JGI
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# Is "Good Enough" Good Enough?

MASSIE SANTOS BALLON

The Department of Energy focuses on a variety of alternative energy sources rather than putting all its eggs in one basket. At the recently concluded 7th annual Sequencing, Finishing, Analysis in the Future (SFAF) Meeting held June 5-7, 2012, a similar message came through as speakers discussed combining sequencing platforms and conducting hybrid assemblies to produce more useful data in the form of finished genome sequences.

Since the first Sequencing in the Future Meeting held in Santa Fe, New Mexico seven years ago, the number of attendees has increased each year from a starting group of 70, reaching a record 310 at this year's meeting. SFAF organizer Chris Detter, head of the Genome Science Group at Los Alamos National Laboratory, noted that the core mission of the meeting has remained the same however. "The focus has been to empower folks to use next-generation sequencing (NGS) and more importantly, to use the data," he said. "It's clear from this meeting and form other related meetings that robust genome analysis is crucial to moving the field forward and that involves figuring out how to leverage the community's capabilities to do the challenging bioinformatics."

In the Meeting's opening keynote, George Weinstock, associate director of The Genome Institute at Washington University, discussed the ongoing processing of finishing a bacterial genome that he and his colleagues considered "finished" back in 1998. He was the first of many to talk about using at least two sequencing platforms and doing hybrid assemblies to finish a genome (see "Aiming for perfection" on page 5 for more details). During the Tech Panel discussion later that same day, representatives from Illumina, Ion Torrent, Pacific Biosciences and 454 answered whether or not there were plans for the companies to collaborate and simplify the genome assembly process.

The responses were unanimous. While the sequencing manufacturers can make it possible for the data to be read on a variety of software assembly platforms, there are no plans to make a cross-platform sequencer. Jim Knight from Roche pointed out that while many of the Meeting's attendees strive for a perfectly finished genome, sequencer manufacturers have far more customers "for whom good enough will in fact be good enough to use a single standardized protocol and standardized analysis." *(continued on page 5)*

**SFAF keynote speakers (left to right) Paul Keim, Rita Colwell and George Weinstock (Rebecca McDonald, LANL)**

## also in this issue



## Omics Response to Deepwater Spill

In the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico two years ago, microbes played a role in dispersing the 4.9 million barrels of light crude oil. To better understand these mysterious organisms, researchers led by Berkeley Lab and DOE JGI senior scientist Janet Jansson collaborated with the DOE JGI and the Advanced Light Source in a demonstration of Ernest Lawrence's pioneering vision of team science.

As described in an article published online June 21, 2012 in *The ISME Journal,* the team used a combination of genomics techniques to study the way the microbes responded to the influx of oil. Jansson said that combining the metagenomic, metatranscriptomic and single-cell sequencing techniques accessed through the DOE JGI provided a uniquely comprehensive perspective.

Researchers used deep sequencing approaches (where nucleotides are read seven times or more) on the DNA and RNA *(continued on page 3)*

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# Clues for Assembling Switchgrass Genome

Assembling the genome of the prospective biofuel feedstock switchgrass poses more challenges than usual because it has multiple copies of its chromosomes. To assist in this effort, the DOE JGI has sequenced genomes of related plants, the candidate bioenergy crop sorghum and the model grass *Brachypodium*, for reference. However, the last common ancestor for sorghum and switchgrass lived more than 20 million years ago, while the last common ancestor for *Brachypodium* and switchgrass lived more than 50 million years ago.

The genome of a much closer switchgrass relative — foxtail millet (*Setaria italica*) with a common ancestor that lived 13.1 million years ago — was described in the May 13, 2012 edition of *Nature Biotechnology*. The DOE JGI sequenced all three genomes, and made them publicly accessible on www.phytozome.net.

