)
- Humanscale Whale mouse

Remember, we can reduce the risk of ergonomics-related injuries through activities such as taking the time to adjust our workstation and frequent "stretch for recovery" breaks that take us out of our working posture. If you have any pain or discomfort, it's important that you report it early to Ira or the DOE JGI EH&S staff (Michael Lee or Stephen Franaszek) and have your current workstation ergonomically evaluated. Ira is easily contacted at janowitz@comcast.net or 925-351-1142, and ergonomic evaluations can be directly requested through the LBNL ERGO Database website: *https://isswprod.lbl.gov/Ergo/Login.asp.*

Ergonomically-correct Nicole Shapiro

Safety Lesson Learned



Phenol

Recently, a DOE JGI employee sustained a chemical burn while returning a bottle of phenol to a chemical storage cabinet. The container cap was loose and the solution spilled onto his arm. We should all learn from this incident. Ensure that the following precautions are taken while handling corrosives:

- Store them below shoulder level (about 4 feet) in an appropriate location, away from incompatible chemicals. Liquid corrosives should have secondary containment.
- When finished using them, tighten the cap and immediately store the container.
- Use appropriate personal protective equipment (PPE) while handling corrosives (as a minimum, tight fitting chemical goggles, lab coat, gloves and closed-toe shoes).
- Before handling corrosives, get the necessary training and know the hazards of these materials. Material Safety Data Sheets (MSDS) are good info sources.
- Know the location of the nearest emergency eyewash/shower and what to do if exposed to corrosive chemicals.

Contact DOE JGI Safety Officer Stephen Franaszek (SMFranaszek@lbl.gov; (925)296-5807) or ES&H Tech Michael Lee (MDLee@lbl.gov; (925)296-5649) if you have questions or need assistance.

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THE HAZARDS OF BEING A MICROBIOLOGIST

BY BRUCE BALFOUR

When the average person hears that you're a microbiologist, they usually picture someone in a lab coat with thick glasses who never leaves the laboratory. However, microbial samples don't collect themselves, and environmental samples have to be collected from…the environment. While toilet bowls and kitchen sinks provide fruitful environments for many lines of research, many biologists are not content to while away their hours slaving over a hot thermal cycler or a recalcitrant centrifuge. The adventurous researcher leaves his lab to lead a life on the edge, traveling to exotic locations in microbe-filled aircraft cabins, drinking bottled water in foreign countries, delivering lectures in front of people who don't speak the same language, and going to extreme measures to collect extremophiles. Even if you don't wear a biocontainment suit to work, sample collection can be hazardous.

Top left: Kamchatka boiling pot hits 97C–boiling at that altitude. Top right: David Mead collects samples at a hot spring. By the end of the day the acid from the hot spring had dissolved the knees of his pants. Bottom left: The bears were ambivalent about sharing their favorite watering hole. Bottom right: Dr. Gennadi Karpov, deputy director of the Institute of Volcanology and Seismology in Petropavlovsk-Kamchatsky, local connection to the many volcanoes on the Kamchatka peninsula.

Hot Enough for You?

What's black and red and white all over? That's right, a biologist collecting microbial samples from a hot spring in Yellowstone National Park. It's an idyllic setting, high in the mountains under a clear blue sky, with birds chirping and small animals watching from the forest, and boiling thermal pools with the odor of rotten eggs that clears your sinuses better than nasal spray. The smell, of course, is the result of anaerobic bacteria converting some of the dissolved sulfur in the water to hydrogen sulfide. David Mead of Lucigen braved the bad smells and boiling waters to collect bacterial samples for metagenomic analysis at the DOE Joint Genome Institute (DOE JGI), and he reports losing several toenails in the process. On another sample collection trip in the backcountry of Yellowstone, Phil Hugenholtz of DOE JGI recalls: "The park rangers warned us that if buffalo charged us, not to try to outrun them. They would win. The trick was to get under a tree, where you were less likely to get killed."

It Smells Like Victory

Speaking of bad smells, Phil Hugenholtz was also drawn to the glamorous environment of wastewater treatment plants so that he could study the microbial population structure in sludge. While hanging from catwalks over sewage tanks to collect sludge samples in Australia and Wisconsin, he recalls being impressed by the strong immune systems of the people who work at wastewater treatment facilities. The cold that he got after his collection trip was probably just a coincidence.

Attacked by Termites

Termites are like little hydrogen factories, where wood goes in one end and hydrogen comes out the other. Termites accomplish this task by working with the 200 different species of microbes that inhabit their guts, where the enzymes produce hydrogen as a byproduct of the fermentation process. However, to study the microbes inside the termite, you have to catch them where they live—in the dense jungles of Costa Rica. Falk Warnecke from DOE JGI, and Jared Leadbetter from the California Institute of

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Webfeeds: What Are They?

René Perrier

BY RENÉ PERRIER

The Internet can be overwhelming. First, there's the challenge of finding good content, and then there's the challenge of keeping up with (and sorting through) the never-ending flood of new content. The same is true (on a smaller scale) for our intranet.

Search engines such as Google help address the first challenge (see article in *The Primer,* Volume 3, Issue 1), but a new kind of online publishing, webfeeds, makes it simple to keep track of the latest content published by your favorite web sites.

What is a Webfeed?

