

# Plant Feedstock Genomics for Bioenergy Joint Awards 2006–2014

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# Plant Feedstock Genomics for Bioenergy

## U.S. Departments of Agriculture and Energy

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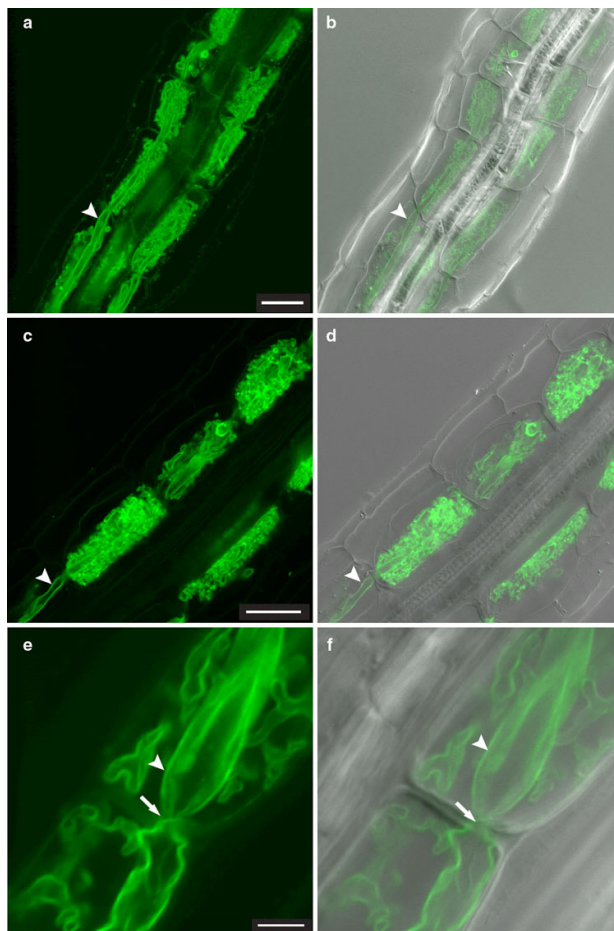
Integrating the U.S. Department of Energy's (DOE) capabilities in genomic sequencing and analysis for production of biofuels with the U.S. Department of Agriculture's (USDA) long experience in crop improvement, DOE and USDA are working together to fund projects that accelerate plant breeding programs and improve bioenergy feedstocks. Since 2006, dozens of projects have been funded by the DOE-USDA Plant Feedstock Genomics program, which is jointly supported by the Office of Biological and Environmental Research within DOE's Office of Science and the USDA National Institute of Food and Agriculture. This program supports fundamental research in biomass genomics to provide the scientific foundation for the use of lignocellulosic and seed oil materials, either primary material or agricultural residues, for bioenergy and biofuels.

Significant advances in breeding, molecular genetics, and genomic technologies provide an opportunity to build upon the existing knowledgebase of plant biology to be able to confidently predict and manipulate the biological function of plant genomes for bioenergy resources. Areas of interest include:

- Characterizing the genes, proteins, and molecular interactions that influence lignocellulosic biomass production and oil seed characteristics.
- Development of new cultivars of regionally adapted bioenergy feedstock crops in public breeding programs using innovative approaches to identify desirable traits and accelerate trait integration.

- Research into the complex interactions between bioenergy feedstock plants and their environment, and how these processes influence plant growth and development, expression of bioenergy-relevant traits, and adaptation to changing environments, particularly on marginal land.

Investigators in these projects are characterizing the genes, proteins, and molecular interactions that influence lignocellulosic biomass production and seed oil characterization, and are using the outcomes to develop novel approaches to improve bioenergy feedstocks.



Diversity of morphology and function in arbuscular mycorrhizal symbioses in *Brachypodium distachyon*. (Courtesy Boyce Thompson Institute for Plant Research. Hong, J. J., et al. 2012. "Diversity of Morphology and Function in Arbuscular Mycorrhizal Symbioses in *Brachypodium distachyon*," *Planta* 236, 851–65. DOI:10.1007/s00425-012-1677-z.)

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Cover photograph courtesy Lawrence Berkeley National Laboratory.

More details including Co-PIs and funding amounts are on the web at <http://GenomicScience.energy.gov/research/DOEUSDA/>.

## 2014 Awards

### Ten Projects Selected for Awards Totaling \$12.6 Million

#### Coordinated Genetic Improvement of Bioenergy *Sorghum* for Compositional and Agronomic Traits

- Patrick Brown, University of Illinois, Urbana-Champaign

**Goal:** Discover and characterize novel genetic variants that affect lignocellulosic composition and saccharification yield in bioenergy feedstock grasses without compromising agronomic performance. This project will characterize genetic variation in compositional and agronomic traits in a panel of 600 diverse *Sorghum* inbreds and identify useful traits and variants that will guide and accelerate the genetic improvement of both bioenergy *Sorghum* and closely related perennial grasses.