**Green foxtail (Daniel Waxler, Donald Danforth Plant Science Center)**

As a model, foxtail millet has several advantages, said Jeremy Schmutz, head of the DOE JGI Plant Program at the Hudson-Alpha Institute of Biotechnology. Its compact genome is roughly half a billion bases in size, and large quantities of the grass can be grown in small spaces in just a few months. Schmutz said that roughly 80 percent of the foxtail millet genome has been assembled using the tried-and-true Sanger sequencing platform, along with more than 95 percent of the gene space — the functional regions of the genome. "The *Setaria* genome is a high quality reference genome," he said. "If you want to conduct functional studies that require knowing all the genes and how they are localized relative to one another, then use this genome."

*Setaria* is a good model for learning how grasses can adapt under various environmental conditions. Additionally, it appears to have independently evolved a pathway for photosynthesis that is separate from that used by maize and sorghum. "With the sequencing of the *Setaria* genome," the team noted in their paper, "evolutionary geneticists now have an annual, temperate, C4, drought- and cold-tolerant grass that they can comprehensively compare to other plants that have or have not yet evolved these adaptions." C4 plants are distinguished by their ability to conduct photosynthesis faster than C3 plants under high light intensity and high temperatures.

Schmutz also noted that the *Setaria* genome is a good experimental model in the lab for studying switchgrass traits such as cell wall formation. As a member of BESC, study first author and DOE JGI collaborator Jeff Bennetzen from the University of Georgia explained how using the Setaria genome could aid one of his team's bioenergy projects: "The biggest cost and bottleneck in biofuel production is converting lignocellulose to simple sugars. We're looking at traits associated with reduced recalcitrance (i.e., easier access to sugars) and looking for variations that alter cell wall composition. If it works in *Setaria* then the same approach will work in switchgrass."

For Tom Brutnell, a co-author on the study and director of the Enterprise Institute for Renewable Fuels at the Donald Danforth Plant Center, the *Setaria* genome is the starting point for his own research interests. "Now that we have the genome sequence, we can kickstart the development of genetic tools for *Setaria*." His proposal under the DOE JGI's 2012 Community Sequencing Program builds off the availability of two *Setaria* genomes, that of foxtail millet and its wild ancestor green foxtail (*S. viridis*), which is also described in the paper.

"What we really want is an *Arabidopsis* for the Panicoid grasses," he said, referring to the ubiquitous plant model used by many researchers.

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# Learning from Picky Fungal Eaters

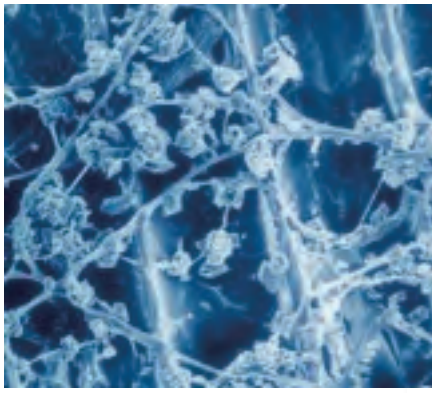
Fungi and microbes break down dead trees and leaf litter in nature; without them, the forest floor might look like a scene from TV's "Hoarders." Those same organisms interest bioenergy researchers because their enzymes that break down plant biomass could be useful for accelerating biofuels production.

In a study published online the week of March 19, 2012 in the *Proceedings of the National Academy of Sciences,* scientists compared genomes of two widespread white rot fungi that the DOE JGI generated and annotated under the Community Sequencing Program. The study revealed substantial differences in the genes in the fungus *Phanaerochaete chrysosporium* and its close relative *Ceriporiopsis subvermispora* involved in lignocellulose degradation, providing further insight into how white rots do their dirty work.

"Very few fungi have the capability to degrade lignin," said study senior author and DOE JGI collaborator Dan Cullen of the U.S. Department of Agriculture Forest Service, Forest Products Laboratory (FPL). "Even fewer fungi have the ability to selectively remove lignin at such an efficient rate. *C. subvermispora* is one exception in its ability to do just that."