In a nutshell, a webfeed (also called RSS feed) is a way for web sites to continuously "feed" you announcements of their latest content, with links to each new content item.

To instantly learn what's new on a web site, just check its feed. When you're trying to follow developments at dozens or hundreds of sites, your time savings can be enormous.

The DOE JGI internal web site (http://www.jgi-psf.org/) updates its content infrequently. By using this site's webfeed, you automatically get notified when a page has been added or updated.

How to Get Started

To read webfeeds, you need a software tool called an aggregator (also called a feed reader). Your aggregator maintains a list of your favorite webfeeds, checks them regularly for updates, and displays updated contents (or a summary of their contents). The main categories of aggregators are:

Online

An online aggregator is a web site service selling or providing free aggregation services. Big Internet companies like Google (Google Reader) and Yahoo! (My Yahoo!) provide such services, as well as companies specializing only in webfeeds, like Bloglines or Newsgator. Online aggregators are often described as personalized newspapers since they allow their users to organize their news feeds on a single page.

The advantage of an online aggregator is that it is available from anywhere (work, home, cyber cafe). The disadvantage is that it will not provide access to feeds that are located on an intranet (like the JGI internal web site, the JGI blog server, or the JGI wiki).

Standalone

Standalone aggregators run on a single computer to perform one task: tracking and displaying webfeeds. Unless you need a tool that is specialized in specific types of feeds like podcasts or vodcasts (aka videopodcasts), this is not the best option.

Integrated Support

Some web browsers and e-mail applications provide integrated support for webfeeds either by default (like Safari or Thunderbird—the e-mail application from Mozilla, which is also approved by the LBNL IT division—or by adding some extension (like Wizz RSS or Sage for Firefox). These allow receiving feeds from an intranet and are nicely integrated with applications that we already use. In fact, when using Thunderbird, the integration is so well done the news feeds look like e-mails. Local (PGF) users can find more info about webfeeds at: http://wiki.jgi-psf.org/cgi-bin/twiki/view/Main/WhatAreWebFeeds.

Softball Season Roundup: G-Nomes Grab Second Place!

The G-Nomes finished in 7th place but managed to make it to the championship game for the second year in a row. This year's team members were (back row) Jason Baumohl, Steve Wilson, Jarrod Chapman, Harris Shapiro, Chris Hack, David Pletcher, Victor Hepa, (front row) Katrin Brand, Kecia Duffy-Wei, Jennifer Kuehl, Beth Lacerna, Susan Lucas, Khela Weiler. Not pictured: Jeremy Brand, Steve Paltiel, Brian Simison, Mark Taylor, Danielle Mihalkanin, Simona Necula, Elena Shapiro, Kristen Taylor.

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Eukaryotic Finishing & Whole-Genome Improvement at Stanford

Group photo: Left to Right; Back row: Jane Grimwood, Michael Young, Kevin Wu, Min Kim, Dave Flowers, James Retterer, Yee Man Chan. Middle Row: Jennifer Chan, Eva Bajorek, Hanz Olonan, Maria Gomez, Ataya Sathirachinda, Catherine Eastman, Xia Liu, Jeremy Schmutz. Front Row: Chenier Caoile, Mirian Denys, Lucia Ramirez, Shelia Hilario, Joan Yang, Ming Tsai, Nu Vo. Unpictured: Richard Myers, Stacey Black, Dea Fotopulos, Sally Fuess, Liz Jackson, Jui Mei Lee, Lajinia Miller, Jennifer Okamoto.

BY JEREMY SCHMUTZ

I'm often asked, "Why are you sequencing the X genome?" These days, the X often refers to obscure squiggly creatures that most Americans don't even know exist. This is not how it has always been at the Stanford Human Genome Center (SHGC). When we began working with DOE JGI on the finishing and assembly of human chromosomes 5,16, and 19 in 1999, the answer to "Why are you sequencing the human genome?" was straightforward. I was even able to explain to my grandmother the potential benefits in being able to screen for, diagnose, and treat genetically inherited diseases. For our current projects, however, it is difficult to explain that *Nectria haematococca* is a fungus that has potential bioremediation uses as it degrades lignins and hydrocarbon chains, or that *Ostreococcus lucimarinus* is a marine algae, a phytoplankton, that plays a significant role in global carbon-fixation in the ocean. When I propose these answers, they inevitably lead to a discussion (rather one-sided) where I explain the global carbon cycle, or using living organisms to reclaim and clean human pollutants from soil, or how it is possible to derive at least some of our energy needs from growing plants. Needless to say, eventually people lose interest as I try to explain "what we are doing at the Center these days."

What we are actually doing these days is working on whole-genome shotgun assemblies (WGSA) after the draft sequencing stage. We still finish clones that are shotgun sequenced at the DOE JGI PGF just like in the days of the human genome, but most of our effort is focused on evaluating WGSA and developing strategies for improving the quality of the eukaryotic genomic sequences produced by DOE JGI. At this time, it is particularly important to produce useful genomic sequences that meet the needs of their respective research communities because the majority of their exposure to genomic sequence has been with the finished human, near-complete mouse, or finished bacterial genomes. What this means is that the average user of a DOE JGI genome has become accustomed to carrying out functional assays from the genomic sequence alone, without having to further refine the underlying genomic sequence. They also expect to be able to identify all of the coding regions in the genome and to have genes that are co-localized on a chromosome appear together in the genomic sequence.

