USDA and DOE to Coordinate Research of Plant and Microbial Genomics

Soybean to Be Sequenced

The U.S. Departments of Agriculture and Energy have announced that they will share resources and coordinate the study of plant and microbial genomics, and DOE JGI will tackle the sequencing of the soybean genome as the first project resulting from the agreement.

“This agreement demonstrates a joint commitment to support high-quality genomics research and integrated projects to meet the nation’s agriculture and energy challenges,” said Dr. Colien Hefferan, administrator of USDA’s Cooperative State Research, Extension and Economics Service (CSREES), who signed the agreement for USDA.

Soybean, or Glycine max, the world's most valuable legume crop, is of particular interest to DOE because it is the principal source of biodiesel, a renewable, alternative fuel. Biodiesel has the highest energy content of any alternative fuel and is significantly more environmentally friendly than comparable petroleum-based fuels, since it degrades rapidly in the environment. It also burns more cleanly than conventional fuels, releasing only half of the pollutants and reducing the production of carcinogenic compounds by more than 80 percent. Over 3.1 billion bushels of soybeans were grown in the U.S. on nearly 75 million acres in 2004, with an estimated annual value exceeding $17 billion, second only to corn and approximately twice that of wheat. The soybean genome is about 1.1 billion base pairs in size, less than half the size of the maize or human genome.

DOE JGI Reviewed by BERAC Subcommittee

On November 16 through 18, 2005, a panel of eminent scientists accompanied by research administrators of the Department of Energy’s Biological and Environmental Research Advisory Committee (BERAC) met at the DOE JGI PGF to conduct a comprehensive review of the DOE JGI. Chaired by Mel Simon from the California Institute of Technology, the committee included Bruce Birren, the Broad Institute; Jane Rogers, The Sanger Centre; Richard Wilson, the Genome Sequencing Center at Washington University St. Louis; Jim Tiedje, Center for Microbial Ecology at Michigan State University; Richard Mural, Windber Research Institute; Bruce Chrisman, Fermilab; Linda Horton, Oak Ridge National Laboratory; and former LBNL Operations Deputy Director Klaus Berkner. The committee was staffed by Dan Drell and Kent Lohman of DOE Office of Biological and Environmental Research. Jane Peterson, the Associate Director from the Division of Extramural Research at the National Human Genome Research Institute of the National Institutes of Health, sat in on the proceedings as an observer. The areas covered in the review included both science and management and operations. A verbal summary of the report was delivered by Mel Simon to BERAC at the beginning of December and was generally very positive, especially about DOE JGI science.
The Laboratory Science Program (LSP) is a new initiative from DOE to leverage JGI sequencing capacity, providing DOE national laboratory researchers with broader access to high-throughput DNA sequencing in support of mission-relevant projects.

The LSP will serve the national laboratories in two major ways. First, it will foster large-scale strategic sequencing projects, across the national laboratory system, that are aligned with future funding opportunities in DOE’s biology programs. Second, it will provide small-scale sequencing that meets the needs of individual investigators at the national laboratories.

The LSP is expected to use 15 to 20 percent of DOE JGI’s sequencing capacity, which is currently over 35 billion bases per year.

Sequencing to be carried out under the LSP will include genomes of entire microbial communities, and individual microbes and plants, useful in decreasing reliance on petroleum and petrochemicals by converting plant materials, such as soybeans, to ‘green’ energy and chemical feedstocks. Sequencing will also focus on characterizing the variation in human susceptibility to nucleic acid damage by ionizing radiation.

LSP’s newly appointed lead is Gerald Tuskan, a senior scientist in the Environmental Sciences Division at Oak Ridge National Laboratory. Tuskan will be responsible for developing, coordinating, and managing the LSP. Tuskan got together with DOE JGI’s Daniel Rokhsar and colleagues from the National Center for Soybean Biotechnology, the USDA Agricultural Research Service, Purdue University, and the National Center for Genome...
Genomic Explorers Seminar Series

BY KENDRA MYERS, LANL STUDENT

Introduced in December 2004, DOE JGI LANL’s ongoing Genomic Explorers Seminar Series features researchers from various institutions who are collaborating with the DOE JGI on the genomic sequencing of a particular organism.

The LANL community looks forward to the monthly seminars, which give researchers and students a chance to learn about microbes that have been selected and prioritized, through a highly competitive review process, for sequencing at the DOE JGI. The seminars also provide an opportunity for the speakers to learn about DOE JGI LANL’s capabilities in genome sequence finishing, and annotation and analysis.

Speakers typically spend a day or two in Los Alamos, enough time to meet with scientists who may have contributed to their sequencing project and with other researchers who may have similar interests.

For the most part, speakers invited for the seminar series are part of either the Community Sequencing Program (CSP) or the Microbial Genome Project (MGP). By welcoming submissions from researchers outside the DOE JGI for genomic sequencing of an organism, these programs make the large-scale sequencing capabilities of the PGF available to the greater scientific community. Organisms are selected based on their potential contributions to the DOE missions of advancing bioremediation, carbon sequestration, alternative energy development, and biotechnology, to name a few. About half of the seminars at LANL focus on bacterial organisms and the other half on fungal organisms.

In February, Teresa Thiel, from the University of Missouri, gave a seminar on a cyanobacterium that was selected for sequencing based on its ability to fix N₂ and CO₂, and to produce H₂.

Teresa Thiel, Professor of Biology, Associate Dean, College of Arts & Sciences, University of Missouri, St. Louis.