Lignin, the second most abundant biopolymer on Earth, represents a significant challenge for the pulp and paper industry. Detailed biochemical analyses conducted by study co-author Angel Martinez's team at the Spanish National Research Council (CSIC) in Madrid, Spain, found that the *C. subvermispora* genome had more manganese peroxidases and laccase — enzymes that may speed the degradation of lignin — than the *P. chrysosporium* genome. Additionally his group found lignin-degrading enzymes that had not previously been found in *C. subvermispora* cultures.

Analyzing *C. subvermispora* and *P. chrysosporium* as part of the diversity of wood-decaying fungi and cataloging



**SEM of *Ceriporiopsis subvermispora* mycelium on wood. (R. Blanchette, University of Minnesota)**

enzymes involved in lignocellulose degradation is one of the goals of the DOE JGI Fungal Genomics Program led by Igor Grigoriev. One of the ongoing projects compares more than 20 fungi genomes sequenced or being sequenced at the DOE JGI to create a better understanding of lignocellulose degradation, its influence on the carbon cycle, and improvements to biopulping.

## Omics and Oil *(continued from page 1)*

samples extracted from deep water microbes from the plume, generating billions of bases of data per sample. Those revealed an abundance of genes involved in the degradation of alkanes and genes involved in degradation of aromatic compounds.

Jansson said that the results suggest that waves of microbes acted on the oil spill. "We probably have a bloom of alkane degraders that were present when we sampled early in the spill history. In later expeditions, they found methane degraders or propane degraders, suggesting that there was a succession in the community and their properties," she said.

A separate group under Microbial Program head Tanja Woyke worked to isolate and sequence three cells of *Oceanospirillales* bacteria, which they co-assembled into a draft genome, the first, deep-sea oil-eating bacterial genome assembled from single-cell sequencing. Analyses later identified *Oceanospirillales* as the predominant bacteria that responded to the plume.

A simulation Jansson and her colleagues reported in a separate study published May 23, 2012 in *Environmental Microbiology* added credence to their hypothesis of a shifting microbial community. They recreated the results found in nature by combining samples of ocean water, oil, dispersants, and deep-water bacteria. Again, the DOE JGI and Berkley Lab's Advanced Light Source helped determine the composition and distribution of the microbes, respectively.

**Study first author Olivia Mason using the CTD Rosette to collect samples from the deep-sea plume (Eric Dubinsky, LBNL).**

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# Single-Cell Genomics

STEPHEN TUNG

For each genome sequenced in the DOE JGI's Single-Cell Genomics lab, researchers accomplish herculean feats: first in identifying and separating a microscopic organism out of myriads of thousands; second, cracking open these tiny organisms to extract their DNA; third, amplifying a single strand of DNA; fourth, identifying what the organism is; and fifth, sequencing its genome.

Those are the challenges that Tanja Woyke's group has been overcoming in the last three years, with requests ballooning from 14 in 2009 to 320 in 2011. The emerging field offers the unprecedented ability to get a partial to full genome from a single cell. And Woyke thinks there's room for growth.

"Right now we're in the hundreds [of genomes per year]. I would like to transition into the thousands," Woyke says. That would help the Genomic Encyclopedia of Bacteria and Archaea (GEBA) project that needs the genomes of 9,000 organisms to cover half of the currently known phylogenetic diversity, according to rough estimates based on the 16S ribosomal RNA tree, she says.

Single-cell genomics is particularly useful for microbes that can't be cultured. "There's a large gap in filling in branches of the phylogenetic tree with sequenced representatives, because for these branches, we don't even have cultured isolates, so we don't have anything to populate these trees," she says. With single-cell genomics, they do.

Furthermore, unculturable cells typically live in microbial communities with thousands of other organisms. The usual tool for the last dozen years has been analyzing the overall DNA of the community.

"Metagenomics provided the first glance into the coding potential of this uncultured majority," Woyke says. "But now, single-cell genomics is an additional technology that I view as completely complementary to metagenomics."

"What you can do with single-cell genomics is link phylogeny with function — in other words say who does what," she says.