At the SHGC, we endeavor to accomplish four goals ***(cont. on page 18)***

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DOE JGI Sequences, Releases Genome of Symbiotic Tree Fungus

The DNA sequence of *Laccaria bicolor*, a fungus that forms a beneficial symbiosis with trees and inhabits one of the most ecologically and commercially important microbial niches in North American and Eurasian forests, has been determined by DOE JGI. The complete *Laccaria* genome sequence was announced July 23 at the Fifth International Conference on Mycorrhiza in Granada, Spain, by an international consortium composed of DOE JGI, Oak Ridge National Laboratory (ORNL), France's National Institute for Agricultural Research (INRA), the University of Alabama in Huntsville (UAH), Ghent University in Belgium, and additional groups in Germany, Sweden, and France.

"The *Laccaria* genome sequence will provide the global research community with a critical resource to develop faster-growing trees for producing more biomass that can be converted to fuels, and for trees capable of capturing more carbon from the atmosphere," said DOE JGI Director Eddy Rubin.

"The woody tissues of trees act as one of the world's most important terrestrial sinks for CO2, making trees an important stabilizer of carbon in the earth's environment," said Francis Martin, INRA's *Laccaria* project leader. "The steady rise of global atmospheric CO2 concentrations suggests that we are on the trajectory for serious environmental problems. This situation could be eased by modeling and actively managing the complex relationships between trees and fungi," said Martin.

Key factors behind the ability of trees to generate large amounts of biomass, or store carbon, reside in the way that they interact with soil microbes known as *mycorrhizal fungi,* which excel at procuring necessary, but scarce, nutrients such as phosphate and nitrogen. When *Laccaria bicolor* partners with plant roots, a *mycorrhizal root* is created, resulting in a mutualistic relationship that significantly benefits both organisms. The fungus within the root is protected from competition with other soil microbes and gains preferential access to carbohydrates within the plant.

Such mycorrhizae are critical to terrestrial ecosystems, Martin said, since some 85 percent of all plant species, including trees, depend on such interactions to thrive. Mycorrhizae significantly improve photosynthetic carbon assimilation by plants and are estimated to fix more phosphate and nitrogen than the world's chemical fertilizer industry produces.

"The study and management of such relationships holds immense potential for the agriculture, forestry, and horticulture industries, as well as far-reaching implications for land management policies and the impact of global climate change on plants," said Gopi Podila of UAH.

"This unique research opportunity enables us to advance the understanding of how functional genomics of this symbiosis enhances biomass production and carbon management, particularly through the interaction with the poplar tree, also sequenced by DOE JGI," Rubin said. "We can now harness the interaction between these species and identify the factors involved in biomass production by characterizing the changes that occur between the two genomes as the tree and the fungus collaborate to generate biomass. It also helps us to understand the interaction between these two symbionts within the context of the changing global climate."

Fruiting bodies of the symbiotic fungus *Laccaria bicolor* associated to seedlings of Douglas fir. Photo © INRA

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Los Alamos Finishes Landmark 50th Genome

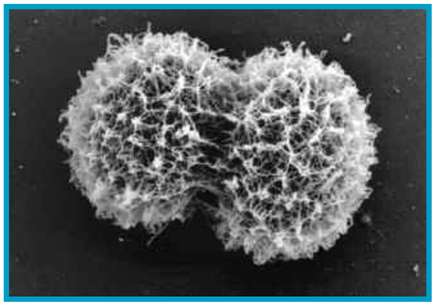
BY KATHERINE HARRINGTON

As DOE's Joint Genome Institute (DOE JGI) celebrated finishing its 100th genome last month, the DOE JGI LANL team celebrated the fact that 50 of those 100 genomes were finished in Los Alamos.

The 50th genome, finished in July, was that of the microorganism *Polaromonas naphthalenivorans*, which is known for its ability to degrade naphthalene, a carcinogen commonly found at DOE's energy production sites. Genetic information about *Polaromonas naphthalenivorans* will help researchers who are seeking insight into how naphthalene-degrading microbes could be used to manage contaminated ecological and industrial environments as well as to understand how organisms survive in highly contaminated environments.

There have been 354 microbial genomes sequenced worldwide since scientists finished the first microbial genome in 1996. The finishing process involves filling gaps that remain after the draft sequencing phase, which occurs at the Production Genomics Facility (PGF) in Walnut Creek, California. The genomes that DOE JGI selects to finish are selected on the basis of their potential to advance research in the Department of Energy's carbon cycling, bioremediation, and bioenergy mission areas.

Since 2003, when DOE JGI LANL first began finishing sequences, the technology and chemistry techniques have evolved significantly, resulting in higher-quality data and faster production rates. When the project began, the team was only able to produce one genome a year—now they are able to finish about one a week. The DOE JGI LANL team also developed a database that has improved the quality of the data and the efficiency with which they are able to finish genomes.