#### Abiotic Stress Networks Converging on FT2 to Control Growth in *Populus*

- Amy Brunner, Virginia Polytechnic Institute and State University, Blacksburg

**Goal:** Uncover divergent and convergent regulatory networks that control growth responses to daylength and nutrient stress in poplar. Regulation of growth and dormancy by such seasonal and episodic environmental factors is of central importance to productivity in temperate tree species. This project will characterize genome-wide gene expression changes in response to daylength and nutrient stress and identify protein-protein and protein-DNA networks that are centered on FT2, a key integrator of multiple abiotic signaling pathways in *Populus*.

#### Exploiting Natural Diversity to Identify Alleles and Mechanisms of Cold Adaptation in Switchgrass

- Robin Buell, Michigan State University, East Lansing

**Goal:** Identify metabolites, alleles, transcripts, and regulatory RNAs associated with cold hardiness in switchgrass that will advance understanding of the biochemical, physiological,

and molecular mechanisms for cold adaptation and provide molecular tools to improve breeding efficiency. Switchgrass biomass yields could potentially be increased by extending the northern range in which lowland ecotypes are grown, but these types are not cold tolerant and have low survival rates outside their adapted range. This project will identify genes and alleles relevant to cold hardiness that can be incorporated rapidly into switchgrass breeding programs to increase biomass and survival in northern latitudes.

#### A Novel Poplar Biomass Germplasm Resource for Functional Genomics and Breeding

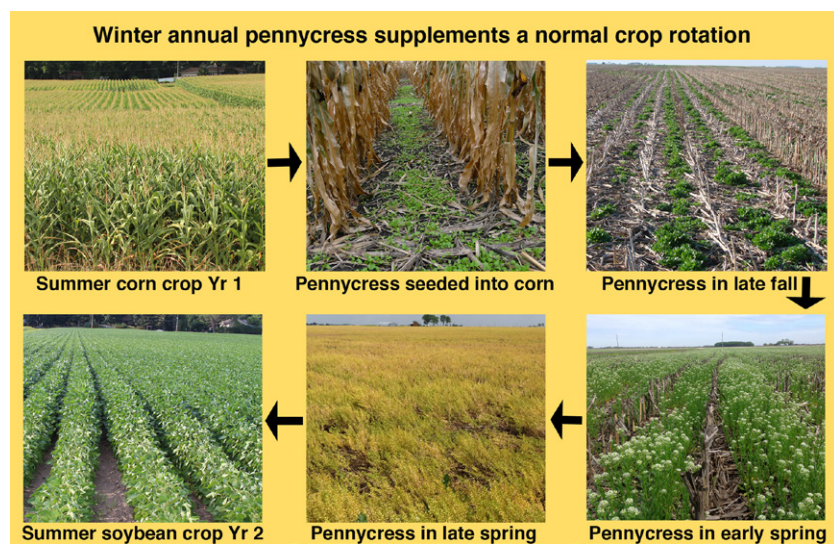
- Luca Comai, University of California, Davis

**Goal:** Further develop the poplar indel germplasm collection and use it to investigate the role of gene dosage in poplar hybrid performance and contribution to bioenergy traits. This project will catalog dosage variation in ~500 *Populus deltoides* × *P. nigra* F1 individuals, use field trials to characterize variation for traits central to sustainable production of biomass with optimal feedstock properties, and identify specific regulatory or functional gene modules underlying phenotypes of interest, ultimately to produce new cultivars directly usable for bioenergy applications.

#### Genetic Dissection of AM Symbiosis to Improve the Sustainability of Feedstock Production

- Maria Harrison, Boyce Thompson Institute for Plant Research, Ithaca, NY

**Goal:** Understand the genetic bases of arbuscular mycorrhizal (AM) symbiosis in feedstocks through studies of a model feedstock species, *Brachypodium distachyon*, and *Sorghum*, a feedstock species. This project will utilize *Brachypodium* to evaluate the function of candidate proteins that potentially control development of the symbiosis and symbiotic P and N transport, and then evaluate AM symbiosis in diverse sweet and cellulosic (bioenergy) *Sorghum* lines. This research will inform feedstock breeding programs and enhance the sustainability of feedstock production.