Single-cell genomics would benefit "any researcher that works with metagenomic samples," she adds.

Currently, single-cell isolation and sequencing at the DOE JGI requires an annual Community Sequencing Program (CSP) application, available at http://1.usa.gov/CSP_UG. If researchers already have isolated the cells and amplified the DNA, the more streamlined single-cell sequencing can be submitted for quarterly CSPs.

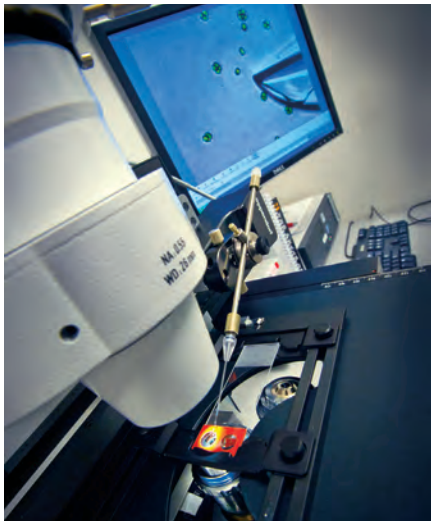
Initially, the sample is stained so that its DNA fluoresces. The cell sorter then physically isolates single cells, based on intensity (a proxy for the DNA amount), and the size of the cell, at a rate of hundreds per second.

But the diversity of samples makes sorting the microbes difficult. A water sample may be rather straightforward, while a soil sample can have too much background noise.

Moreover, to open up the cell to extract its DNA may require more work. "We use alkaline lysis to lyse these cells, right?" says Woyke. "We get samples from highly alkaline environments. Well, how are we going to lyse these cells? They are resistant to it. They live in it, they love it. They're not going to lyse."

"So we have to try the opposite, or try to heat it," she says. A team led by Rex Malmstrom, head of the microscale applications group, actively develops new techniques to extract DNA from tens of thousands of these tricky cells.

Once the DNA is obtained, it's amplified. Researchers look at the 16S rRNA gene sequences to identify an organism and determine whether it should be entirely sequenced. "For example, researchers might say, 'We're really interested in this particular *Chloroflexus* from permafrost,'" says Woyke, "There's no culture representative that's closely related. We need this as a reference genome."



**Micromanipulator (Roy Kaltschmidt, LBNL)**

The process reads anywhere from a few to 100 percent of the genome, if researchers are lucky. Malmstrom's team is looking for better ways to isolate and identify organisms, and amplify its DNA.

In the future, Woyke thinks single-cell genomics and the DOE JGI's substantial resources would be able to put a dent in the GEBA project. "We have so many interesting, phylogenetically diverse samples that come through JGI," she says. "We're in a good position to select some of these samples and say, 'Hey let's go back. Let's get some more material to capture some of these genomes using the single-cell approach.'"

"We can populate the tree of life with these references, and that will vastly improve our metagenome data analysis," Woyke says.

And it won't stop there. Woyke believes that the DOE JGI should be looking to expand beyond bacteria and archaea: "Algae, fungi, plants, you name it," she says.

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## "Good Enough" *(continued from page 1)*

Aside from the tech panel discussion and short technology talks, this year's SFAF Meeting also included breakout sessions focused on the core areas of microbial communities, genome assembly and improvement and human sequencing. Attendees had productive dialogues in these smaller groups in areas ranging from sample prep, standards and databases to clinical and diagnostic genomics.

Another theme of the Meeting was expanding the use of genomics in other areas of study. In his speech, Paul Keim of Northern Arizona University and the nonprofit Translational Genomics Research Institute talked about the application of genomics to track a pathogen, in this case plague, across space and time around the world and over the course of nearly 1,600 years. The closing keynote delivered by Rita Colwell, the noted cholera researcher from the University of Maryland, focused on her group's efforts to learn more about the pangenome of *Vibrio cholerae,* and her desire to conduct further studies using single-cell genomics.