Polaromonas naphthalenivorans

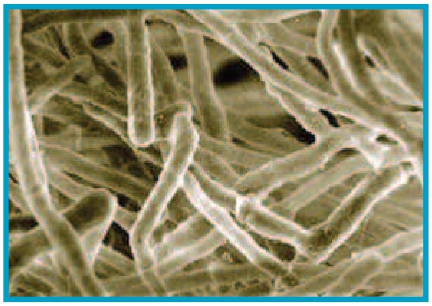
As the team at DOE JGI LANL continues to increase efficiency and automation in sequencing simple microbial genomes, such as bacteria, they will be able to tackle more complex genomes, including yeast and other fungi. "That's what is on the horizon for us" said David Sims of the Genomic Sequencing and Computational Biology Group at DOE JGI LANL.

Of the 50 finished, some of the organisms Los Alamos has sequenced are used in bioremediation to clean up oil spills or other such pollutants from the environment. For instance, *Pseudoalteromonas atlantica* has been studied off the coasts of California, New Jersey and Florida, and has been shown to absorb twenty to forty percent of trace metal lead in ocean environments. Having the finished sequence of *P. atlantica*, researchers will be able to better understand the microbe's function in controlling toxic metal concentrations.

Microbes might also help carbon cycling researchers better understand the fate of greenhouse gases and may help them develop techniques for reducing the buildup of these gases in the atmosphere.

One microbe, *Sphingopyxis alaskensis*, is found in ogliotrophic (nutrient deficient) waters near Alaska, the North Sea, and the North Pacific. It is able to survive in these nutrient-deficient waters because of its ability to feed on atmospheric carbon. Researchers are interested in studying these types of microbes in order to predict how various ecosystems might respond to nutrient deficiencies caused by global warming.

Microbes useful in bioenergy work are attractive for their ability to convert organic matter into renewable energy. *Syntrophomonas wolfei Gottingen*, which recently finished at Los Alamos, produces hydrogen and methane as byproducts of its metabolism. Both of these materials might be burned as clean fuels or used in fuel cells. By studying these microbes, scientists also have begun developing technology to imitate their processes to create renewable, less expensive energy sources.



Trichoderma reesei
Photo: Irma Salovuori, VTT Biotechnology

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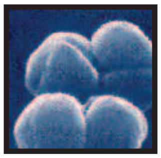
DOE JGI Finishes 100th Microbial Genome, Tapping the Power of the Unseen World

Mighty microbes, thriving in even the world's most extreme environments, have learned over billions of years to perform myriad biological functions. Those lessons are among the secrets encoded in their DNA sequence, and this information can be applied to national needs for clean renewable sources of energy and the repair of damaged environments.

On May 23, at the largest gathering of microbiologists in the world, the 106th general meeting of the American Society for Microbiology (ASM) in Orlando, Florida, ASM President Stanley Maloy announced that the scientific community has embarked on the third golden age of microbiology, one that requires a blend of old and new approaches—including DNA sequencing.



***Methanosarcina barkeri* Fusaro, a methanogenic Archaebacterium. From Kevin Sowers, University of Maryland Biotechnology Institute (UMBI)**

Maloy highlighted the most recent milestone achieved by the Department of Energy Joint Genome Institute (DOE JGI): its finishing 100 microbial genomes—a diverse portfolio of the vast and mostly uncharacterized microscopic world.

"DNA sequencing is mining a particularly productive vein in expanding the frontier of microbiology. Especially where, through conventional culture methods, we are unable to shed light on the metabolic profiles of these microorganisms and their environmental implications, DNA sequencing provides us a welcome set of tools."

DOE JGI has sequenced over 380 organisms, more than any other institution in the world. As microbes range in size from typically five million to tens of millions of letters of code, several microbes could be sequenced in one day at DOE JGI's Production Genomics Facility (PGF). However, the sequencing process is an iterative one, requiring six- to eight-times coverage of each genome. The term, "finished," refers to the rigorous standard of accuracy established for the Human Genome Project—tolerating no more than one mistake in 50,000 letters of genetic code without gaps.

The DOE JGI 100 list can be found at *http://genome.jgi-psf.org/microbial*.

Olavius algarvensis

cont. from page 1

Olavius algarvensis

are taken up by these symbionts—not only aiding the host in the removal of these toxic waste products, but also conserving valuable nitrogen, further maintaining the microbial community.

"It's an excellent example of outsourcing energy and waste management, where this worm and the microbes living under its skin are enjoying a mutually beneficial relationship," said Eddy Rubin, DOE JGI Director. The work was conducted by postdoctoral fellow Tanja Woyke and colleagues from the Rubin lab at DOE JGI and Lawrence Berkeley National Laboratory, and collaborators led by Nicole Dubilier, Head of the Symbiosis Research Group in the Department of Molecular Ecology at the Max Planck Institute for Marine Microbiology in Bremen, Germany.

"The microbes, floating around in the sea, strike up a bargain with the worm—in exchange for housing, the microbes take care of energy production and handling the waste," Rubin said.

The team uncovered the unique method of waste management employed by *Olavius algarvensis* by metagenomics, a strategy pioneered by DOE JGI and its collaborators. This technique entails isolating, sequencing, and characterizing DNA extracted directly from environmental samples to obtain a profile of the microbial community residing in a particular environment. This is the first

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Olavius algarvensis

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instance of such a symbiotic relationship being analyzed by using a metagenomic shotgun sequencing approach, heralding a renaissance in symbiosis research.

