

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 conven-

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tional 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

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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 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.

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Save the Date



**First Annual
DOE Joint
Genome
Institute
User
Meeting**

March 29-April 1

<http://www.jgi.doe.gov/usermtg/index.html>

SPOTLIGHT ON DOE JGI ORNL

MICROBIAL GENOME ANNOTATION THE ORNL WAY

As hundreds of genomes churn through DOE JGI sequencers, efforts are underway to make their annotation—the determination of what biological functions these sequences actually encode—faster, more accurate, and cheaper. One of the challenges in assigning function to sequence is that the generation of raw sequence has far outpaced experimental data-gathering. To help close this gap, the

annotation process is guided by the principle that, if genes found in two different organisms look similar, there is a high probability that they will function in a similar way.

Loren Hauser, of Oak Ridge National Laboratory, explains: “If the sequenced DNA contains a gene that looks like another gene which has been experimentally proven to be responsible for

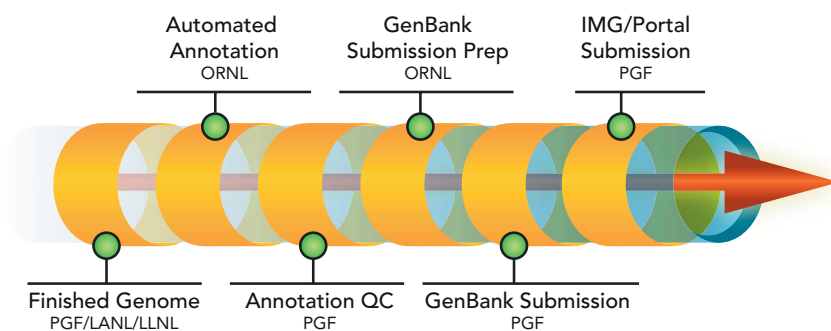
the production of a specific protein, there is a high probability that our gene has the same function.”

Two types of organisms are sequenced by DOE JGI: microbes and eukaryotes. The two groups differ dramatically, and are handled at DOE JGI through two distinct pipelines. Igor Grigoriev leads the eukaryote annotation effort at the DOE JGI Production Genomics Facility (PGF). Microbes, meanwhile, are sent out for automated annotation to Hauser and Miriam Land at Oak Ridge. A look at the microbial pipeline illustrates how automation is helping to speed up the annotation process.

The microbial annotation pipeline begins with Sam Pitluck at the PGF uploading data from completely assembled microbial genomes to the DOE JGI FTP website, where Miriam and Loren pick it up. Then, with the help of such

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DOE JGI Microbial Genome Annotation Pipeline



ORNL's Tuskan to Lead LSP

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 pro-



grams. 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 con-

verting 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

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SPOTLIGHT ON DOE JGI LANL



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



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

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 N_2 and CO_2 , and to produce H_2 . This

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Detter Joins Team at DOE JGI LANL

BY REBECCA E. MCINTOSH

After six years at Walnut Creek, Chris Detter has decided to make a move to greener, nope, browner pastures. Chris, who led the cloning technology group at the DOE JGI PGF, recently accepted a position at DOE JGI LANL and left sunny California for northern New Mexico.

New Mexico isn't a completely new place for Chris. Over the past four years, Chris has collaborated with researchers at LANL to develop more efficient DNA cloning protocols for the Bioscience Division. In his new position, Chris will contribute to the finish-

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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-

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JGI FACES

Tijana Glavina del Rio—Biomedical Ballerina

Tijana Glavina del Rio has been part of the DOE JGI Production Genomics Facility (PGF) since shortly after the doors opened back in 1999, but Tijana's path to the PGF was anything but conventional.

Growing up in Croatia, she was in the water practically before she could walk. "I got a BS with a concentration in marine biology because I was hoping to go work with the whales and dolphins, explore the oceans. I was really in love with Jacques Cousteau and his explorations since I was a little kid.