DOE JGI Assists in Plant Pathogen Consortium Research

BY KATHERINE HARRINGTON AND REBECCA E. MCINTOSH

Plants are essential commodities for many industries, from food to biofuel, textiles, and building materials. Scientists at Los Alamos National Laboratory (LANL) are protecting our nation’s agricultural investments using methods originally designed to identify human pathogens. The Plant Pathogen Consortium was formed a little over a year ago to tackle the problem of pathogens that threaten major crops—pathogens that, because of the economic importance of these crops, are in some cases considered potential biothreat agents. The Consortium’s efforts focus on developing the early detection techniques and effective treatments essential for the quick response needed to prevent major agricultural losses.

Lacking the ability to produce antibodies or other adaptive responses to pathogens, plants have limited defenses against disease. Therefore, the priority for farmers is to prevent plants from becoming sick in the first place. When this fails, there is a need for early detection and for non-toxic treatments safe enough to use alongside healthy plants. Naturally, scientists would like to identify the pathogen when it has affected only a few plants instead of the entire crop, thus preventing the economic hardship of losing an entire har-
Naturally, Tijana is a believer in the power of genes. “I look at my parents and see what kind of traits I’ve gained from each of them.”

While she tried to concentrate on her studies, the war rumbled on. “A lot of young boys went to the war and got killed. There were times when the action got close to downtown. One day a bomb fell—it was one of those grenades that split into many pieces—right inside this rehearsal room. It had a glass ceiling so people got hurt. Girls I know got shrapnel all over their feet. Can you imagine, for dancers!”

The event was sufficient motivation to make the move to California. She had relatives in the Golden State and came for a visit in 1990. “I liked it a lot in Walnut Creek and San Francisco. I mean, what’s not to like? I decided I was in love with California and one day I was going back.”

The part of the state where Tijana ended up moving to was far from the capital city of Zagreb. “Zagreb’s a beautiful city. It was part of the Austro-Hungarian Empire, so it has very similar architecture to Vienna and Prague. The whole of Croatia is breathtaking.”

“I started attending classical ballet at the age of five and was quite serious. With the ballet schools over there, you either sink or swim. They are very competitive. My mom kept me in there because I was doing well. I graduated from classical ballet school and earned a diploma at the same time as my high school diploma. My school schedule was much regimented; I never had any free time. After schooling, I started working in the National Theater of Croatia, where I danced as part of an ensemble for three years. I played roles in many beautiful ballets, from Don Quixote to Swan Lake, the Nutcracker—anything you could imagine.

“When I enrolled at the University of Natural Sciences in Zagreb as a freshman it was very difficult and challenging to keep up with schoolwork and dancing, so eventually I had to make a career decision. I chose biology, but never lost the love for ballet. We call it ‘bloody work,’ because your feet bleed. You work so hard and they pay you so miserably. But, nevertheless, I really enjoyed every minute of it.”

Tijana drew her inspiration and tenacity from her parents. Her mother is a professor of English and French, and her father is a mechanical engineer and main production manager of a big natural gas company. “All his life he’s been involved in management. I get a lot of my management skills from him, particularly the ability to work with people, understand where they are coming from and coach them to ensure success.”

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A Berkeley Lab Outstanding Performance Award (OPA) has been presented to a team, consisting of Dana Alcivare, Jason Baumohl, Patrick Hajek, Aaron Porter, and Arkady Voloshin for their creative and exceptional accomplishment in porting the legacy Venonat LIMS from a proprietary, obsolete, unsupported platform to an industry-standard Oracle 10g database and Apache web server. Common wisdom held that it was not possible to upgrade WebDB applications; the team was confronted with a 10+ person-year effort to rewrite the whole application. The porting breakthrough and implementation saved a huge amount of time and effort that was better spent on improving the application.

JGI New Faces (Sept 2005–March 2006)

PAUL BARALE
Software Developer 3, Instrumentation
MATT BLOW
Computational Biologist Postdoc Fellow, Vertebrate Program
BILL CANNAN
Sr. Recruiter, Human Resources
ARYK GROSZ
Software Developer 1, Instrumentation
ELIZABETH (BETTE) HERRERA
Operations Administrator, Operations
NATALIA MIKHAILOVA
Systems Analyst 2 Genome Biology Group
ROBERT OTILLAR
Systems Analyst 3, Genome Annotation
MICHAEL PHILIPS
Biosciences Technician, Production
ROTEM SOREK
Computational Sciences Postdoc Fellow, Vertebrate Program
ALICIA TOLIBAS
HR Asst III, Human Resources
RAY TURNER
Ops Dept Head, Operations
PARAG VAISHAMPAYAN
Computational Biologist Postdoc Fellow, Vertebrate Program

NEW OPS HEAD RAY TURNER

In October 2005, Ray Turner assumed the helm of the DOE JGI Operations Department, where he oversees finance, human resources, facilities, and administration at the PGF. Ray received his BS in Finance from the University of Utah and was commissioned into the United States Navy in 1980. After completion of flight training he served in various senior management and training positions within the Navy. In 1995, he received a Masters Degree in Financial Management from the Naval Post Graduate School and served as the Controller at Naval Air Station Alameda during the base closure process. After completion of a 21-year Navy career, he joined a San Francisco Bay Area healthcare information technology company where he served as the Vice President of Finance and Vice President/General Manager of the Health Information Management Division.

Tijana—Biomed Ballerina

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beach and the whales: on a campus, Cal State Stanislaus in Turlock, that’s landlocked in California’s great Central Valley. The school is known by locals as “Turkey Tech,” because of Turlock’s reputation as a leading poultry producer.