In this experiment, Woyke donned scuba equipment and dove into the Mediterranean to sift through tons of sediment to uncover enough of the worms and their microbial contents, which, like the vast preponderance of microbial life, cannot be grown in the laboratory.

What they found particularly unusual about this worm/bacteria relationship was that several different microbes came into play, unlike most symbiotic interactions between larger organisms and microbes where usually only one microbial species is present.

For the study in question, a total of 204 million bases, or units of genetic code, were generated. Through a process known as "binning," a protocol developed by collaborating author Frank Oliver Glöckner from the Max Plank Institute, these sequences were then organized by specific DNA signatures to reveal the identities of particular microbial species.

"By sequencing the genomes of the microbes we were able to discern how they cope with the needs of the worm and why such a diversity of microbes is warranted," Rubin said. It turns out that the worm isn't just housing for the microbes, but is also transportation. The worm burrows into the sediment and as it heads for different environments, each with different chemical constituents, provides energy adapted to the capabilities of a particular group of microbes. As the worm doesn't have a mouth, the microbes use chemical energy from the sediment, perfusing through the skin, to convert organic material into the stuff that nourishes the worm.

"It's not unlike a car with a hybrid motor that can run on both electricity and gas depending on the situation," Rubin said. "In certain places the worm is powered by specific bacteria that can exploit the chemical energy abundant at a specific location, while in other strata, where a different chemical energy source is abundant, the worm switches its energy production to resident bacteria that can exploit that available energy source."

The work was conducted under the auspices of the DOE JGI's Community Sequencing Program (CSP).

Tanja Woyke, lead author on the *O. algavensis* Nature paper.

Better Sludge

cont. from page 1

the largest microbially-mediated biotechnology process on the planet. When it works, it is a microbial symphony in tune with humanity. When it fails, the consequences can be dire. DOE JGI researchers and collaborators at the University of Wisconsin-Madison and the Advanced Wastewater Management Centre, University of Queensland, Australia, have published the first metagenomic study of an activated sludge wastewater treatment process. The research appeared online in the September 24 edition of the journal Nature Biotechnology *(http://www.nature.com/nbt/journal/vaop/ncurrent/abs/nbt1247.html).*

The metagenomic strategy entails generating DNA sequence information directly from samples of sewage sludge to provide a blueprint of the genes and hence the metabolic possibilities of the wastewater environment.

"This is a first step in a much broader strategy employing a systems biology approach to the study of microbial communities with the goal of designing predictive models to understand how these communities function," said Hector Garcia Martin, lead author of the study and post-doctoral fellow with Phil Hugenholtz in the DOE JGI's Microbial Ecology Program. "With this information now available, there are opportunities to bioengineer the process to make it more reliable."

Removing phosphorus is best done by an environmentally friendly process known as enhanced biological phosphorus removal (EBPR). The researchers obtained a nearly complete genetic blueprint for a key player in this process, the bacterial species *Accumulibacter phosphatis*.

Activated-sludge

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Lindquist

cont. from page 4

applied to graduate school at Berkeley and accepted a position with Richard Stephens, whose lab focused on *Chlamydia*, the world's leading cause of preventable blindness. She emerged from Berkeley as a newly-minted PhD scientist, and knew she wanted to focus on bioinformatics. She started her scientific career working on gene annotation at Celera Genomics, and later moved to Incyte Genomics as a project leader for splice-variant editing and annotation. Following that, she set up the Biowatch Program in the Bay Area, which was sponsored by the Department of Homeland Security and managed by the Centers for Disease Control. The program was to implement a system designed by LLNL to monitor for and respond to airborne biological pathogens on a daily basis. "Although I thoroughly enjoyed working in biodefense, when Dee Catino, DOE JGI human resources head, called regarding an open position at JGI, I jumped at the chance to interview."

Erika is currently the cDNA and EST group lead, working for Paul Richardson. The position is responsible for all components of cDNA projects, including definition of the project, discussions with the collaborator, sequencing, and publication of the data in GenBank. Part of this process is informatics, which is where Harris Shapiro's group comes in.

"I love working at JGI. I believe in our mission and what we do. In particular, coming from industry where genomics companies sold access to information, JGI provides free access to all researchers. Collaborators consistently communicate how appreciative they are and how important the JGI-provided information is to their research. I'm proud to be a contributing part of the JGI team, and every day I feel honored to be working here!"

Erika and her partner Mayumi live in a floating home in Alameda, across from Jack London Square.

Erika Lindquist at her post during Operation Desert Storm.

Better Sludge

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wastewater treatment is used throughout the world to purify trillions of gallons of sewage annually. Many treatment plants use specialized bacteria to remove phosphorus, to protect lakes and rivers from eutrophication, a deterioration of water quality characterized by excessive algae blooms. *Accumulibacter* play a vital role in wastewater management, accumulating massive amounts of phosphorus inside their cells.

Hector Garcia Martin

"Engineers and microbiologists have been trying for 35 years to grow this microbe, with no success," said Trina McMahon, Assistant Professor, Department of Civil and Environmental Engineering, University of Wisconsin-Madison, and one of the study's authors. "Remarkably, through metagenomic techniques, we were able to isolate and acquire the genome sequence of *Accumulibacter phosphatis* without a pure culture of the organism, which, like most microbes, eludes laboratory culture. We expect that clues hidden in the genome will lead to domestication of this mysterious organism, enabling further studies of its habits and lifestyle.