**SFAF meeting organizers (left to right) Donna Muzny, Baylor; Mike FitzGerald, Broad; Johar Ali, OICR; Patrick Chain, LANL; Chris Detter, LANL; Alla Lapidus, Fox Chase Cancer Center; Bob Fulton, Washington University; David Bruce, LANL**

Colwell had spoken at the 2010 DOE JGI Annual Meeting on lessons learned from combining environmental and genomic studies of *V. cholerae*, the bacteria that cause global outbreaks of the disease. The Meeting wrapped with an extra half-day session as attendees from various governmental agencies and NGS leaders discussed the growing need and available applications of NGS for the field of forensics.

Videos from the SFAF Meeting, and from previous Meetings, are on the DOE JGI's SciVee channel at http://www.scivee.tv/user/7476.

## Aiming for perfection

REBECCA MCDONALD

In his Keynote address on the first day of the 2012 SFAF Meeting, George Weinstock raised the question of achieving a "perfect genome." With all the advances in speed and throughput over the last decade, he wondered if researchers have been too accepting of draft genomes, and suggested that by using multiple platforms and cheaper sequencing the community could seek a higher standard of perfection.

Although the availability of next generation sequencing has grown, Weinstock explained that these new platforms have different "idiosyncrasies," making it difficult to pick one approach over others. He listed the main challenges as "closure, assembly accuracy, base accuracy, and how repeated sequences are dealt with" and suggested that a hybrid approach could be the solution to his quest for the "perfect genome." Weinstock explained that, "in the end, one may have different tiers of quality." This topic was the product of a previous SFAF Meeting, and a team led by LANL's Patrick Chain later published the work in an October 2009 issue of *Science*.

Weinstock's message was echoed in the presentations that followed, as many researchers laid out descriptions of their recipes for sequencing. For instance, Sergey Koren from the Department of Homeland Security showed comparisons of various combinations of 454, Illumina and PacBio RS data; and Dan Ader from Monsanto described a "platform bake-off" including combinations of 454, Illumina, Ion Torrent, SOLiD and PacBio RS.

Many participants took advantage of breaks and the poster session to discuss their lab's strategies and compare notes on the best approaches. Keynote speaker Rita Colwell noted that "most pleasing was the very large attendance of young scientists, an indication that the field of genomics and next generation sequencing has genuinely taken off."

Multiple talks discussed the valuable real-time genomic analysis of the *E. coli* outbreak in Germany last year, and there were even talks about diagnostic sequencing and single-cell sequencing to help develop cellular targeted therapies for cancer patients.

The SFAF Meeting *(continued on page 7)*

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# JGI Highlights

## GLBRC's first hot patent

STEPHEN TUNG

It sounds like a dream vacation: hang out in hot springs all day, converting sugar to alcohol. That's precisely what researchers are looking for in microbes to break down plant matter more efficiently into fermentable sugars for biofuel. They found one such microbe, *Dictyoglomus turgidum*, in samples collected from Obsidian Hot Spring in Yellowstone National Park and from remote hot springs in the Russian Kamchatka Peninsula. The DOE JGI sequenced the bacterium's genome in a project started in 2005.

Owing to the DOE JGI's and Great Lakes Bioenergy Research Center's contributions, a patent has been issued to biotech company C5•6 Technologies for the heat-tolerant enzyme that powers the deconstructive capability of *D. turgidum*. C5•6 plans to deploy the novel enzyme in biomass processing for biofuel production where leftover plant material is heated up to high temperatures to weaken the polymers that hold the cell walls together. Most enzymes that break down polysaccharides are inactivated at temperatures over 130 degrees Fahrenheit (54 degrees Celsius), but *D. turgidum*'s performs up to 200 degrees Fahrenheit. This enzyme also deconstructs a variety of sugar-rich plant materials, possibly simplifying the cocktail of enzymes needed to break down different polymers.

