

# ***Biological Systems Science Division (BSSD) Update***

Biological and Environmental Research Advisory  
Committee Meeting

February 16-17, 2012  
Washington DC

**Dr. Susan Gregurick**



U.S. DEPARTMENT OF  
**ENERGY**

Office  
of Science

Office of Biological  
and Environmental Research

# **Update Outline**

- Programmatic Activities
- Biosystems Design Initiative
- Response to Committee of Visitors
- Science Highlights
- Facility Highlights

# ***Programmatic Activities***

## **(Completed)**

### **Reviews:**

- Bioenergy Research Center Reviews (on site) (Fall, 2011)
- Joint Genome Institute Review (on site) (December 5-6, 2011)

### **Workshops:**

- Biosystems Design (July 17-20, 2011)  
Draft workshop report released January 9, 2012 and is accessible at <http://genomicscience.energy.gov/biosystemsdesign>

### **Reports:**

- Switchgrass Research Group: Progress Report  
<http://genomicscience.energy.gov/pubs/switchgrassreport.pdf>

### **Program Meetings:**

- USDA-DOE Plant Feedstocks Genomics for Bioenergy annual Principal Investigators Meeting (Jan.13, 2012)

# *Programmatic Activities*

## (On-Going)

### *Program Meetings*

- Genomic Science Principal Investigator's Meeting (Feb. 26-29, 2012) Bethesda North Marriott, MD

### *Solicitations*

- Joint DOE-USDA Plant Feedstock Genomics Notice (**DE-FOA -0000598**)
- Integrated Nuclear Medicine Research and Training Projects of Excellence (**DE-FOA-0000646**)
- Genomic Science: Biosystems Design to Enable Next-Generation Biofuels (**DE-FOA-0000640**)
  - Innovative genome-scale engineering techniques for development of modules and platform organisms for production of advanced biofuels by microbes
  - Large-scale genome engineering of biomass feedstock plants and identification of biosystem elements needed to re-design plants for sustainable bioenergy production

# Biosystems Design

- Research to **establish biological design rules** will enable the predictive design of innovative natural and hybrid systems for clean energy production.
- **Discovery and synthetic redesign of plant and microbial systems** pushes science frontiers and paves the way for sustainable production of advanced biofuels and bioproducts. Leverages strong U.S. resources and leadership in fundamental biological research and industrial biotechnology.

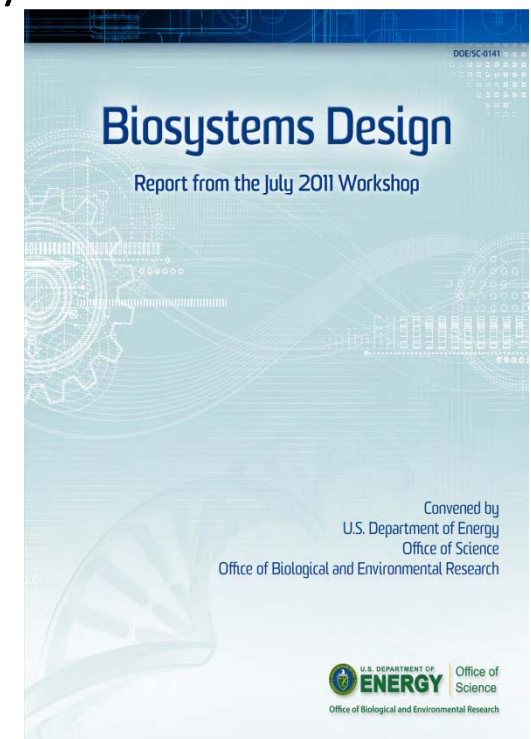
## Research Focus:

### Synthesis:

- New synthetic biology methods
- New genetic toolkits
- Predictive integration of components and processes

### Characterization & Testing:

- Verify & validate computer-aided design toolkits
- New testbeds to prototype performance and function



# Biosystems Design to Enable Next-Generation Biofuels (DE-FOA-0000640)

- **Microbial systems design for biofuels:** From computer modeling to experimental validation: To develop modeling algorithms and innovative biosystems design technologies to define, build, and test functional biological modules for the generation of novel biological systems that advance toward the production of biofuels
- **Plant systems design for bioenergy:** To develop novel technologies to re-design bioenergy crops that can grow in marginal environments while producing high yield of biomass that can be easily converted to biofuels.

Applications should also address potential societal implications of engineered organisms (bio-containment challenges, unexpected outcomes, etc); up to 5% of budget can be devoted to these activities.