"The genome sequence will also enable biologists and engineers to understand why and how these organisms accumulate phosphorus, and it will lead to major advances in optimizing and controlling the EBPR wastewater treatment process," McMahon said. "In particular, it makes possible further research into why some wastewater treatment plants occasionally fail. These failures often result in serious pollution of lakes, rivers, and estuaries."

When things go wrong, the environment can be inundated with untreated phosphorous, carbon, and nitrogen, necessitating costly and environmentally taxing remedies and exposing the public to potential disease hazards. The scale is daunting—more than 31 billion gallons of wastewater are treated daily in the U.S. alone. Even a marginal improvement in the process would translate into huge savings and spell relief for environmental engineers.

The work was conducted under the auspices of the DOE JGI's Community Sequencing Program (CSP).

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Energy-Rich Portfolio of New Sequencing Targets


A farmer tends his cassava field.

Bioenergy crop plants switchgrass and cassava, other important agricultural commodities such as cotton, and microbes geared to break down plant material to render biofuels, round out the roster of more than 40 projects to be tackled by DOE JGI over the next year. Drawing submissions from DOE JGI's more than 400-strong user community, the genomes of these organisms will be sequenced and characterized as part of the Community Sequencing Program (CSP).

The CSP selections echo recommendations outlined in the "Breaking the Biological Barriers to Cellulosic Ethanol" report issued by DOE on July 7 (*http://www.doe.gov/news/3804.htm*).

In his 2006 State of the Union Address, President George W. Bush specifically cited the promise of switchgrass as a bioenergy crop. A tall perennial grass, and a dominant species of the North American prairie, switchgrass (*Panicum virgatum*) is particularly compelling because of its relatively low production costs, minimal nutrient and pesticide requirements, perennial growth habit, and its ability to adapt to a broad range of growing conditions. The net energy gain for ethanol production from switchgrass is exceptionally favorable, coupled with low greenhouse gas emissions. The switchgrass project, which entails sequencing the gene transcripts, or Expressed Sequence Tags (ESTs) of the plant, is led by Christian Tobias and researchers at the U.S. Department of Agriculture Western Regional Research Center in Albany, California, and Gautam Sarath at the University of Nebraska, Lincoln.

"Switchgrass has enormous potential as an energy crop, and environmental benefits that are associated with its cultivation," said Chris Somerville, Professor of Biological Sciences at Stanford University and Director of the Carnegie Institution's Department of Plant Biology. "I envision that switchgrass will be an important feedstock for the emerging lignocellulose-to-ethanol industry. An enhanced understanding of gene structure and diversity at the molecular level may lead to new approaches to enhance both biomass productivity and feedstock quality for bioenergy production."

In complement to switchgrass, DOE JGI will be sequencing *Brachypodium distachyon*, a temperate grass model system with a simple genome more amenable to sequencing. This choice responds to the urgent need for developing grasses into superior energy crops while improving grain crops and forage grasses for food production. *Brachypodium* will be undertaken via a two-pronged strategy: the first, a whole-genome shotgun sequencing approach, is a collaboration between John Vogel at the USDA laboratory, colleagues at the John Innes Centre in England, and the University of Minnesota; and the second, an expressed gene sequencing effort, is led by Todd Mockler and Jeff Chang at Oregon State University, with Todd Michael of The Salk Institute for Biological Studies, and Samuel Hazen from The Scripps Research Institute.

Another major CSP project is the selection of cassava (*Manihot esculenta*), an excellent energy source and food for approximately one billion people around the planet. Its roots contain 20 to 40 percent starch, from which ethanol can be derived, making it an attractive and strategic source of renewable energy. Cassava grows in diverse environments, from extremely dry to humid climates, acidic to alkaline soils, from sea level to high altitudes, and in nutrient-poor soil.

The cassava project will extend broad benefits to its vast research community, including a better understanding of starch and protein biosynthesis, root storage, and stress controls. It will also enable crop improvements for related plants, including the rubber tree and castor bean.

The cassava project is led by Claude M. Fauquet, Director of the International Laboratory for Tropical Agricultural Biotechnology and colleagues at the Danforth Plant Science Center in St. Louis, and includes contributions from the USDA laboratory in Fargo, North Dakota; Washington University St. Louis; University of Chicago; The Institute for Genomic Research (TIGR); Missouri Botanical Garden; the Broad Institute; Ohio State University; the International Center for Tropical Agriculture (CIAT) in Cali, Colombia; and the Smithsonian Institution.

For a full list of the CSP 2007 sequencing projects, see: *http://www.jgi.doe.gov/sequencing/cspseqplans2007.html.*

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Stanford

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with a DOE JGI genome. The first is to increase the long-range contiguity (scaffold contiguity). Ideally, we would like every eukaryotic genome sequence to accurately reflect the number and organization of chromosomes that the actual creature of interest contains. However, WGSA usually are in many scaffolds: these range from the smaller side of the simple yeast *Pichia stipitis* with 16 scaffolds to the tree *Populus trichocarpa* with 22,000 or more scaffolds. We improve scaffold contiguity by sequencing clones that extend from the ends of the scaffolds and attempting to join scaffolds with this new sequence. We also sequence into telomeres and centromeres to provide a boundary for the euchromatic sequence. We integrate mapping data from genetic maps or optical maps, when it is available, to reinforce sequence joins and create chromosome-size genomic pieces. We have also recently begun sequencing BAC end libraries from genomes to add jumbo-size linking clones to DOE JGI eukaryotic genome assemblies.