**Obsidian Hot Spring in Yellowstone National Park (mfwhite2 via Flickr/CC 2.0 License)**

## CLIP-PE method for improving genome assembly

Assembling a genome is often compared to assembling a puzzle, and the increasing use of next-generation sequencing technologies has made the process more challenging as the pieces of DNA sequence generated are much smaller and far more numerous than those produced by the Sanger platform.

DOE JGI researchers led by Deputy Director of Genomic Technologies Len Pennacchio and Advanced Sequencing Group head Feng Chen have developed a way to more efficiently assemble the short DNA segments.

Their approach detailed in an article published January 9, 2012 in *PLoS ONE* involves the use of mate pairs, two short segments of DNA separated by an insert that can help align and orient the sequences across perceived gaps in a genome during the assembly process.

Chen and his colleagues developed a novel Cre-LoxP Inverse PCR Paired-End (CLIP-PE) methodology of quickly generating mate-pair libraries, which allow for a wide variety of possible combinations of the short sequences so that researchers can assemble a genome with fewer gaps, which in turn helps reduce the finishing costs. The team tested the method by generating Illumina mate pair libraries of 5 kb, 12 kb and 22 kb.

The CLIP-PE technique has been submitted to the Lawrence Berkeley National Laboratory Tech Transfer division and has a patent pending.

## Short-read assemblies from metagenomes

In an article published in the April 2012 issue of *The ISME Journal*, researchers from the Georgia Institute of Technology and the DOE JGI determined the impact of short reads from next-generation sequencing (NGS) platforms on assembling individual genomes from complex microbial communities. They used Illumina data consisting of 100-basepair paired-end sequences from soil and freshwater metagenomes, as well as simulated datasets for their studies. For example, they "spiked" a dataset from a freshwater planktonic community sampled from a lake in Atlanta, Georgia with a reference bacterial genome and then compared the results of deriving that reference genome against assembling it from the genome reads alone.

The genome assembly challenges posed by short sequence reads from sequencing platforms such as 454/Roche and Illumina are well-documented. The team reported that they were able to accurately assemble a single genome from the complex community when the NGS platform used had at least 20X coverage. At less coverage, they added, they found more errors and problems with individual genome assembly.

"The results presented here reveal the errors and limitations as well as the strengths of metagenomics for population analysis, and provided practical standards and guidelines for experimental design and

analysis," they concluded. "Some of our results should be independent of the NGS platform used and therefore broadly applicable to short-read sequencing."

## Characterizing a TCE-degrading metagenome

**ANAS (Telstar Logistics/Todd Lappin)**

Groundwater sites contaminated with compounds such as trichloroethene (TCE), a pervasive organic groundwater pollutant often used by industry as cleansers or degreasers. In an article published online March 1, 2012 in *The ISME Journal* a team of researchers including DOE JGI Metagenome Program Lead Susannah Tringe conducted a metagenomic analysis of a stable dechlorinating community derived from sediment collected at the Alameda Naval Air Station (ANAS) in California.

The analysis allowed the team to characterize the members of this microbial community, particularly those involved in dechlorination such as *Dehalococcoides* bacteria, often found in a microbial community at contaminated groundwater sites contaminated. The *Dehalococcoides* bacteria can break down TCE and convert it into ethene, a harmless chemical compound often used to help ripen fruits.

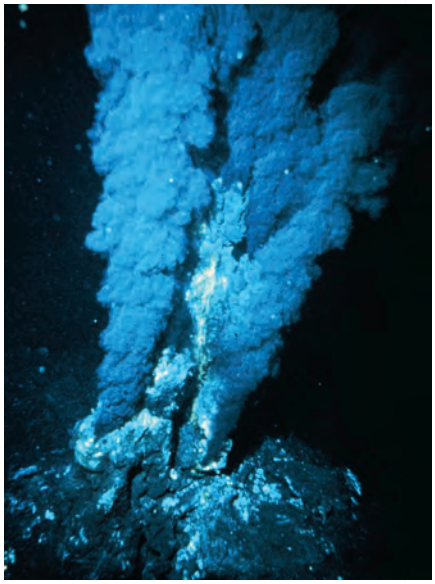
The researchers' data suggested that all of the genes that code for enzymes involved in dechlorination were associated with *Dehalococcoides*, suggesting its importance as the dominant dechlorinating microbe in the ANAS microbial community. Additionally, they found that though the various microbes in the community had widely varying roles as hydrogen producers and consumers, "in this community, they appear to have developed working syntrophic [mutually dependent] relationships, allowing stable long-term dechlorination activity."

