

SEMINAR CALENDAR*

JAN 14

Stephen West, Cancer Research UK
"Double-strand Break Repair in Mammalian Cells"

JAN 18

Tom Hei, Columbia University
"Radiation Induced Bystander Effects: Mechanisms and Implications for Radiation Risk Assessment"

JAN 21

Thomas Jenuwei, The Research Institute of Molecular Pathology - IMP
"The Indexing Potential of Histone Lysine Methylation"

JAN 25

Eric Reiman, Translational Genomics Research Institute
To be announced

FEB 1

Mark D'Esposito, University of California, Berkeley
"Is Functional MRI the New Phrenology?"

FEB 8

Gregory Wray, Duke University
To be announced

FEB 15

Karlene Cimprich, Stanford University
"Sensing and Responding to DNA Damage"

FEB 22

Howard Ochman, University of Arizona
"The Evolution of Defective Genes and Genomes"

*all seminars at 4pm, LBNL B-66 Auditorium

New Names/Transitions

Eric Abbott
contract Laboratory Technician

Ranjana Ambannavar
contract Laboratory Technician

Mariana Anaya
Biomedical Scientist

Shane Brubaker
Computer Scientist

Sandra Chaparro
Career appointment

Jarrod Chapman
Post Doc appointment

Vanessa Chiu
Scientific Technician

Peg Folta
Acting Department Head for Informatics

Navjett Kaur
Laboratory Technician

Victor Kunin
Post Doc

Konstantinos Mavrommatis
Post Doc

Donna McCown
contract Secretary

Brenda Mitchell
contract Secretary

Hope Tice
Career appointment

Jennifer White
Sequencing Supervisor

Matthew Zane
Scientific Technician

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JGI Hosts Poplar Annotators

Thirty-five researchers from as far away as Canada, the United Kingdom, Belgium, Finland and Sweden gathered the week of December 13th at JGI Walnut Creek to plumb the depths of the first tree genome to be sequenced, the black cottonwood or poplar, *Populus trichocarpa*.

With just over 480 million bases, the poplar genome contains over 40,000 genes. The whole genome is duplicated with some 40% of the poplar genes found in two recently diverged copies and the remaining duplicates having been lost in the more than 60 million years since the genome doubled. This observation framed the discussion of the gene content relative to the mustard weed *Arabidopsis thaliana*—the most closely related plant genome sequenced to date. Among the other discussion topics were transcription, transport, and metabolic regulation,

micro RNAs, cytochrome P450s and regulation of other genes, including those involved with insect and disease resistance, as well as novel cell wall features of plants.

"The preparatory work by folks at JGI and elsewhere created an environment where discussions and interactions dealt with hypothesis generation as opposed to rote editing of draft sequence," said Jerry Tuskan, who leads the poplar annotation effort from Oak Ridge National Laboratory. "At the end of each day we had to drag the participants from the room. That's a good sign."

Plans for a manuscript describing the genome were put in place, with submission targeted for March 2005.

Other JGI Jamborees coming up in 2005 include the frog, the lancelet amphioxus and the sea anemone *Nematostella*.

MegaBACE 4500s Now On-line

BY CHRIS DAUM

The MegaBACE DNA analysis platform has been an indispensable component of the JGI production sequencing line for several years. With the inception of the capillary based MegaBACE 1000 platform in 1999, significant advances were made over the slab gel instruments in the areas of automation, workflow, and operation; the MB1000 platform went on to play a crucial role in the Human Genome Project. Once more, with the launch of the updated MegaBACE 4000 platform in late 2001 a new standard for high-throughput sequencing was set with its

384-capillaries accelerating the process.