“I had no idea where I was going but I was so desperate to leave Croatia that it did not matter. When I got there, I couldn’t smell anything but cows. I cried the first night. I was thinking that I wanted to go back home. The new environment was a shock. Where’s the beach? Where is all the beautiful stuff I’d seen on my first visit? I had no friends. It took me some time to adjust. But luckily for me, it only took two days to meet my soul mate.” Her husband Daniel, originally from Spain, spent most of his life in Tenerife, in the Canary Islands. Ten years later they have two American kids, Nicolas and Isabel, who are destined to be perfectly trilingual in Spanish, English, and Croatian. But will they dance?

New Safety Officer Stephen Franaszek has a Bachelors in Mechanical Engineering and a Masters in Environmental Health Science from University of California at Berkeley. He has 15 years’ experience in Safety and Industrial Hygiene at LLNL and on the UC Berkeley Campus. For the last three years he has been LLNL Engineering Safety Officer.
SPOTLIGHT ON SAFETY

Keeping the PGF on the SafetyTrack

Implemented last December, SafetyTrack is the Production Genomics Facility’s centralized safety issue tracking system. Through SafetyTrack, you can report a safety incident or register a safety concern which could result in an incident if not addressed. You can also anonymously track the status of anonymous submissions.

Check out http://venonat.jgi-psf.org/safetyTrack to:
- Report incidents
- File requests
- Voice concerns
- Leave feedback
- Audit concerns

FOCUS ON ERGONOMICS

To increase awareness of ergonomics as part of the safety culture at the PGF, production groups will be putting together Ergonomics posters for their working areas in the production facility. Look for posters in each of the labs as each staff member has identified ergonomic risks, ergonomic changes, and possible solutions to help keep their work area safe and efficient. Everyone is getting involved with the goal to keep our facility a safe place to work.

PROMOTIONS—NEW PRODUCTION SHIFT LEADS

“We have taken a major step with these internal appointments to implement a structure that allows us to offer internal leadership opportunities, increase production capacity, and provide the necessary support structure to operate a production staff seven days a week, two shifts in a safe work environment,” said Susan Lucas, DOE JGI Sequencing Department Head.
Welcome to the first edition of the “Critter in the Queue” column. The goal of the column is to explore the “what” and “why” behind the organisms the DOE JGI is sequencing. It is an opportunity to turn the cryptic names scribbled on the side of a sequencing plate into real living creatures, and to understand why the DOE JGI has chosen to sequence their genomes. The first Critter in the Queue is Trichoplax adherens.

This is a Community Sequencing Program (CSP) project that is being completed in collaboration with Stephen L. DellaPorta (proposer) and Leo Buss, Yale University; Dan Rokhsar (proposer), DOE JGI; Bernd Schierwater, Tierärztliche Hochschule, Germany; Rob DeSalle, American Museum of Natural History; Peter Holland, University of Oxford; and George Weinstock, Baylor College of Medicine.

**Trichoplax Basics**

At first glance, this critter looks a lot like an amoeba, but it is very different. While an amoeba is a single cell, *Trichoplax* is a true animal, albeit a very simple animal.

At only a few millimeters in diameter, it has the simplest body plan of any animal, consisting of only four cell types that are arranged in only three cell layers. Based on its appearance, zoologist Franz Eilhard Schulze in 1883 named this animal *Trichoplax adherens*, which in Greek means sticky-hairy-plate.

*Trichoplax* is found in tropical and subtropical oceans around the world, where researchers have been able to collect these animals from the water column. They have also been found living on a variety of subtidal surfaces, including on other animals such as fish. Because studying *Trichoplax* in its natural environment has not yet been possible, very little is known about it.

Some cool facts:

Unlike most other animals it lacks a basal lamina, a layer of extracellular material that is used to organize tissues in most other animals. Some groups of sponges also lack a basal lamina.

Unlike all other animals it seems to lack an extracellular matrix, the material that exists between cells that assists in cell-to-cell adhesion and communication.

**Why is DOE JGI sequencing it?**

**PHYLOGENETICS**

*Trichoplax* is so unusual that it has been placed in its own phylum, the Placozoa. Furthermore, it has been hypothesized that this animal diverged from other animals soon after multicellularity evolved. Although its exact position in the tree of life remains uncertain, it seems clear that *Trichoplax* represents an early branch of the animal tree of life. Therefore, understanding similarities and differences between *Trichoplax* and other animal lineages will give us unique insight into animal evolution. It will also provide a new standard basal genome for comparative analysis.

**Minimal Genome**

*Trichoplax*’s genome is 50 megabases, the smallest of any animal. This is only ten times larger than the genome of the bacterium *E. coli* and three times larger than yeast.

**Cancer and Aging**

*Trichoplax* has only been observed to reproduce asexually, either by dividing in half or by budding of small “babies” called swarmers. There is evidence that this animal had the ability to reproduce sexually in its recent evolutionary past, but whether or not it retains this ability presently remains uncertain.

*Trichoplax* is the first animal to have its genome sequenced that can reproduce asexually. Some of its laboratory cultures have been maintained continuously via asexual reproduction for more than 20 years. Without sexual reproduction, which provides special DNA repair mechanisms, it would be expected that this culture would suffer from the same problems our bodies experience with time that lead to aging and cancer, yet this is not seen in these cultures. So how do they keep from aging or getting cancer? Do they have special DNA repair mechanisms or other ways to protect themselves from oxidative damage and telomere degradation? Once the genome is sequenced, we will be better able to answer these questions.

**How Far Have We Gotten?**

The *Trichoplax* genome draft is expected to be completed this summer.
Transferring Technology at the PGF

Every Moment Spent Planning Saves Three or Four in Execution

The Technology Transfer group at the PGF, supervised by Nancy Hammon, facilitates the planning, coordination, and implementation of new technologies, protocols, and workflow into the production sequencing line while continuously communicating the status of these transitions effectively across many DOE JGI groups. The Technology Transfer group is also responsible for production line project implementation. Projects include any new instruments, protocols, reagents, hardware, software, or workflows. Project responsibilities begin at the earliest formation of an idea and extend through the production adoption, but are primarily concerned with a project’s transition from working model to production line ready.