## Protein studies on extremophile palm worms

Like geysers at a national park, except for the fact that they erupt deep underwater in the Pacific and Atlantic Oceans, hydrothermal vents behave just like geysers at a national park. Though the water that shoots out of these vents can reach temperatures as high as 300 degrees Celsius (572 degrees Fahrenheit), many animals and other organisms thrive in the surrounding area.

Two of these extremophiles are "palm worms," named for their resemblance to tiny palm trees. To learn more about these worms' tolerances for the high temperatures around the deep-sea vents, researchers led by DOE JGI collaborator Peter Girguis of Harvard conducted a series of experiments on two examples, *Paralvinella sulfincola* and *P. palmiformis*, collected from hydrothermal vents located 2.2 kilometers underwater and off the Pacific Northwest coast.

In the paper published online May 2, 2012 in the *Proceedings of the Royal Society B* journal, the researchers found that while the two worms had overlapping thermal tolerances, they employed different strategies to deal with high temperatures close to their tolerance limits. For example, *P. sulfincola*'s strategy involves adapting to varying oxygen concentrations in its environment so it can decrease its aerobic metabolism.



**Black smoker at a mid-ocean ridge hydrothermal vent (P. Rona/OAR/National Undersea Research Program (NURP); NOAA)**

Girguis is also leading a DOE JGI 2011 Community Sequencing Program project to develop a metagenomic database of the microbial communities that thrive around hydrothermal vents. The database is expected to provide an inventory of novel gene sequences that will aid research in renewable energy, carbon and heavy metal sequestration, and environmental remediation.

## Perfection *(continued from page 5)*

is a widely-attended international gathering of leaders in the genome sequencing arena and their industrial counterparts to converge and discuss new strategies for sequencing, finishing, assembly, annotation, and analysis, as well as applications for the use of high quality data. "Hands down one of the best conferences I've been to," stated Daniel Bozinov, CEO and Founder of Genimbi, Inc. He added that not only were most of the talks "highly interesting and moreover relevant" to his area of research but he felt the free registration made it "genuinely scientific and altruistic."

*Rebecca McDonald is the communications specialist for the Bioscience Division at Los Alamos National Laboratory.*

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## A Retiring Colleague

**Sam Pitluck, a fixture since the DOE JGI's doors opened in Walnut Creek in 1999, is retiring after nearly 35 years with Lawrence Berkeley National Laboratory. The Philadelphia native with an undergraduate degree from Temple University and a Ph.D. in physics from Brown University joined LBNL's radiotherapy project at the 184-inch cyclotron and at the Bevatron — targeting therapy tumors in patients — writing treatment planning software in the late 1970s. When the bioinformatics group moved out from Berkeley to the DOE JGI, he eventually took over the entire sequence archiving effort and became the point man for submission of all assembled data to GenBank, effectively the last person to touch the data before it went out to the world. Joining him in retirement is his stalwart, signature Atomic Energy Commission oak desk, which he officially acquired.**

# EIGHTH ANNUAL
## Genomics of Energy & Environment Meeting

MARCH 25-29, 2013 • WALNUT CREEK, CA

Presentations and poster sessions on DOE-relevant science including microbial ecology and bioprospecting; genomic analysis of biofuels crops; single-cell genomics; and synthetic biology. Also bioinformatics and new genome sequencing technology tutorials, tours and more.

Be there!

**http://1.usa.gov/JGI-Annual-Meeting**

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