With the advent of a newly improved platform, the MegaBACE 4500 sits poised to yet again shift the paradigm of high-throughput DNA sequencing. Development work and onsite testing of the Amersham Biosciences (now part of GE Healthcare) new MegaBACE platform has been ongoing at the JGI since February 2003. During this time the MB4500 system has gone through several modifications, reagent reformulations, and software changes. In July of this year, three official release versions of the MB4500s were installed here at the JGI for proof of **cont. on page 4**

inside this issue

2. JGI Faces—Phil Hugenoltz
3. Birds of JGI
4. Tech Tech—Rolling Circle Amplification (RCA)
5. Tanja and the Gutless Worm
6. Genetics & Plate Tectonics
7. JGI OpsNews
8. Seminar Calendar

ORNL DIRECTOR VISITS JGI PGF

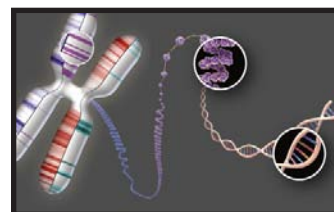
Oak Ridge National Laboratory Director Jeff Wadsworth made a whirlwind tour of the JGI Production Sequencing Facility (PGF) on Thursday, December 16th. Dr. Wadsworth will be among those laboratory directors, along with his counterparts at LLNL, LBNL, LANL, and PNNL, who will be meeting in March to discuss the memorandum of understanding governing on-going operations of JGI.

JGI senior managers Susan Lucas, Paul Richardson, Eddy Rubin, and Jim Bristow, show ORNL Director Jeff Wadsworth (fourth from left) around the PGF.



Chromosome 16 Sequence Caps DOE HGP Contribution

Culminating a 16-year effort, DOE has completed its share of the Human Genome Project (HGP) with JGI's publication of the DNA sequence and analysis of chromosome 16 in the journal *Nature* **432**, 988-994 (23 Dec 2004). A poster highlighting the DOE HGP effort can be acquired by contacting David Gilbert (gilbert21@llnl.gov).



JGIware now on sale—Polo, tee, Oxford and Hawaiian shirts, ball caps and more, care of the JGI Employee Activities Committee (EAC) on the JGI internal website.

JGI FACES

Phil Hugenholtz—Bug Hunter from Down-Under

For a guy whose name in Dutch means “hill wood” it seems only fitting that he spend his days characterizing the microbial world from hill to dale, hot spring to acid mine, farm to wastewater plant.

Phil Hugenholtz, head of JGI’s microbial ecology program, was born in Auckland, New Zealand son of a Dutch immigrant and a mother who is fifth generation Kiwi. At the age of 12, his family made the trip across the Tasman Sea to Australia where he spent the rest of his youth.

“My two favorite subjects in high school were biology and art. Out of the two, biology was the one that I could probably make more of a living out of. I later discovered that I wasn’t very good at chemistry or biochemistry, so I was left with microbiology. But I have always been fascinated with the microbial world.

“As is often the case, you meet someone who gets you fired up about something in particular.” In Phil’s case it was Professor Erko Stackebrandt when he was at the University of Queensland, Brisbane, Australia. Erko now serves as the Director of the German Collection of Micro-organisms and Cell Cultures in Braunschweig.

“In 1989, when I was just starting my Ph.D., at University of Queensland, Erko introduced me to the notion that we’ve hardly scratched the surface of what’s out there in the microbial world. It fired up my imagination that we have this huge, vast evolutionary tract that we didn’t know anything about.”

In his graduate work, Phil started out working on “bugs” (microbes) in air



While conducting molecular surveys in Yellowstone, they found 12 new divisions of life. “We took out the 16S small sub-unit ribosomal RNA (16S) marker gene, which codes for functional RNA, or the production of protein. It’s a bit like the early explorers discovering a whole new continent. The first step in ecology is to find out who’s living there, then find out what they’re doing. You can then compare those sequences to each other, make a phylogenetic tree, and see how the sequence

conditioning systems. Efforts to culture them on plates were slow so he later helped develop culture-independent methods based on 16S ribosomal RNA to characterize them.

Phil isolated Methylobacteria, which utilize methane derivatives as carbon sources.

From there, Phil did a short post-doc in Australia, working on bacterial methods for removing phosphorous from sludge in the wastewater treatment process. “If you want study microorganisms, it’s a great system,” says Phil. “They’re happy bugs.”

Phil later joined Norm Pace’s lab as a post-doc at the University of Indiana in Bloomington from 1995 to the summer of 1997 and then followed Pace when he relocated out to U.C. Berkeley.