- Up to \$20 million available in annual funding, award sizes expected to range from \$1-5 million
- Issued Jan. 13<sup>th</sup>, Required Preliminary Applications were due Feb. 13<sup>th</sup>, Encouraged Proposals are due April 2<sup>nd</sup>.
- [http://science.doe.gov/grants/pdf/SC\\_FOA\\_0000640.pdf](http://science.doe.gov/grants/pdf/SC_FOA_0000640.pdf)

**BER Point of Contact: Pablo Rabinowicz (Pablo.Rabinowicz@science.doe.gov)**

# ***Programmatic Activities***

## **(Upcoming)**

### **Reviews**

- Human Subjects Protection Program reviews at LBNL and Sandia
- Scientific Focus Areas Research, in FY12
- DOE-USDA Plant Feedstock Genomics Notice, in Spring FY12
- Integrated Nuclear Medicine Research and Training Projects of Excellence, in Spring FY12
- Biosystems Design to Enable Next-Generation Biofuels, in Spring FY12

### **Symposia/Conferences**

- PittCon 2012 *“Mass spectrometry advances conversion of biomass to biofuels”*  
(R. Hirsch and A. Katz organizers; Mar. 14, 2012)
- ASM 2012 *Microbial Powers to Tap Earth's Energy*  
(J. Graber, co-organizer; June 18, 2012)
- ACS 2012 : *“Glenn T. Seaborg Award for Nuclear Chemistry”*  
(P. Srivastava, Chair, March 27, 2012)

### **Workshops**

- JGI Strategic Planning for Genomic Sciences Invitational Workshop, May 30 and 31, 2012  
(D. Drell, organizer)

## BSSD Response to Committee of Visitors (June 2011)

COV Recommendation	Action Plan
<b><i>Transparency for preapplication processing</i></b>	Include standard language on preapplication.
<b><i>Recommendations for scientific advisory committees</i></b>	No new advisory committees will be organized by the Division
<b><i>Improved documentation for future COVs</i></b>	Standardization of documentation will be available for future COVs.
<b><i>Continue community outreach for Kbase</i></b>	Provide public updates on progress
<b><i>Improve public outreach of the BRCs</i></b>	BRCs shall improve their public websites.
<b><i>Increase transparency of BRC interactions with JGI</i></b>	Provide website access for BRC genomes sequenced.
<b><i>Conduct a community workshop including the BRCs and the broader research community</i></b>	Continue BRC involvement in Genomic Science principal investigators meeting
<b><i>Convene a workshop addressing future directions in the genomic sciences to facilitate strategic planning for the JGI</i></b>	BSSD program staff will conduct a workshop in FY2012

[http://science.energy.gov/~media/sc-2/pdf/cov-ber/BER\\_COV\\_2011\\_BSS\\_Response.pdf](http://science.energy.gov/~media/sc-2/pdf/cov-ber/BER_COV_2011_BSS_Response.pdf)



# Designing Low Lignin, High Biomass Yielding Plants

## Objective

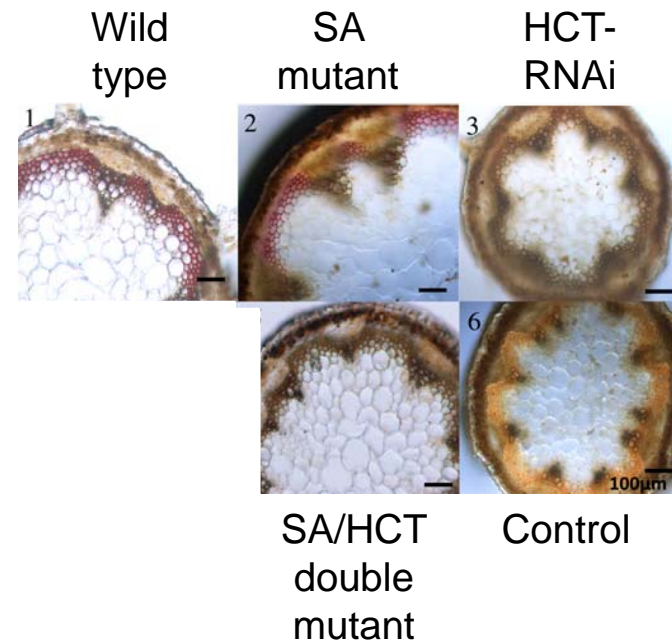
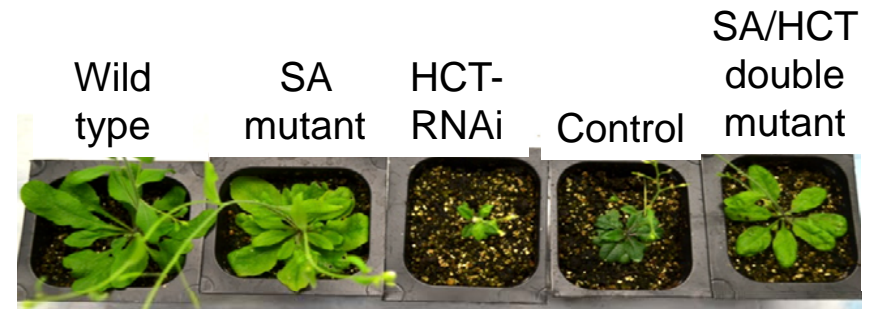
➤ Overcome recalcitrance barrier by producing low-lignin plants without negatively affecting growth.