"However, when I graduated, there were just too many marine biologists and not enough jobs, and a BS didn't prepare me enough for those jobs. So I started looking for anything that had to do with biology. I stumbled upon the Human Genome Project, which caught my attention. Then I saw an application for a lab tech position at Lawrence Livermore National Laboratory."

She never actually worked in Livermore, though, because she was needed straightaway in Walnut Creek. "Nancy Hammon was my coach during the first couple of weeks at JGI. She trained me on cleaning up DNA samples using the SPRI [Solid Phase Reversible Immobilization] original bead cleanup method. At the time, we were just forming a production line. Our throughput was just one 96-well plate per day. Next, they brought the CRS robots and I was tasked to work on automating the SPRI process using the new robots."

Tijana's responsibilities have grown by leaps and bounds over the last five years, and today she is the Sequencing Prep Group Leader. She now supervises, in addition to sequencing prep, the Freezer Group and the Fosmid Group. It's quite a complicated dance, but she's up to the task.

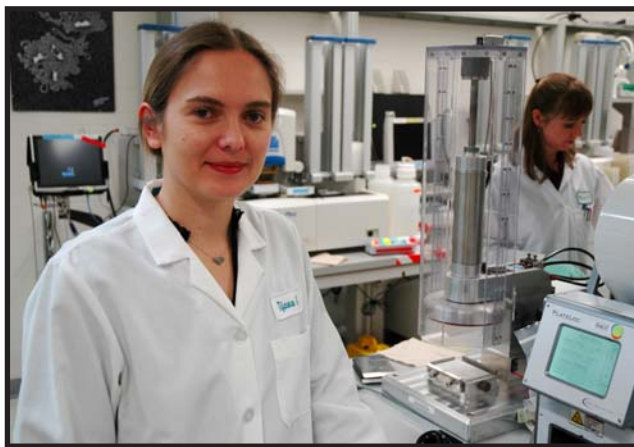
From an early age Tijana, an only child, attended a classical ballet school

in the Croatian 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 natu-



PGF Sequencing Prep Group Leader Tijana Glavina del Rio

ral 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."

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

JGI New Faces (Sept 2005–March 2006)

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

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 Comptroller 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 health-care information technology company where he served as the Vice President of Finance and Vice President/General Manager of the Health Information Management Division.

OPA FOR PORTING VENONAT

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.

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 envi-

ronment 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



A show of hands for SafetyTrack by Tijana Glavina del Rio, Chris Hack, and Terri Jackson.

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.

Ergonomically Designed Products



PROMOTIONS—NEW PRODUCTION SHIFT LEADS



Amber Nivens



Matt Zane



Eric Abbott



Cailyn Spurell

"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.

CRITTERS IN THE QUEUE

BY WENDY SCHACKWITZ

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*.

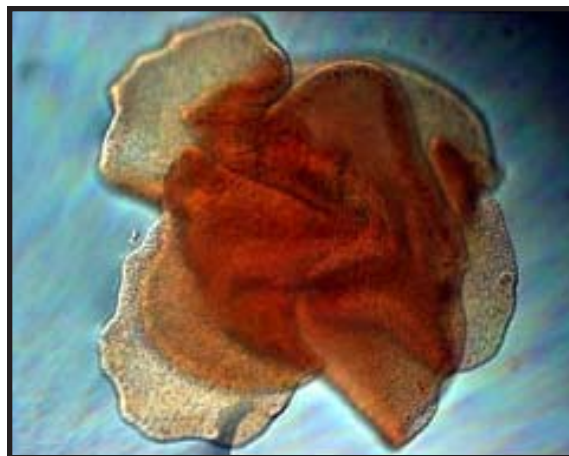
This is a Community Sequencing Program (CSP) project that is being completed in collaboration with Stephen L. DellaPorta (proposer) and Leo Buss, Yale University; Dan Rokhsar (proposer), DOE JGI; Bernd Schierwater, Tierärztliche Hochschule, Germany; Rob DeSalle, American Museum of Natural History; Peter 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 *Tricoplax 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 extra-cellular 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*)

Soybeans

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genomes.

"The 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."

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.

BERAC

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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.