“I worked on hot springs, which compared to air conditioning systems or sewage, was much more sexy stuff. We got to go off-road, sampling in the back-country of Yellowstone at the Obsidian Pool. The park rangers warned us that if buffalo charged us, not to try out run them. They would win. The trick was to get under a tree, where you were less likely to get killed.”

that you have isolated is close to a bug that has been described before—or not—and from that you can make inferences about what they might be doing. For instance, sulfate reduction is common in the delta proteobacteria, so if the sequence in question is similar, then you can infer which players are involved in sulfate reduction.

“The main thing that came out of that study is that there are all these completely novel lineages in life that have been missed. So what’s the point? You’re stamp collecting, but who cares? Imagine that for whatever reason, you couldn’t see all vertebrate life forms. Then somebody found a method to visualize them using a probe and stuck it under a microscope. To give you some context, on a scale of evolution, all the vertebrates together represent less diversity than one of the lineages that we characterized.”

In 1998, Phil got homesick and went back to Australia where he was offered a post-doc. Even though he just scraped by in mathematics in high school and had no math in college, Phil was hired as an **cont. on page 3**

JGI OPERATIONS UPDATE

BY HANK GLAUSER

The JGI has been looking for additional space for the last nine months. This space was to house the Informatics Department and associated functions. As you may have heard, negotiations for a new lease to occupy this space have fallen through. In brief, this occurred because the parties (DOE, UC, and the landlord) could not agree on terms limiting liability. It is unlikely in the current environment of review that LBNL, on behalf of the JGI, will be able to successfully negotiate a contract for additional space.

It is important to revisit the issues leading to the space strategy. In the

first quarter of last fiscal year we received a report that our computational infrastructure and staffing was not sufficient for our needs. We also received a report that our emergency power system was about to be over-tasked with the addition of potentially 20 more sequencers and more freezers. You may recall that at that time, sequencers were spread out around B400. We developed a plan to consolidate sequencers and address computational infrastructure needs while simultaneously reducing emergency power loads at B100 through the Data Center and Sequencer Bay Projects. Anticipating personnel growth we also started negotiations for additional office space.

At this point, the Room 122 Project has been completed, the Data Center Project is through design review and sub-contractor bidding with construction scheduled to start in January. The Sequencer Bay Project has completed preliminary scope with the design review scheduled for February. All sequencers will be consolidated into rooms 120 and 122 to enable Operations to relocate into 149 and 150. This will allow

the construction of JGI’s new Conference Center.

Operations is in the process of developing a plan to accommodate new hires while allowing for all projects to be completed. As a result, we are reconfiguring our existing space to accommodate growth and our evolving business needs.

Safety & Ergonomics News

Tried of mousing around? Studies have shown that a person who works at a computer for 8 hours could accumulate upwards of 6,000-8,000 mouse clicks with their index finger. This makes your index finger the most over used finger on your hand. You avoid excessive mousing by learning keyboard shortcuts. A handy list of keyboard shortcuts for PC and MAC can be found on the JGI internal site:

<http://www.jgi-psf.org/EHS/PCMacKeyboardShortcuts.pdf>

AWARDS AND ACCOLADES

JGI Finance & Materials Manager **Sandra McFarland** and Senior Resource Analyst **Jeannie Chan** were recognized in December for their efforts in researching and identifying billing discrepancies that resulted in vendor overcharges and ultimately led to JGI recovering \$225,000 from a supplier. In the process, they significantly improved the JGI’s blanket order process. For their respective contributions Sandra received an LBNL Outstanding Performance Award and Jeannie garnered the LLNL equivalent.

JGI Human Resources Manager **Dee Catino** received a SPOT award in recognition of her dedicated work and substantial contributions as part of the HR Center’s team efforts in support of the Berkeley Lab’s 2004 performance review and salary management process.

JGI TECHNOLOGY GARNERS AWARD

David Humphries, whose efforts in Berkeley Lab’s Engineering Division contribute to the advancement of the instrumentation systems at JGI PGF, was among the 2004 LBNL Award for Excellence in Technology Transfer recipients. David, on the right, shown here with Berkeley Lab Deputy Director Pier Oddone, was recognized for his work on the High Performance Hybrid Magnet for DNA Separation at a ceremony on November 17. The technology has been successfully implemented in the post

sequencing reaction clean-up step at the PGF.