## Approach

- Plants genetically modified to have less lignin usually exhibit stunted growth.
- The plant stress hormone salicylic acid (SA) influences plant growth and development; levels are inversely proportional to lignin levels.
- Genetically removing SA from Arabidopsis plants modified to produce low lignin restored normal growth without impacting lignin level.

## Results/Impacts

- Growth defects in these plants due to high SA rather than low lignin.
- Provides strong support for the possibility of engineering plants with low lignin but high biomass yields.



Gallego-Giraldo L et al. *Salicylic Acid Mediates the Reduced Growth of Lignin Down-Regulated Plants*. 2011, *Proc Natl Acad Sci*. 10.1073/pnas.1117873108

## Objective

Develop consolidated biomass processing capabilities in *Escherichia coli* for production of cellulose degrading enzymes and conversion to a range of biofuel compounds.

## Approach

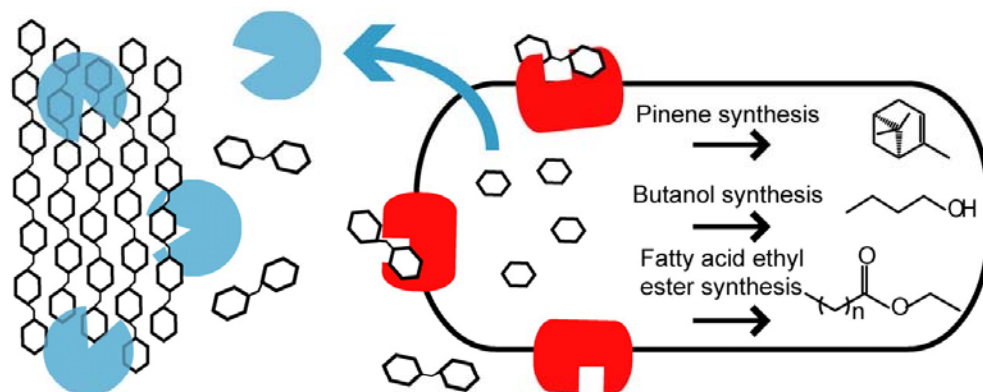
- Built two plasmids encoding cellulase and hemicellulase enzymes and associated secretion mechanisms
- Developed three additional plasmids encoding metabolic pathways for synthesis of fatty acid ethyl esters (i.e. biodiesel), butanol, or pinene (a monoterpene jet fuel precursor)
- Flexibly combined plasmid sets in *E. coli* to allow conversion of cellulose and hemicellulose released from ionic liquid pretreated switchgrass into the three biofuel compounds

## Results/Impacts

Demonstrates potential to develop consolidated biomass processing capabilities in non-biomass degrading microbes and develop tools for the flexible expression of biomass degradation machinery and biofuels synthesis pathways in *E. coli*.

Bokinsky et al. *Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using engineered Escherichia coli*, 2011. *Proc. Nat. Acad. Sci.* doi/[10.1073/pnas.1106958108](https://doi.org/10.1073/pnas.1106958108)

# Microbial Conversion of Switchgrass to Multiple Drop-In Biofuels



## Using –Omics to Understand Impacts of Soil Warming on Microbial Carbon & Nitrogen Cycling

### Objective

To understand the impacts of long term warming effects on microbial communities in a grassland setting.

### Approach

➤ Using a combination of metagenomics and functional gene arrays for HTP analysis of soil to understand microbial community composition and gene expression in warmed vs unwarmed plots

➤ Activities of key enzymes, substrate utilization, and microbial community biomass were assayed and correlated with functional data and observed shifts in biogeochemical processes.

### Results/Impacts

➤ Significant shifts observed in soil microbial community composition under warming, with correlated increases in expression of genes involved in degradation of labile (but not recalcitrant) carbon compounds and nitrogen fixation and cycling.

➤ Microbes thus appear to play substantial roles in increased CO<sub>2</sub> production and accelerated nutrient cycling tied to increased ecosystem productivity.