Current projects include:
FOSMID 384 PROTOCOL (Jamie Jett, Diana Lawrence, Julita Madejska, Maryam Waheed, and Duane Kubischta)

A new fosmid protocol has been developed and implemented in production. This protocol introduces a 384-well plate format for the fosmids, which will account for a 30 percent fosmid reagents and consumables cost savings. The new protocol also gives us a theoretical maximum throughput of 64 fosmid plates per day, over twice the current maximum. We are currently running Biomeks 5 and 6 with the fosmid 384 protocol. A new version of the Sprint Prep reagents is being tested side-by-side with the current version of Sprint Prep. The two versions will continue to run in parallel as we refine, evaluate, and optimize the workflow.

1/32ND BIG DYE CHEMISTRY (David Hillman, David Robinson, and Mingkun Li)

In December 2005, a coordinated effort lead by the Technology Transfer group set out to implement the newest version of the 1/32 BigDye sequencing reaction handed down from Genomic Technologies. Based on a statistical analysis performed by Mingkun Li, the 1/32nd BigDye samples have at most a loss of 24 Q20 bases. The library being sequenced and the polymer lot on the sequencer are significant contributors to this loss in quality. With the consent of the Sequencing Preparation Supervisor and QC, Technology Transfer launched the full-scale production use of the 1/32 BigDye chemistry reaction on January 25.

NEW PCR PLATE FOR PRODUCTION SEQUENCING (Victor Hepa, Dave Robinson, Nora Nichols, Sharon Ropes, and Simon Roberts)

Several new PCR plate types have been brought in for evaluation in order to address an ergonomic concern of plates sticking in PE Thermocyclers and warping of the PCR plate due to heat. These plates have been the Axygen M2 plate, the Eppendorf twin.tec plate, and the Axygen Hard Shell plate. After some initial evaluations, the M2 plate was not found to significantly reduce ergonomic stress on the operators and suffered from the same warpage as production’s current Axygen plate. The Eppendorf twin.tec plate is still under investigation. So far, this plate shows promise in both reducing ergonomic stress and plate warpage. Negotiations are ongoing to make this plate cost competitive. The Axygen Hard Shell plate failed its initial testing phase due to severe plate warping. The vendor is currently addressing this issue. Some changes to workflow and instrument heights have been made in the lab to reduce the overall ergonomic concern for this task while the new plates are under investigation.

Soybeans

cont. from page 1

“A soybean represents an excellent example of how DOE JGI is playing a key role in ‘translational genomics,’ that is, applying the tools of DNA sequencing and molecular biology to contributing to the development of new avenues for clean energy generation and for crop improvement,” said DOE JGI Director Dr. Eddy Rubin. “Effective application of translational genomics to soybean requires detailed knowledge of the plant’s genetic code. With this starting material in hand, researchers in academia, industry, and agriculture will be better positioned to optimize soybean for the broadest range of uses.”

BERAC

cont. from page 1

Among the highlights cited by Simon on the BERAC website were:

- The experts were quite satisfied.
- Appropriate and inspiring vision.
- Driving and exploiting sequence-based science.
- Have “cornered the market” on sequencing for energy, carbon sequestration, and environmental remediation.
- DOE JGI line managers are very good, especially the sequencing lead.

The written draft report now goes to the full BERAC committee, which is expected to convene in May for review and (probable) approval of the report.
Thousands of students, families, and teachers gathered at Orinda Intermediate School on October 6, 2005, to extract DNA, gaze at the stars, use liquid nitrogen to make ice cream, observe farm animals, and join in many other activities. Designed to use “way cool science stuff” to raise awareness among middle schoolers of science in general and careers in science in particular, the event combined classroom demonstrations, outdoor booths, and large presentations. Teachers, community members, and scientists from Bay Area companies, the University of California, Stanford University, LLNL, and LBNL captivated attendees with their enthusiasm for science and the lively topics covered in a total of 36 inventive demonstrations. The atmosphere was electric, as kids swept through the carnival of scientific experiments and presentations, pulling along parents and friends from one display to the next.

Volunteers from the DOE JGI, led by David Gilbert, worked with genome education outreach coordinator Karen Kelly of Clayton Valley High School to engage students of all ages in a hands-on experiment to extract DNA from blueberries, strawberries, and raspberries. A legion of Miramonte High School volunteers helped prospective scientists into lab coats, gloves, and goggles, and DOE JGI volunteers Damon Tighe, Julita Madejska, and Cailyn Spurrell instructed each table of four or five participants. The berries were first mashed using a mortar and pestle. They were then combined with an extraction buffer consisting of meat tenderizer, detergent, and salt before being gently mashed some more. The reaction mixture was separated from the seeds and pulp of the berries using cheesecloth. Ethanol was slowly added on top of the filtrate. Instantaneously, small bubbles formed in the alcohol layer, and eyes shone as thin white strings of DNA precipitated. Students were able to collect the DNA using an unfolded paperclip and take it home in a small vial of ethanol. A continuous stream of children and parents were eager to try their hand at recovering DNA from a small berry, and were intrigued when a comparatively large stringy mass precipitated in the ethanol. Orinda Intermediate students, with their younger siblings and friends from nearby schools (and a few parents), tried the protocol. Some mashed with vigor, messily combining the fruit extract and ethanol or violently shaking up the mixture. However, the experiment was extremely forgiving, and all reactions produced some amount of DNA.