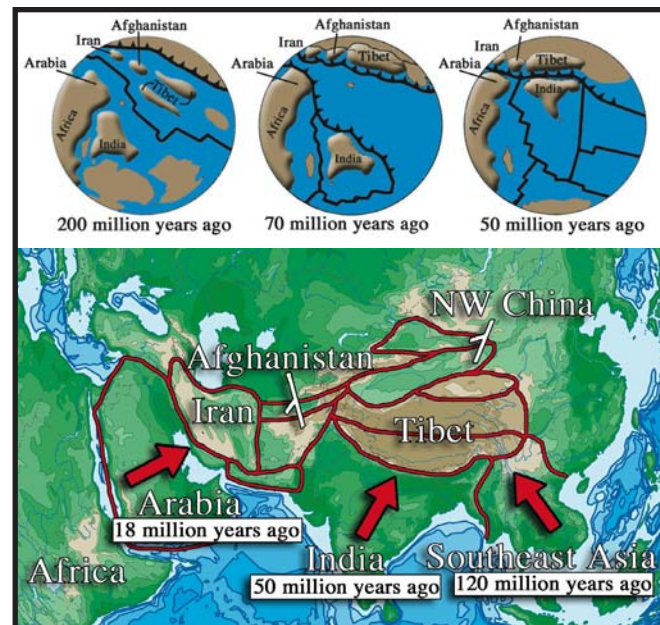
THE GENETICS BEHIND PLATE TECTONICS IN THE MIDDLE EAST AND CENTRAL ASIA:

A Lizard's Perspective

BY ROBERT MACEY

Although the vast desert landscape of the Old World is famous for places like the Sahara, it actually stretches from the Atlantic Ocean in Morocco to the Pacific Ocean in China, creating a region of dynamic ancient history.

Some hundreds of millions of years ago a super-continent called Gondwanaland broke apart and pieces started migrating north as small tectonic plates. These plates started to smash into Europe and Asia. In fact, Turkey, Iran, Afghanistan, western China and Tibet are composed of small plates that came from the south. Then 50 million years ago India smashed into Afghanistan and Tibet, and 18 million years ago Arabia smashed into Turkey and Iran. What followed is like bumper cars at an amusement park, with micro-plates being shuffled around within



A simplified tectonic map of Asia's associated tectonic plates.

Asia. Plate boundaries became mountain ranges such as the mighty Himalaya and internal regions of plates became basins such as the Iranian Plateau.

Some of the lizard species studied:
Toed-Headed Agama (*Phrynocephalus mystaceus*) from Turkmenistan (left).

Small-Scaled Frog-Eyed Gecko (*Teratoscincus microlepis*) from the Iran-Afghan border (below).



The JGI Evolutionary Genomics Department is examining the mitochondrial genomes of lizards from 25 countries that were there to witness it all. Mitochondrial DNA is maternally inherited from mother to daughter and therefore not subjected to recombination like the nuclear genome. This molecule is compact with almost entirely coding sequence for 37 genes making it the ideal marker to reconstruct historical relationships among animals. The molecule also evolves in a clock-like manner. Well-dated geological events such as the rise of a mountain range can be used to calibrate the "molecular clock", as it is called, for use

across genetic divergences of lizards occurring in regions where the geology is less understood.

The research team includes JGI's Robert Macey, Theodore Papenfuss of the Museum of Vertebrate Zoology at U.C. Berkeley and Alan Leviton of the California Academy of Sciences, and Natalia Ananjeva, the first woman zoologist to be admitted to the Russian Academy of Sciences.

A primary focus of this research is the identification of "biodiversity hot spots," regions of the highest amount of genetic diversity for conservation priority. As new governments form in the Middle East and Central Asia, choices will need to be made as to which regions to focus conservation efforts.