Zhou et al. *Microbial mediation of carbon-cycle feedbacks to climate warming*  
2011 **Nature Climate Change** DOI: 10.1038/nclimate1331



## Maize juvenility gene enhances biofuel production in bioenergy crops

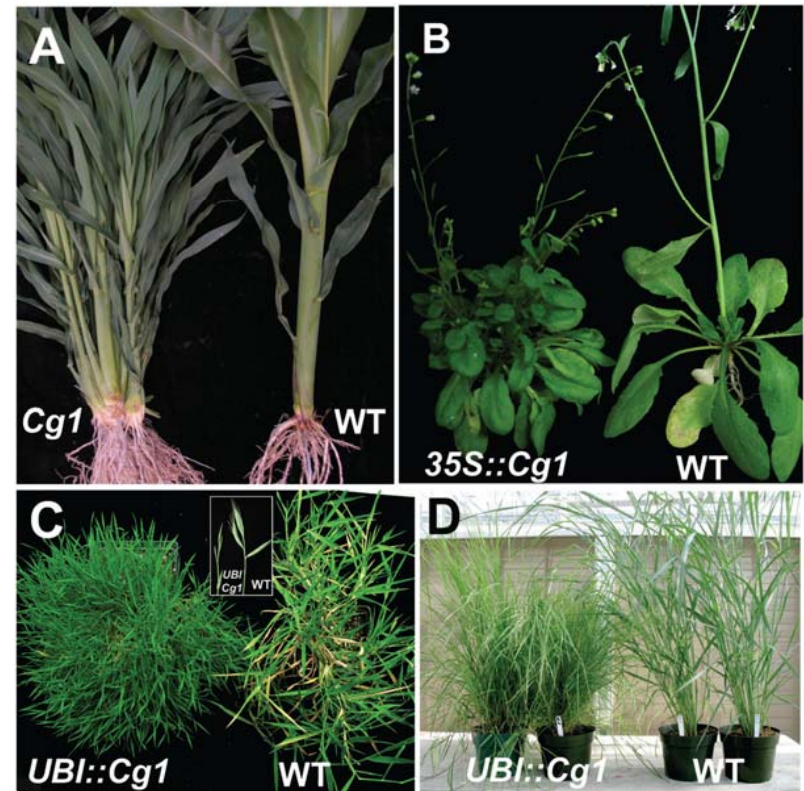
**Objective** Identify a gene, Corn Grass 1 (Cg1), in Maize that maintains the plant in a juvenile state, with less lignin and a higher starch content which is more easy to converted into biofuels.

### Approach

- Maize *Cg1* plants maintain juvenility, contain less lignin , more convertible starch.
- Transgenic switchgrass containing maize *Cg1* had significantly higher glucose release than wild type plants without expensive pretreatment.
- Complete inhibition of flowering in *Cg1* plants prohibits transgene escape.

### Result/Impacts

Transferring the *Cg1* gene to bioenergy crops could result in reduced recalcitrance and lowered conversion costs, offering a promising new approach for the improvement of dedicated bioenergy crops.



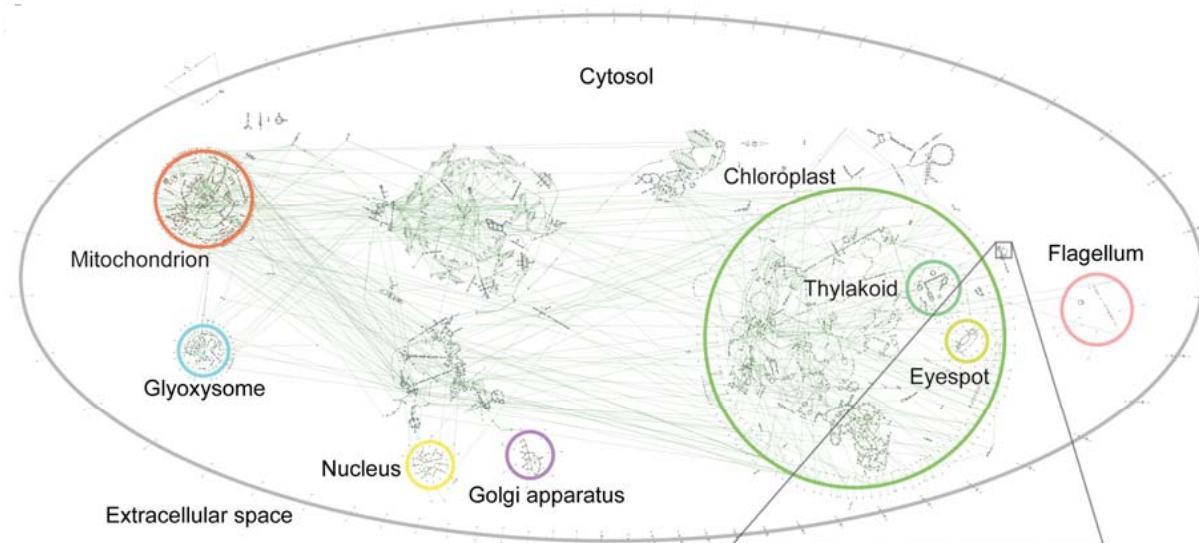
Chuck et. al. 2011. "Overexpression of the maize *Corngrass1* microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass" *Proc Nat Acad Sci* 10.1073/pnas.1113971108.