After the extraction was performed, DOE JGI volunteers engaged the students in short discussions about the extraction to illustrate that the same molecule of DNA is present in all living organisms and that it is a real, tangible thing. Each group also learned some quick cell biology, discussing what each step of the reaction accomplished in the cell and how the DNA was extracted. Seeing the excitement and awe on the students’ faces as they became scientists, extracting berry DNA, was an enjoyable and valuable experience. If you would like to participate in future events, contact Karen Kelly (kkelly@lbl.gov).
The Google search engine uses a technology called PageRank. PageRank relies on the uniquely democratic nature of the web by using its vast link structure as an indicator of an individual page’s value. In essence, Google interprets a link from page A to page B as a vote, by page A, for page B. But Google looks at more than the sheer volume of votes—or links—that a page receives; it also analyzes the page that casts the vote. Votes cast by pages that are themselves “important” weigh more heavily and help to make other pages “important.”

However, it is worth understanding two things about the Google search engine:

Google tends to rank web documents higher than other types of documents. This is simply because web (HTML) documents are more likely to be referenced by other web documents than other types of documents, like PDF files or Excel spreadsheets. HTML makes linking easier and Google uses HTML links to rank pages. An analogy might be that you are more likely to receive junk mail in your email mailbox than in your postal mailbox, since it’s easier to advertise a magical dietary supplement by sending 100,000 emails than via the postal services.

The more documents indexed, the better the ranking. The official Google site (www.google.com) indexes the whole internet. When indexing a smaller network, like an intranet, it is more difficult to produce a good ranking, because there’s less information available for calculating the ranks. Like a poll, the bigger the sampling is, the more accurate the results.

Google at the DOE JGI

DOE JGI has its own Google search engine, which indexes documents stored on some local machines (see list below). It can be accessed via http://search.jgi-psf.org/ or via the Search the DOE JGI Intranet box located on the top of pages on the internal website and on the DOE JGI wiki.

More Than Just a Search Engine

The main Google site also provides special features to help you find answers. Using www.google.com, you can:

- Evaluate mathematical expressions (5 + (2 * 2)).
- Define words (define:species).
- Find out about the weather (weather concord, ca).
- Convert units in plain English (16 oz in liter or 2.3 USD per gallon in EUR per liter).

More examples are at http://www.google.com/help/features.html.

Google Advanced Search Operators

The DOE JGI search engine doesn’t have the features we’ve just mentioned (calculator, definition, weather, etc.); however, it does provide advanced search keywords that will help DOE JGI users refine their searches (these search keywords are also available on www.google.com). Keywords allow you to refine your search to specific types of documents, to specific websites, or to document titles only. Here are some useful examples for searching the DOE JGI intranet:

- Search for Microsoft Word documents containing the word “hybrid” (filetype:doc hybrid).
- Search for web pages that have the word “ramorum” in their title (filetype:html intitle:ramorum).
- Search for Microsoft Word documents on the DOE JGI wiki that have the word SOP in their title (filetype:doc inurl:wiki intitle:SOP).
- Search for all the pages that reference (link to) the Chlamydomonas reinhardtii genome portal page (link: http://genome.jgi-psf.org/chlre2/chlre2.home.html). Note: the title of a word document is not the printed title, but the title in the document properties.
- Search for all the pages that reference (link to) the Chlamydomonas reinhardtii genome portal page (link: http://genome.jgi-psf.org/chlre2/chlre2.home.html). Note: the link operator should be used alone. No other operators or keywords should be part of the search string.
- Search for all non-html and non-pdf documents containing the word “Halloween” located somewhere besides the DOE JGI wiki (Halloween -inurl:wiki -filetype:html -filetype:pdf).

Other Considerations

ALTERNATIVE SPELLING(S)

If your query didn’t return any results (or not the results you were expecting), try searching again with a different spelling (and the OR operator). For instance, if searching for “eRoom” didn’t find the document you were looking for, try “eRooms” (or “eRoom OR eRooms”).

Note 1: the www.google.com site will often suggest another search if you didn’t spell one of the keywords correctly.

Note 2: Google doesn’t take into consid-
cont. on page 11
cyanobacterium, *Anabaena variabilis*, has a unique system of N₂ fixation that involves three different nitrogenase genes—two molybdenum-dependent and one vanadium-dependent. The fixation of atmospheric nitrogen into a form that plants can readily use is an essential component of the global nitrogen cycle, and is of interest in itself. Of particular interest in sequencing *A. variabilis*, however, is the production of H₂ that occurs as a byproduct of N₂ fixation. A better understanding of the genome of *A. variabilis* may contribute to the development of strains capable of generating large amounts of H₂ for use as a renewable energy source.

Thiel was open to questions and input during her seminar, and said more than once that she was “here to learn as much as to teach.” Following the seminar, Thiel had the opportunity to meet with many different researchers involved in multiple aspects of sequencing and analysis.

Of particular aid to speakers who come to LANL is comparative analysis of their organism with the genome of one or more similar or related organisms. DOE JGI LANL, in addition to assisting in these analyses, has welcomed students and other researchers to come and learn how to do their own comparative analyses.

Many extended collaborations have formed as a result of the Genomic Explorers Seminar Series. Following the first seminar, given by Tom Inzana of the Virginia Polytechnic Institute in December 2004, Inzana and Jean Challacombe (JGI LANL) continued to work together on *Haemophilus somnus* 129 Pt, comparing this non-pathogenic bovine commensal to *H. ducreyi*, a sexually transmitted localized pathogen, and *H. influenzae* RD, a non-pathogenic lab strain. They are currently finishing a paper reporting the insights that these comparative analyses have provided into metabolism and host colonization. They also plan to continue their collaboration, comparing the non-pathogenic strain *H. somnus* 129 Pt to a pathogenic strain (*H. somnus* 2336) that DOE JGI LANL just recently finished sequencing.