BIRDS OF JGI

BY WENDY SCHACKWITZ



BLACK PHOEBE

To kick off this feature I have selected one of my favorite birds, the Black Phoebe. It is one of the most common birds seen on the campus of JGI Walnut Creek, often perching on the lamp posts out on the lawn between buildings 100 and 400. This little black flycatcher, that looks like it has dipped its belly in cream, can be seen making incredibly acrobatic flights as it chases down its insect prey. They are particularly fond of habitat that is near water, and are often seen near stream sides and ponds. To find out more about the Black Phoebe, visit the Cornell

Lab of Ornithology link listed below.

http://www.birds.cornell.edu/programs/AllAboutBirds/BirdGuide/Black_Phoebe.html

To go and see the Black Phoebe and other common birds of our area, pack up your binoculars and a field guide and head to Don Edward's National Wildlife Refuge. My favorite field guides include Ken Kauffmann's *Birds of North America*, David Sibley's *The Sibley Guide to Birds*, and Roger Tory Peterson's *Western Birds*. Directions to Don Edwards NWR can be found at: <http://desfbay.fws.gov/directions.htm>. Or attend the flyway festival on Mare Island Jan 21-23: <http://desfbay.fws.gov/Tideline/Flyway.htm>.

Recently, a great milestone was achieved which will assist avian researchers studying topics such as phylogenetics, population structure, and conservation genetics; the chicken genome was finished. There are many gaps in our knowledge of our feathered friends, and having the complete sequence for the chicken greatly increases the number of tools that will be available for avian researchers.

Good Birding!

JGI MYSTERY PIC



What and where is this? Send your answer to degilbert@lbl.gov

Phil Hugenoltz
Susses Sludge

cont. from page 2

assistant professor in the mathematics department at the University of Queensland doing bioinformatics. "Up until that point in my career, I had mainly been talking with biologists, then I was stuck in a situation when I interacted only with mathematicians and programmers. I loved it. It was a very productive year with lots of papers. Then I had to leave because I would have had to teach math students. I would have been revealed," says Phil.

"So I had a very cunning exit strategy. I bumped into Jill Banfield who was doing a sabbatical on campus from Berkeley. She was telling me about this great system of microbial study, acid mine drainage. I was just learning about metagenomics, sequencing communities directly and I thought that would work on the acid mine drainage system. So she invited me to write a DOE/NSF grant with her and if we got it, she offered me a staff scientist position in her lab and boom, it got funded for a good chunk of change."

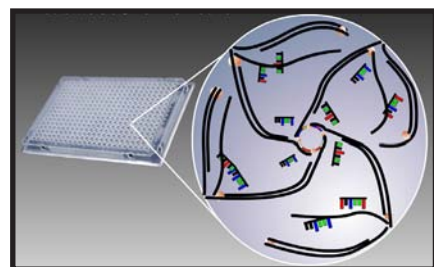
After some major soul-searching—and having to separate his kids from the grandparents—Phil relocated to Berkeley, where he still lives on the edge of the Gourmet Ghetto, with wife Penny Boys, an Irish folk music singer from Australia, and their two kids, Sam (four years old) and Lucy (one year old)—their token American citizen.

At JGI, Phil is working on sludge again, back collaborating with Linda Blackall (with whom he did his first post-doc in Australia) and Trina McMahon at Wisconsin. Phil's proud of the fact that he has the smallest, but rapidly-growing, program at JGI with three post-docs: Victor Kunin, Hector Garcia-Martin, Falk Warnecke, representing (respectively) Israel, Spain, Germany.

RCA: Rolling into a Genomics Lab Near You

BY DAMON TIGHE AND JEFF BOORE

Rolling Circle Amplification (RCA) provides the basis for one of the hottest and most costly pieces of the sequencing process. The technique amplifies minute amounts of circular DNA 10,000 fold which cuts out the normally time



intensive process of growing, isolating and concentrating DNA into yields high enough to work with for sequencing.

Templphi is similar to polymerase chain reactions (PCR) in that they both

utilize primers, dNTPs and a polymerase to carry out amplification of DNA. Templphi utilizes the phi-29 polymerase, which was isolated from a phage that infects *B. subtilis*. A set of random exonuclease-resistant hexamers are used in place of normal sequence specific primers to increase the kinetics of the reaction.

In the production genomics line, 384 well plates of *E. coli* containing circular vectors carrying DNA fragments of interest from other organisms are heated up to pop the *E. coli* and release their vectors. The heat lysis of the of the *E. coli* leaves the *E. coli*'s circular genome attached to the cell wall where it can not be acted on upon the phi-29 polymerase. RCA mix is then added to the 384 well plates after they have cooled and the plates are incubated at 300C for 18 hours. In this time the random hexamers attach all over the circular vector

and act as priming sites for the phi-29 polymerase to start its replication of the DNA. The phi-29 polymerase is able to displace double stranded DNA ahead of it which allows a single polymerase molecule to make its way around the circular vector many times and thus make many copies of the vector. The displaced DNA strands are able to have hexamers bind to them and act as sites for DNA replication. This system of displacement and replication allows the amplification of the DNA to be geometric. By the end of the 18 hours there are so many copies of the original vector that the reaction is sticky with DNA.