## Understanding Genome Scale Metabolic Networks of Algae

### Objective

Understanding integrated metabolic networks of algae is critical for predictive modeling and more effective engineering of strains for optimal biofuels production.



### Approach

Coupling gene expression analysis, validation of gene function, and computational modeling, an improved genome scale metabolic reconstruction has been produced for the alga *Chlamydomonas reinhardtii*.

### Results/Impacts

This reconstruction allows for a much clearer picture of the relationship between photosynthesis and production of lipids, precursors of biodiesel and other potential biofuel compounds.

R. L Chang et. al 2011 "Metabolic Network Reconstruction of *Chlamydomonas* offers insight into Light Driven algal Metabolism" **Molecular Systems Biology** 7:Article 518 (doi:10.1038/msb.2011.52)

# Identification of fluorescent compounds with low non-specific binding for live cell imaging

## Objectives

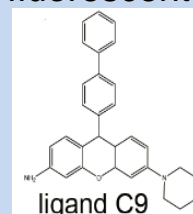
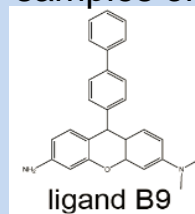
Compounds exhibiting low non-specific intracellular binding are required for direct *in vivo* imaging of mRNA and intracellular receptors.

## Approach

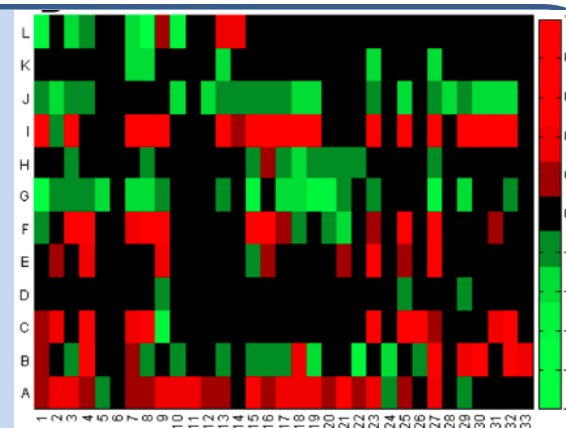
Three mammalian cell lines, with diverse genetic backgrounds, were used to screen a combinatorial fluorescence library via high throughput live cell microscopy. The hits, shown in different shades of green, are further validated on plant root hair (bottom figure).

**Results/Impact** First chemoinformatics study that links chemical structures to the flux property with a dual labeled compound that is functional as fluorescent and radiolabeled.

Two samples of 300+ Combinatorial fluorescent library



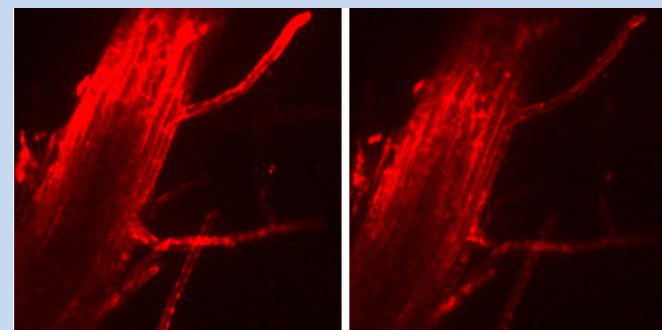
Hits on 3 mammalian cell lines are labeled as shades of green



In-flux

out-flux

Hits are validated on plant root hairs



Nath, et. al, 2012 "Identification of fluorescent compounds with non-specific binding property via high throughput live cell microscopy," **PLoS One**, doi:10.1371/journal.pone.0028802