The second goal is to improve and fix the base pair accuracy of the genome (sequence accuracy). This includes correcting false joins within scaffolds, validating all joins across the scaffolds, resolving repetitive sequence, closing all sequence gaps, and raising the quality of and verifying poor-quality base pairs. This is usually referred to as sequence finishing.

The third goal is to integrate orphan gene sequences into the assembly. Usually, a WGSA is missing a minimum of 5% of known coding sequences, and this number can go as high as 15%, depending on the complexity of the genomic sequence organization and the available amount of gene-based evidence. These missing genes can sometimes be found in very large captured sequence gaps, which we recover with clone-based sequencing, but they also appear in repetitive sequence arrays and duplications. Using the EST resources developed at the DOE JGI PGF, we locate as many of these genes as possible and represent the surrounding genomic sequence in the finished genome release. The final goal is to provide the resources necessary for the research communities to use genomic sequence effectively. We provide additional genomic resources such as EST sequences, full-length cDNA sequencing, genomic mapping assistance and targeted-clone finishing for DOE JGI genome projects, including quality control sequences to aid in genome assembly.

For now, as DOE JGI focuses on biological energy sources, I finally have something that is easy to describe. Because everyone back in Illinois, where I am from, knows what a soybean is, and how expensive it is to fill their truck's gas tank, they see the connection and how our work at SHGC fits into their daily lives.

Promotions

Julita Madejska
Scientific Technician to Biomedical Scientist
Cailyn Spurrell
Biosciences Technician 2-Shift Lead to Biomedical Scientist
Matt Zane
Biosciences Technician 1 to Biosciences Technician 2-Shift Lead
Eric Abbott
Biosciences Technician 1 to Biosciences Technician 2
Edwin Kim
Student Assistant to Computer Systems Engineer Trainee
Sanna Anwar
Student Assistant to Senior Research Technician
David Robinson
Senior Research Technician to Research Associate
Miranda Harmon-Smith
Scientific Technician to Sequencing Supervisor
Chris Daum
Senior Scientific Technician to Sequencing Supervisor
Susan Lucas
Production Department Head to Production Division Leader
Arshi Khan
Biosciences Technician 1 to Biosciences Technician 2-Shift Lead
Susannah Tringe
Special Postdoctoral Fellow to Biologist Scientist

Hazards

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Technology, traveled over 3,000 miles and hiked through lush rainforests to accost the local insects, but the tables were turned when the insects accosted them. Seeking *Nasutitermes corniger*, a special "higher" wood-feeding termite devoid of protozoa that would complicate DNA samples, the researchers hunted for likely volunteers among the local insect population. The basketball-sized termite nests were easy to find, and so were the angry occupants—up to a million termites in a single nest. *Nasute* termite soldiers have nozzle-shaped heads that squirt a clear sticky fluid intended to immobilize the threat to the colony. Warnecke was never immobilized when he hacked into a nest with his machete, but it was an exciting experience nonetheless. Leadbetter said that these soldiers "run up your arms and spray you with what smells like lime, which is why we let the post-docs do the sample collection."

Bears Collect Samples, Too

Tamas Torok and Glen Dahlbacka of the Lawrence Berkeley

(cont. on next page)

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Hazards

cont. from page 18

National Laboratory were drawn to the hot springs, fumaroles, and acid lakes of Siberia's Kamchatka Peninsula to seek undiscovered microbes. Unfortunately, the local brown bears—about 6,000 of them, some of the largest in the world—are also drawn to the hot springs and geysers because they like to keep warm. The local Institute of Volcanology provides armed escorts to protect researchers from the bears that roam the landscape, but Torok says that evenings are the most dangerous period—"when they're hungry." At night, the bears watched the researchers through the windows of their remote cabin. Who doesn't like a midnight snack?

Data Mining Where Nobody Can Hear You Scream

When you're studying ways to clean up toxic waste sites, the answers may lie in the deep gold mines of South Africa, where microbes living at high temperatures and pressures can tolerate an environment rich in uranium and organic carbon. When gold prices warrant the effort, miners will descend over three miles beneath the earth, and it's hot down there. Jim Fredrickson of the Pacific Northwest National Laboratory says he only had to go down about two miles to collect the anaerobic microbes he was seeking, where the temperature in the mine shafts was about 60 degrees Celsius (140 degrees Fahrenheit). For added excitement, mining-induced earthquakes would occasionally rock the shafts. Commenting on the conditions deep in the mine, he said they were "as close to hell as I care to get."

At the end of the day, it seems that microbiologists should be happy just to avoid being boiled alive or eaten by a bear, but when they get back to their labs, they have to face the greatest hazard of all—the paperwork required to maintain their funding.