RCA is not only a fantastic boost to Production Genomics, but also to the Evolutionary Genomics Department for sequencing mitochondrial and chloroplast genomes, where often the limiting factor is getting enough of a suitable template for library creation.

MegaBACE 4500s Now On-line cont. from page 1

principle sequencing validation tests. By mid-October the decision was made to upgrade our current MB4000s to the new MB4500 platform. Currently, 18 instruments have been upgraded and are running full time in production (see their stats on our external page <http://www.jgi.doe.gov/sequencing/statistics.html>). The remaining 18 MB4000s will be upgraded in early 2005.

While the outside looks strikingly similar to the previous versions of the platform, the internal components of the MegaBACE4500 present an assortment of new technologies. From the more reliable and powerful solid state laser offering longer lifetimes and improved detection sensitivity when

mated to an enhanced optical system, to the reformulated V2E separation matrix giving better sequence resolution and containing a copolymer additive



Don Miller (left) and author Chris Daum, integral components of the 4500 conversion team.

that dynamically coats the capillary walls thereby extending array lifetimes, to the advanced internal environment allowing for consistent airflow and uniform cooling, and to the overall instrument efficiency that requires less electrical power and environmental cooling/air-handling systems to keep running. Taken as a whole, these developments allow for a more efficient system that when running the same sample prep as used on the MB4000 platform provides better pass rates (4-6% higher than MB4000), significantly higher Q20 base collection (150-200bp higher Q20>50bp average read-length than MB4000), and superior sequence quality all achieved with a considerably reduced run time allowing for six sample runs per day.

Critters in the Queue

Tanja & the Gutless Worm—JGI Staff Scours the Mediterranean

BY TANJA WOYKE

The Tuscan island Isola d'Elba is one of the very few places the gutless worm *Olavius algarvensis* has been found to date. These small worms are truly gutless, as they have completely reduced their digestive, as well as their excretory systems. Under their skin, they harbor a microbial community of five bacterial endosymbionts in an obligate manner and with quite different metabolic pathways and evolutionary origins, which makes them an attractive system to study.

Sequencing of the gutless worm symbionts is one the projects in this year's Community Sequencing Program (CSP). Although heavily inhabited by the bacterial symbionts, the worms are only 0.1 mm in diameter and 10-20 mm long and therefore large amounts of samples are needed. Tanja Woyke, JGI postdoctoral fellow, was sent to the island to collect and process the sample worms.

The worms are found at the northwest shore of the island in a semi-exposed bay close to Capo di Sant' Andrea. They live in the shallow sediment at a water depth of approximately 6 meters, and 15 centimeters below the sediment surface. In the mornings, Tanja and collaborators from the Elba field Station Centro Marino Elba would



Tanja on the hunt.

go out to the Sant' Andrea Bay and collect containers full of sediment. The highest abundance of worms were generally found 1 meter from the sea grass meadows. All afternoon was then spent screening through the sediments looking for *Olavius*. The accompanying fauna consisted mostly of nematode worms such as thiotrophic Stilbonematinae and Linhomoeidae. Once a small dish full of worms was collected, they were cleaned under the dissection scope to remove plant and other material. Because of *Olavius*' small size, and its rather low population density, the process was very tedious.



The field Station Centro Marino Elba is located in the small village of Fetovaia in the Southwest of the island. On a clear day, the surrounding islands of Montecristo, Corsica and Pianosa were visible. Elba, historically known as the place that saw Napoleon Bonaparte's first exile, is only 86 square miles (223 square kilometers) in area and about 6 miles (9.7 km) away from the Italian mainland. Since Etruscan and Roman times, iron ore has been mined on the island and there are still ironworks at the island's main town, Portoferraio. Wine, olive oil, as well as fruit are produced on Elba, and there is a large tourist industry.