## DNA repair center formation is greater at lower doses than at higher doses

### Objective

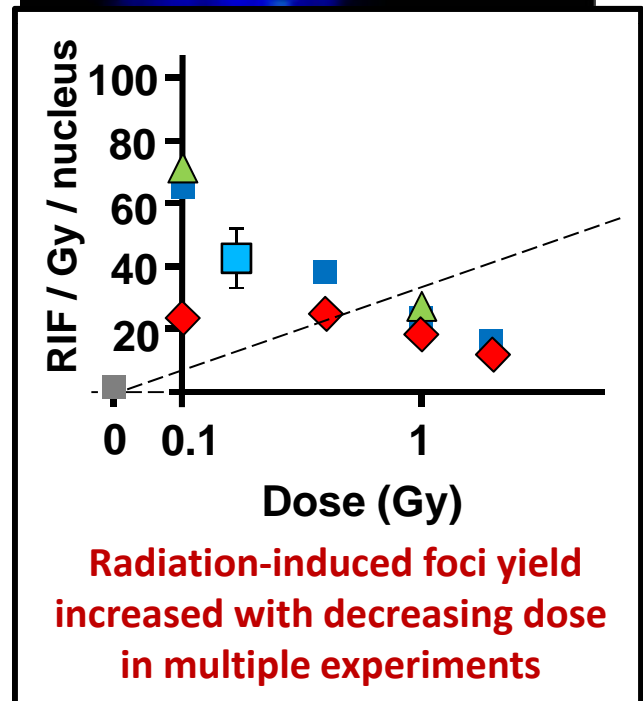
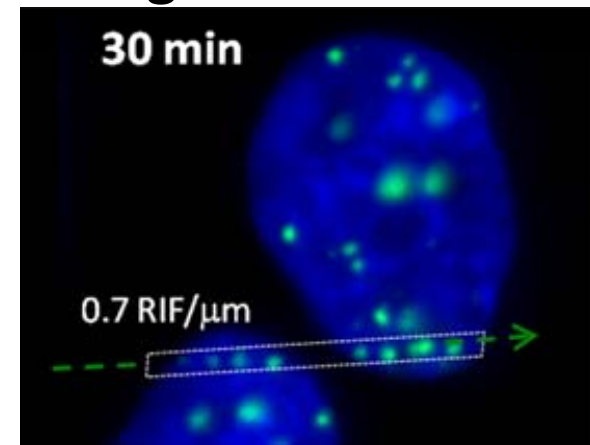
A critical question in radiation biology is how efficiently radiation-induced damage is repaired as a function of dose. This study investigates the kinetics of radiation-induced DNA damage and repair in human cell cultures

### Approach

- MCF10A cells were exposed to various doses of X-rays or heavy ions and incubated for one hour
- Cells were immuno-stained for 53BP1 to image and count the radiation-induced foci (RIF) repair centers

### Results/Impact

- The absolute number of repair centers (RIFs) is 4-fold larger at lower doses than at higher doses
- This discovery adds critical new confirmation that DNA damage repair at low radiation doses is more efficient than at higher doses, and
- Provides further evidence that cancer risk from exposure to ionizing radiation is not linearly proportional to dose in the low dose range



Neumaier, et al. 2011, Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. *Proc Natl Acad Sci*. doi: 10.1073/pnas.1117849108

## **Investigation of Ca<sup>2+</sup>-activated K<sup>+</sup> channel action**

### **Objective**

To understand the way that high-conductance voltage- and Ca<sup>2+</sup>-activated K<sup>+</sup> channels function in many physiological processes that link cell membrane voltage and intracellular Ca<sup>2+</sup> concentration.