Another collaboration resulted from a seminar given by Randy Berka in January 2005 on the fungus *Trichoderma reesei*. Berka, who works for Novozymes, Inc., in Davis, California, and Diego Martinez of DOE JGI LANL expect to publish a paper this year on *T. reesei*, which is of particular interest for its efficient production of cellulase, an enzyme that breaks down cellulose. The fermentation product of cellulose degradation can be processed to yield fuel-grade ethanol, so a better understanding of cellulase production may eventually help lower the cost of cellulose degradation and help make bioethanol financially viable as an alternative fuel.

In March, Cindy Nakatsu will come to LANL from Purdue University in Indiana to give a seminar on *Arthrobacter* sp. FB24, a bacterium with a great potential application in bioremediation because of its extremely high degree of chromium tolerance, its resistance to some other metals and to radiation, and its ability to degrade certain hydrocarbons. Nakatsu’s seminar is guaranteed to open with the same statement that Paul Gilna, leader of the Genomic Sequencing and Computational Biology Group at LANL, makes at the beginning of every seminar: “The DOE JGI is here to serve the scientific community....”

For more information contact: Chris Detter at (505) 667-1326 or cdetter@lanl.gov.
computational tools as CRITICA (Coding Region Identification Tool Invoking Analysis) and GLIMMER (Gene Locator and Interpolated Markov ModelER), they generate a master list of genes. This helps in visualizing gene positions and resolving trouble points in the initial draft sequence. The proteins encoded by the found genes are then compared against a microbial sequence database using a set of algorithms contained in the BLAST (Basic Local Alignment Search Tools) suite of programs.

“We use these criteria to resolve overlaps,” says Loren. “For example, if you have a situation where one has a BLAST hit and one doesn’t, you keep the one with the BLAST hit and throw the other one away.” In this manner the list is updated and revised. It is then compared to the spectrum of annotated sequences available from other databases in an attempt to assign functions to genes that have been identified within the sequence.

“After we go through all the database searches and predictions, we have then a hierarchy of information that we give a function call, or what I call a product description. When you do a BLAST search...you get back your hit: an alignment, a score—the product description. So we need to be the most accurate with...the product description because that’s what everybody sees when they do a BLAST job.”

The annotated genome is sent back to the PGF, where the information is uploaded into the Integrated Microbial Genomes (IMG) data management system, the DOE JGI portals, and the National Center for Biotechnology Information’s (NCBI’s) GenBank for use by the global research community.

Shewanella is a major microbial player in the bioremediation of environments contaminated with toxic metals and radioactive waste. Its characterization for the Shewanella Federation, which is led by Margie Romine at Pacific Northwest National Laboratory (PNNL), illustrates how automation tools can speed up the annotation process. Under the auspices of the DOE Office of Biological and Environmental Research (BER) Microbial Genome Sequencing Program, DOE JGI is contributing sequences from 14 different species of the bacterium to this effort.

“Once we annotate as accurately as we can, we then transfer all of that information directly to the other species, making the initial time investment well worth it,” says Loren.

The whole process would be quick and easy if only all the microbial organisms were structured in a similar way. One of the important aspects of how they differ is the GC content, or the proportion of G and C bases in the sequence. Genomes with low GC content (below 60 percent) can be annotated fairly quickly using the available process-automation tools. High GC content genomes still require a significant time investment.

When can you say that a genome has been fully annotated?

“E. coli is not fully annotated yet,” Loren said. “It is the best annotated, because it has the most experimental data, but it is not fully annotated yet.”

“So a lot of the annotation, filling holes, still depends on future experimental data,” adds Miriam. “That’s one of the biggest problems right now is that we can generate sequence data and get preliminary annotation a heck of a lot faster than we can provide even high throughput experimental data to verify that these things are real, or their function.”

Miriam, Loren, and their colleagues in the bioinformatics community are striving to establish a set of rules that would allow them to more easily describe the features of the genome and eventually automate the process. According to Miriam, “Hopefully, within a year or two we will have a list of product descriptions that would be standard operating procedure that would apply to all genomes. The advantage of this is that all of our genomes will be annotated in a consistent fashion.”
Mourning doves (Zenaida macroura) are a common site at the DOE JGI Production Genomics Facility. They can be seen foraging on the ground for grains and seeds, and perching on the power lines. They are among the most abundant birds in North America, and thrive in the environment that we Homo sapiens provide. The DOE JGI Vertebrate Genomics Program’s Anna Ustaszewska was lucky enough to have a pair of mourning doves choose her balcony as a site to raise their babies. I asked Anna to share her experience with us in this issue’s Birds of DOE JGI article.

There is a beautiful, huge redwood tree right outside my Walnut Creek apartment. Over the summer, a pair of mourning doves decided to start a family on one of its branches. At first, the female was just trying out the spot, sitting for an hour or two on the branch while the male watched over from close by. Both would then fly away for the rest of the day. Doves make a characteristic whistling noise with their wings when flying, so it was quite easy to hear them come and go. After a few days of this, they decided to stay and build a flimsy nest of twigs and dry leaves. Soon two eggs appeared. The parents took turns incubating. After two weeks, the eggs hatched! (At this point, picture me rolling on the balcony with the camera, every chance I got, trying to capture the best view into the nest.)

The young were naked, blind little things, unable to hold their heads up for the first few days. Both parents fed the young, first with pigeon’s milk, a white fluid produced in the bird’s crop that’s similar to the milk of mammals, then solid food. The babies were never left unattended in the nest (to the frustration of this photographer), except for brief moments during the parental “switch off.” After two weeks, the young were ready to leave the nest. The last picture was taken the evening before they fledged.