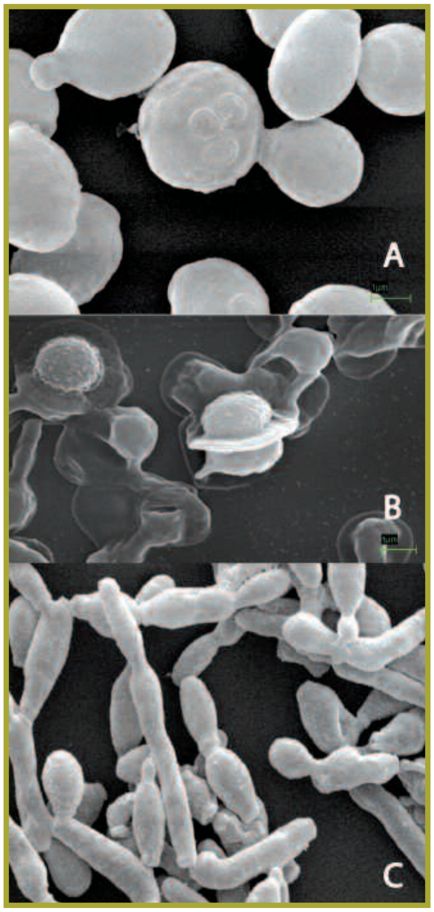
Pichia stipitis Genome Released

DOE JGI has recently released the complete genomic sequence of the yeast *Pichia stipitis*, which is known as one of the best native microbes for the fermentation of xylose to ethanol. Completion of the *P. stipitis* genome has greatly advanced our knowledge of how to genetically modify this yeast and other microbes for improved xylose utilization.

Xylose is a major component of agricultural residues and fast-growing hardwoods that could be used for bioconversion. Its economic use is critical for the conversion of biomass into renewable fuels. Fermentation of corn starch presently produces more than four billion gallons of fuel ethanol per year. This could be increased to about 80 billion gallons, or 40 percent of automotive fuels, if the problem of lignocellulose conversion can be solved.

Yeasts are especially useful for industrial ethanol fermentation because they have thick cell walls that facilitate processing and they resist infection by bacteria and viruses. Genomic sequencing has revealed features in addition to xylose fermentation that make *P. stipitis* important for bioconversion.

Pichia stipitis is an unusual yeast that is found in the gut of passalid beetles. In nature, it apparently helps these insects survive on rotted wood. In the laboratory, it has been adapted for the commercial production of ethanol from byproducts of wood processing and paper manufacture. A recent start-up company, Xethanol, has licensed the use of genetically modified P. stipitis for the fermentation of hemicellulosic sugars to ethanol.



Pichia stipitis **morphology under various conditions. (A)** ***Pichia stipitis*** **growing exponentially with bud scars; (B)** ***P. stipitis*** **hat-shaped spores seen from top and side; (C) Pseudomycelia formed under carbon-limited continuous culture. Photo by Thomas Kuster, USDA, Forest Products Laboratory**

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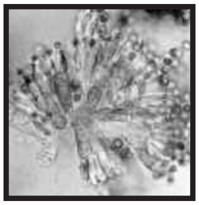
Aspergillus niger Genome Sequence Released



Aspergillus niger

DOE JGI and the Fungal Biotechnology Team of the Chemical and Biological Process Development Group at Pacific Northwest National Laboratory (PNNL) have released the first public sequence of *Aspergillus niger*, an important filamentous fungus used to manufacture industrial enzymes and organic acids. Scott Baker, Senior Research Scientist (PNNL) and Igor Grigoriev (DOE JGI), made a joint announcement of the public release of the *A. niger* genome sequence (*www.jgi.doe.gov/aspergillus*) at the 3rd Meeting of the *Aspergillus* Genome Community in Vienna, Austria. The announcement of the completed sequence included an invitation to participate in the annotation jamboree following the European Conference on Fungal Genetics in Vienna. On April 13, 2006 an international team of scientists began the process of annotating more than 11,000 genes.

The filamentous fungus *A. niger* has been used for nearly 100 years by industry to make organic acids, notably citric acid, and several biomass-processing enzymes. At PNNL, *A. niger* is used as a model microbial system to make value-added products from lignocellulosic biomass to augment the product portfolio of the anticipated biorefinery industry. The sequence of this organism provides information that will accelerate the rate of progress toward the Office of Biomass Program goal to enable the development of economical processes for converting biomass to fuel ethanol. Just as the petroleum refinery produces a diversity of products from oil, filamentous fungi will be used to convert complex biomass via their specific hydrolyzing enzymes and/or co-products, such as organic acids and platform molecules for the biopolymer industry. The sequencing of *A. niger* is an example of the collaboration between the Office of Science (OBER), the EERE Office of Biomass Program, and PNNL, all of which provided direct or indirect support of this project.

DOE JGI Wins Pollution Prevention Award

DOE JGI was one of the recipients of the Central Contra Costa Sanitary District 15th Annual Pollution Prevention Awards. This award recognizes a local institution's outstanding job in protecting our environment by integrating effective pollution prevention strategies into its business practices. DOE JGI Safety Coordinator Stephen Franaszek accepted the award for DOE JGI at Central San's September 21 board meeting in Martinez.

Stephen Franaszek receives pollution prevention award from Central Contra Costa Sanitary District's Mario Menesini (left), President of the Board of Directors, and Tim Potter, Source Control Superintendent.

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We're looking for contributors.

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