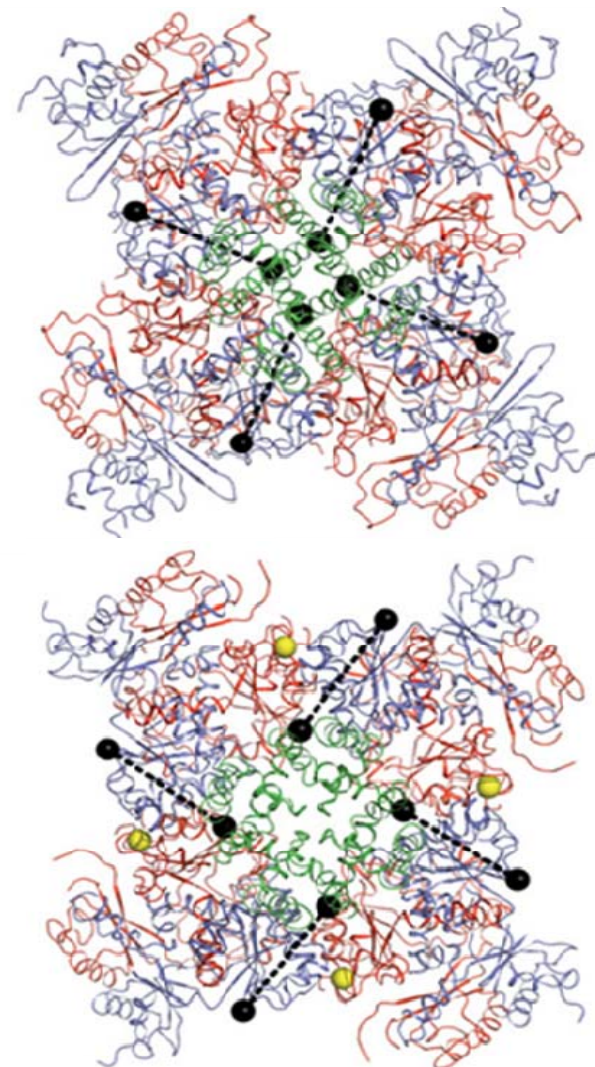
### **Approach**

Use the PXRR's flexible access and automation system to solve the structure of the Ca<sup>2+</sup>-bound, open conformation. Then compare with the known structure of the Ca<sup>2+</sup>-free, closed, conformation of the cytoplasmic-side gating ring, showing how one layer of the gating ring, in response to the binding of Ca<sup>2+</sup>, opens like the petals of a flower, opening the pore that lies below.

### **Results/Impacts**

“These findings present a molecular basis for Ca<sup>2+</sup> activation of K<sup>+</sup> channels and suggest new possibilities for targeting the gating ring to treat conditions such as asthma and hypertension.”

Yuan et. al, 2012, *Open structure of the Ca<sup>2+</sup> gating ring in the high-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel*, *Nature* doi:10.1038/nature10670.

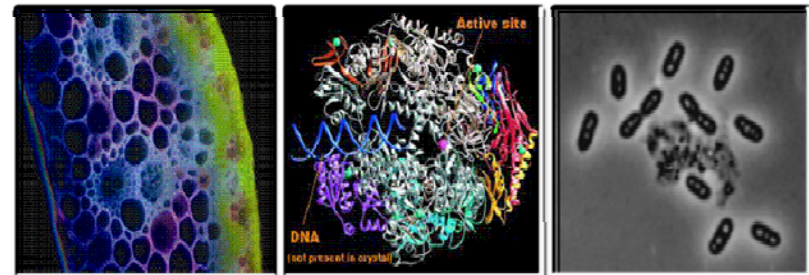


*A Ca<sup>2+</sup>-gating model  
for the BK channel.*



*Utilize existing commercial software technology and leveraging DOE internet resources (ESNet) and DOE cloud computing platforms (Magellan)*

➤ ***Kbase is a framework for data collection, integration and analysis tools to enable the simplified use of large scale genome and genome enabled information***

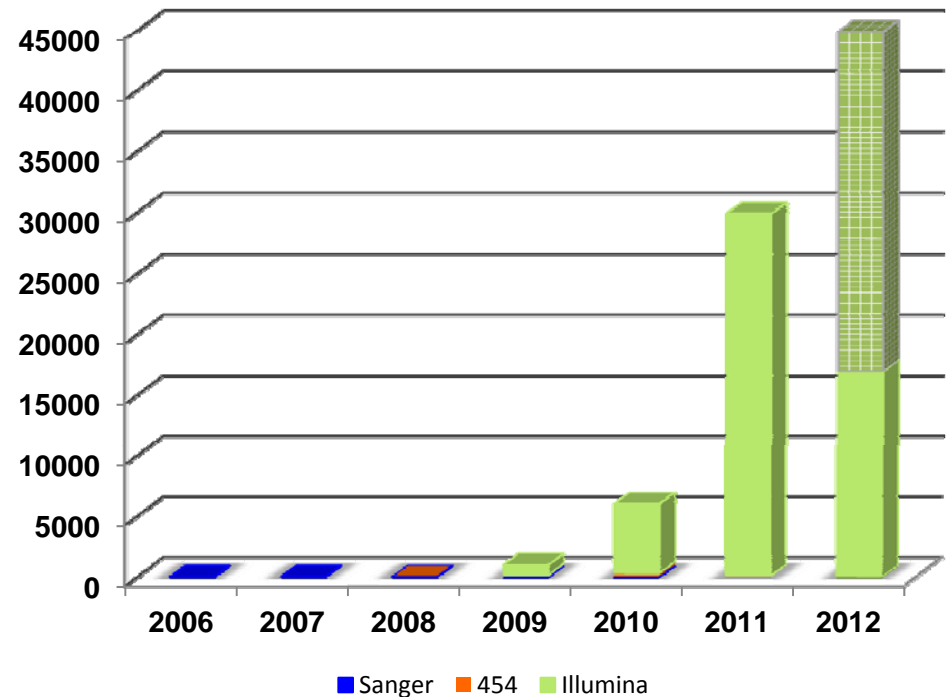


### **By 2013 Deliver on the Initial Goals:**

- Kbase Infrastructure firmly established at 4 laboratories including high performance and cloud computing with routine 10+ Gb/s data transfer over ESNet between all Kbase sites
- First public release will include:
  - Integration of data to reconstruct and predict metabolic and gene expression regulatory networks for up to 1,000 microbes to manipulate microbial function.
  - Integration of phenotypic and experimental data for bioenergy plants to predict metabolic and regulatory genotypes enabling manipulation of biomass properties.