Some facts about mourning doves:
- Both males and females are about 12 inches in length, with a 28-inch wingspan. They each have a plain brown, plump body, and a small, pinkish head with a black spot on the lower cheek. Their long tails taper to a point, and they have black spots on their upper wings. The male has a small, iridescent blue patch of feathers on the back of its head and a pinkish breast.
- The bird is named for its mournful call, “Oowooo-woo-woo,” which is often mistaken for the hoot of an owl.

According to some research, mourning doves mate for life. They are very successful breeders. Parents may have several broods per year, each consisting of two or three chicks.

Their nest is usually a flimsy bowl of twigs. During nest building, the female stays at the nest and the male collects sticks. He stands on her back to give her the nest material. She takes it and weaves it into the nest. Maybe that’s why the nests are so poorly built!

They are the most widespread and abundant game bird in North America. Despite being hunted throughout most of their range, they remain among the 10 most abundant birds in the United States.

For more information about mourning doves and to listen to their cry, visit Cornell’s website: http://www.birds.cornell.edu/programs/AllAboutBirds/BirdGuide/Mourning_Dove.html

For more on the evolutionary relationships of birds or any other organism, visit the Tree of Life website: http://tolweb.org/
Detter Heads South

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...ing capability at DOE JGI LANL and lead the lab team as part of a cooperative arrangement between the Walnut Creek and Los Alamos facilities. According to Tom Brettin, Acting Group Leader for the B-5 Genomic Sequencing and Computational Biology Group, “Chris is filling a critical position at DOE JGI LANL that has been vacant since David Bruce accepted the position of Project Manager for the DOE JGI Microbial Program in the fall of 2004.”

One of the goals Chris has set for the finishing team is to increase the consistency and efficiency of its sequencing procedures. Toward this end the group is investing in new technology to rebuild their infrastructure. For example, a new Biomek FX liquid handler was acquired recently to help automate sequencing reactions.

“I like technology,” says Chris. “I like making things bigger, better, and faster.” But in addition to updating equipment to increase efficiency, Chris wants to improve coordination between the lab team and the computational team. By encouraging the lab team to be more involved with the computational analysis of the raw sequence before protocols are ordered, Chris hopes that all parties can contribute to deciding which protocols are best. This can save precious time by eliminating some of the redundancy in the process.

“Chris brings significant technical skills to these high-throughput lab operations,” says Brettin. Chris hopes to share the knowledge he has amassed at DOE JGI PGF with his new group. So far, he has worked to get to know the DOE JGI LANL team members and to evaluate how to achieve his goals. Chris is pleased that his new colleagues have given him the benefit of the doubt and given his ideas and suggestions a positive reception.

Although New Mexico is a little cold for someone who’s spent most of his life in either Florida or California, Chris is enjoying the change thus far. Los Alamos is nestled up against the mountains, making it a great place for hiking, mountain biking, and skiing. And it’s only five minutes from work!

He’s even had time to get out and have some fun. Chris, who’s been riding motorcycles since he was ten, quickly found fellow riders in B Division with whom to explore the “Land of Enchantment.”

LSP Lead

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Resources to make the soybean project a reality.

“Sequencing the soybean genome will create new opportunities for the advancement of biodiesel as an economically viable transportation fuel,” says Tuskan. “Having the catalog of all soybean genes at our fingertips will facilitate the discovery of metabolic processes that will lead to improved oil production in the seeds and possibly throughout the entire plant. Greater yields of oil per unit area of land will reduce the cost of production and move soybean-based biodiesel closer to your local gas station.”

More information on the LSP can be found at: http://www.jgi.doe.gov/materials/LSP/index.html.

SPRING JAMBOREE ROUNDUP

Diatoms are eukaryotic photosynthetic microorganisms found throughout marine and freshwater ecosystems. Major players in the carbon cycle, diatoms are responsible for about 20 percent of global production of new biomass by photosynthesis.

*Phaeodactylum tricornutum* is the second diatom sequenced. This 30 million base genome, with the diatom *Thalassiosira pseudonana* (also sequenced by DOE JGI), provides the basis for comparative genomics studies and will serve as a foundation for interpreting the ecological success of these organisms.

Forty-plus biologists will gather March 22-24, 2006, at the Embassy Suites, Walnut Creek, Calif., for the *Phaeodactylum* Jamboree led by the principal investigator Chris Bowler of the Ecole Normale Superieure and the DOE JGI annotation team of Alan Kuo, Asaf Salamov, and Igor Grigoriev. Other Jamborees include:

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<th>Jamboree</th>
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<tr>
<td>Laccaria bicolor</td>
<td>April 4-5</td>
<td>Nancy, France</td>
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<td>Aspergillus niger</td>
<td>April 12-14</td>
<td>Vienna, Austria</td>
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<td>Xenopus tropicalis</td>
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<td>Walnut Creek, Calif.</td>
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For more information, contact Igor Grigoriev: IVGrigoriev@lbl.gov or (925) 296-5860.
Plant Pathogen
cont. from page 3

vest. A collaboration of LANL, several universities, the USDA Agricultural Research Service (ARS), and representatives from the wine, almond, and citrus industries, the Plant Pathogen Consortium will develop and apply modern technology to diagnose and combat plant diseases of concern to the agricultural industry.

According to Project Leader Goutam Gupta of the Bioscience Division at LANL, the first step in this process is to create a knowledge base about plant pathogens. The more researchers can learn about how the organisms work, the better equipped they will be to determine pathways for detection and prevention, and to adapt existing technologies to these problems. Variability, for example, is a major issue. As Gupta explains, some plant pathogens have various strains, and the biggest problem is figuring out which strain is affecting the plant.