## JGI Latest News



➤ Collaboration with NERSC enables JGI to provide the community with access to a billion genes and counting (in IMG/M)

➤ Release of *Phaseolus vulgaris* (common bean) through **Phytozome v8.0**

➤ **JGI User Meeting:** March 20 - 22, 2012  
[www.jgi.doe.gov/meetings/usermeeting/](http://www.jgi.doe.gov/meetings/usermeeting/)

➤ **Next Deadline for CSP Microbial Sequencing Proposals:** March 12, 2012.  
<http://proposals.jgi-psf.org/>

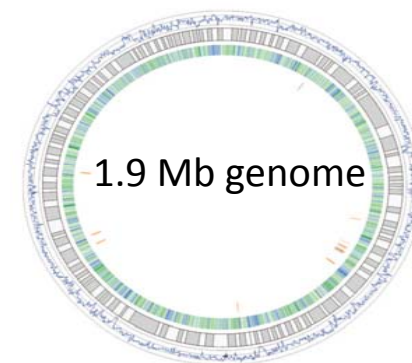
**FY2012 anticipated output:  
45, Trillion base pairs of DNA  
(~ 15,000 human genomes)**

## Selected JGI High Impact Publications

- Godoy-Vitorino, F., et al. (2011), *Comparative analyses of foregut and hindgut bacterial communities in hoatzins and cows*, **ISME**, DOI: 10.1038/ismej.2011.131
- Foston, M., et al. (2011), *Chemical, ultrastructural and supramolecular analysis of tension wood in *Populus tremula x alba* as a model substrate for reduced recalcitrance*, **Energy Environ. Sci**, DOI: 10.1039/c1ee02073k
- Hardoim, S., et al. (2011), *Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis*, **Molecular Plant-Microbe Interactions**, DOI: 10.1094/MPMI-08-11-0204
- Berka, R., et al. (2011), *Comparative genomic analysis of the thermophilic biomass-degrading fungi *Myceliophthora thermophila* and *Thielavia terrestris**, **Nature Biotech**, DOI:10.1038/nbt.1976
- Mackelprang, R., et al. (2011), *Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw*, **Nature**, DOI:10.1038/nature10576
- Grbic', M., et al. (2011), *The genome of *Tetranychus urticae* reveals herbivorous pest adaptations*, **Nature**, DOI:10.1038/nature10640
- Markowitz, V., et al (2011), *IMG/M: the integrated metagenome data management and comparative analysis system*, **Nucl Acids Res**, DOI:10.1093/nar/gkr975
- Nawabi, P., et al (2011), *Engineering *Escherichia coli* for biodiesel production utilizing a bacterial fatty acid methyltransferase*, **Appl & Environ Microbiology**, DOI: 10.1128/AEM.05046-11
- Persson, T., et al. (2011), *Genome sequence of *Candidatus Frankia datiscae* DGI, the uncultured microsymbiont from nitrogen-fixing root nodules of the dicot *Datisca glomerata**, **J. Bacteriology**, DOI: 10.1128/J.B.06208-11
- Roberts, E., et al. (2011), *Isolation and Characterization of the *Prochlorococcus* Carboxysome Reveals the Presence of the Novel Shell Protein *CsoSID**, **J. Bacteriology**, DOI: 10.1128/JB.06444-11
- Schuster, A., et al. (2012), *A versatile toolkit for high throughput functional genomics with *Trichoderma reesei**. **Biotechnology for Biofuels**, DOI:10.1186/1754-6834-5-1

**188 Total articles published with JGI acknowledgement in 2011**

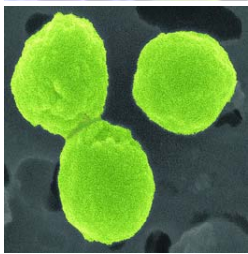
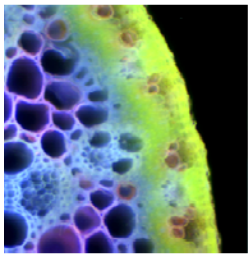
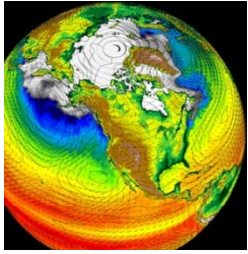
<http://www.jgi.doe.gov/News/pubs.html>



Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw



Dark-field photo of *Frankia* vesicle clusters (single plant cells filled with the filamentous organism) that have broken free from nodules in the plant *Coriaria arborea*. (Image courtesy of David Benson, University of Connecticut)



***Thank You!***



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