This is where the DOE Joint Genome Institute (DOE JGI) will be especially helpful. Through gene sequencing and annotation, and subsequent analysis, Consortium researchers can develop a knowledge base for these pathogens. Using sequence information and bioinformatics, along with analysis of pathways and gene expression, they can evaluate the specific characteristics that make each pathogen unique. One approach may be to distinguish between the strains by looking for specific genetic markers and virulence factors. Another method goes one step further and looks at macromolecules such as proteins and carbohydrates that may be displayed by the host as early indicators of infection. This kind of investigation can help delineate the early steps of plant-pathogen interactions, which will then lead to therapeutic techniques.

The Consortium picked two common, but destructive, organisms to begin researching: Citrus tristeza virus (CTV) and Xylella fastidiosa (Xf). Citrus tristeza virus is a single-stranded RNA (+) filamentous virus that is transmitted by aphids. It is the most destructive virus in citrus trees and manifests in many ways: it can cause the tree to yellow and wilt rapidly (commonly called “quick decline”) or can result in pitted trunks, stunted growth, and irregular fruit. X. fastidiosa is a gram-negative bacterium that is transmitted by insects called sharpshooters. It infects a variety of plants, causing numerous diseases, such as variegated chlorosis in citrus trees, Pierce’s disease in grapevines, and leaf scorch in almond trees. These diseases can cause problems such as weakened plants, dead leaves, and disfigured, tasteless fruit. The Consortium is already looking at adding a new organism, citrus greening Liberibacter, to the research plan.

Two X. fastidiosa strains isolated from diseased almond trees in the southern San Joaquin Valley of California were recently sequenced by DOE JGI. Libraries were compiled at DOE JGI LANL, sequencing was done at DOE JGI Production Genomics Facility, and the finishing will be completed at DOE JGI LANL. These two X. fastidiosa isolates have different genotypes based on single nucleotide polymorphisms in the 16S DNA. In addition, they exhibit phenotypic differences through different colony-forming properties in vitro.

“The relationships of these variants to the pathogen biology and disease epidemiology are unknown. We expect that the genomic sequences of these two variant Xf strains will provide insight into the genetic basis of their biology or microbial ecology, as well as their relative roles in the epidemiology of almond leaf scorch disease,” says Gupta.

Identification of key signatures such as these genotypic variations may be the best strategy for early detection; waiting for symptoms could be too late. The Plant Pathogen Consortium hopes that by starting with the genetic information and later studying macromolecules and host-pathogen relationships, they will be able to develop modern diagnostics and effective therapeutics for these pathogens. Once the Consortium has successfully developed strategies for these organisms, they will look to expand their investigation to other problem pests.
CYBIO COMBINED CHEMISTRY
EQUIPMENT (Simon Roberts, Steve Wilson, Kecia Duffy-Wei, Simona Necula, and David Robinson)

On December 12 we received the first of two combined chemistry instruments from CyBio. These machines will replace the Hydra-Twisters as well as the Cavros (some of the PGF’s oldest equipment) in the sequencing preparation lab. The CyBio instrument will integrate the template aliquot and the sequencing cocktail dispense into a single automated run. In addition, these instruments will embrace a new “web services” technology from the production informatics group that will improve speed, flexibility, and reliability when communicating between lab instruments and the database. Production operators will no longer hand-scan plate barcodes into the database, availing more time to multi-task. The CyBio instruments will eventually utilize vendor applied barcodes, eliminating the need for operators to hand-apply printed barcodes. The first CyBio Combined Chemistry instrument was moved into the production lab 140 and began daily processing of production plates on a limited scale on February 22. Production scale-up of the first instrument will continue through March 2006. The second CyBio Combined Chemistry instrument is scheduled to come online in April 2006.

The CyBio CyBi-Well Vario and CyBi-Drop height-adjustable table was purchased from Lista Toolboxes in Menlo Park, CA, with a 21-inch MIN to 31-inch MAX height adjustment range. This was specified to give an ergonomic working height for all operators, both short and tall, based on an Ergonomic Risk Reduction Analysis called STEER. The standard fixed lab bench section was removed to install the system and table. With casters on the table, it also has the advantage of enabling easy access for maintenance if required.

New Senior Recruiter Bill Cannan has joined the DOE JGI Human Resources team. In this role, he will provide comprehensive recruitment, staffing, and outsourcing support, including representing the JGI in various recruitment efforts. Bill’s recruiting experience includes over eight years with California State Automobile Association, West Valley Engineering, and Kelly Services Onsite @ Lifescan. He brings strong knowledge of California employment labor laws, demonstrated success in high-volume recruiting, and over thirteen years of client service experience.

The 2007 DOE JGI Community Sequencing Program call for Letters of Intent generated:

- 234 letters of intent which break down into:
  - 25 large genomes/8 given the go-ahead for full proposal submission
  - 118 microbes/88
  - 91 small eukaryotic genome projects/65

147 full proposals were received on March 10 and will be reviewed in early May.

CSP PROJECTS IN PROGRESS

The tropical grain Sorghum bicolor, proposed by an international consortium led by the University of Georgia and Rutgers, will complement the knowledge gleaned from rice, the only other monocot grain sequenced to date. The U.S. provides 70 to 80 percent of world sorghum exports. The worldwide annual economic value of the crop exceeds $69 billion. With a relatively compact genome of approximately 736 million bases, Sorghum will serve as a valuable reference for analyzing the fourfold larger genome of maize, the leading U.S. fuel ethanol crop. About 12 percent of the U.S. grain sorghum crop goes into ethanol production. Sorghum is an even closer relative of sugarcane, arguably the most important biofuels crop worldwide.