Pennycress produces a valuable oilseed containing up to 40% oil that is suitable for conversion to biofuels. By fall planting into standing corn with spring harvest before soybean planting, pennycress fits into corn-soybean crop rotation and reduces soil erosion and nutrient runoff. (Courtesy Kevin Anderson, University of Minnesota: pennycress seeded into standing corn; Dr. Winthrop Phippen, Western Illinois University: pennycress in late fall, early and mid spring; Kirk Amundson, University of Minnesota: layout assistance)



## Advancing Field Pennycress as a New Oilseed Biodiesel Feedstock That Does Not Require New Land Commitments

- Michael Marks, University of Minnesota, Minneapolis

**Goal:** Genetically improve the agronomic traits of field pennycress (*Thlaspi arvense* L.) for its use as a new winter annual oilseed/meal/cover crop in the Upper Midwest. Pennycress can be double-cropped on the same land during the time between the traditional corn harvest and subsequent planting of soybeans the following spring. This oilseed plant holds much agronomic promise, but improved domesticated varieties remain to be developed. The project will lead to superior, higher-yielding pennycress varieties grown as a winter oilseed crop integrated within corn-soybean rotations throughout the Midwest.

## Biofuels in the Arid West: Germplasm Development for Sustainable Production of *Camelina* Oilseed

- John McKay, Colorado State University, Fort Collins

**Goal:** Facilitate the development of *Camelina* as an oilseed feedstock crop that can be grown on marginal farmland with relatively low fertilizer inputs and no irrigation. *Camelina* has many optimal qualities as an oilseed feedstock, but its performance as a fuel with minimal processing can be improved. Leveraging the newly available genome sequence of *Camelina sativa*, this project will use forward and reverse genetics and natural variation to combine optimal qualities in *Camelina* as an oilseed feedstock for the Great Plains and Western United States (see next page for photograph of crop).



## The *Brachypodium* ENCODE Project—From Sequence to Function: Predicting Physiological Responses in Grasses to Facilitate Engineering of Biofuel Crops

- Todd Mockler, Donald Danforth Plant Science Center, St. Louis, MO

**Goal:** Identify and characterize the functional elements associated with progressive drought response in the *Brachypodium distachyon* genome sequence and develop integrated genome feature maps that will enable advanced modeling of complex pathways in plants. Using a model grass, the *Brachypodium* ENCODE (for Encyclopedia of DNA Elements) project will elucidate the molecular mechanisms and gene regulatory networks underlying drought stress, information which will aid basic and applied research on a wide range of bioenergy grasses and accelerate deployment of improved bioenergy grass feedstocks.

## Genomics of Energy *Sorghum*'s Water Use Efficiency / Drought Resilience

- John Mullet, Texas A&M University, College Station

**Goal:** Increase the water use efficiency, drought resilience, and yield of high biomass energy *Sorghum* and other C4 bioenergy grasses. This project will use field analysis to identify traits and molecular responses that improve water use efficiency and drought resilience of energy *Sorghum* and characterize genetic variation, and then test the utility of modulating these traits in energy *Sorghum* hybrids through marker-assisted breeding.

## Quantifying Phenotypic and Genetic Diversity of *Miscanthus sacchariflorus* to Facilitate Knowledge of Directed Improvement of *M. × giganteus* (*M. sinensis* × *M. sacchariflorus*) and Sugarcane

- Erik Sacks, University of Illinois, Urbana-Champaign

**Goal:** Facilitate the rapid development of *Miscanthus* as a bioenergy crop by obtaining fundamental knowledge about *M. sacchariflorus* (*Msa*) genetic diversity, population structure, and environmental adaptation. This project will conduct field trials with ~600 individuals of *Msa* from throughout its natural range to evaluate yield potential and adaptation. It will develop molecular markers associated with traits of interest that will enable plant breeders to quickly develop improved biomass cultivars of *M×g* as well as the closely related sugarcane and energycane.

*Camelina*, an oilseed feedstock crop that can be grown on marginal farmland with relatively low fertilizer inputs and no irrigation. (Courtesy Jean-Nicolas Enjalbert, Colorado State University)

## 2013 Awards

### Seven Projects Selected for Awards Totaling \$8.1 Million

#### Functional Manipulation of Root Endophyte Populations for Feedstock Improvement

- Jeffrey Dangel, University of North Carolina, Chapel Hill

**Goal:** Utilize genomics, genetics, and physiology to understand how endophytic bacteria alter plant growth and productivity, ultimately to manipulate plant performance for feedstock production. A variety of plant functions and traits are co-dependent on the surrounding microbial community, especially those associated with the plant root system (rhizosphere). This project will investigate whether plant performance phenotype in association with microbial communities translates across plant species in a predictable manner.

#### Unraveling the Genetics of Two Key Biomass Traits that Differentiate Upland and Lowland Tetraploid Switchgrass Ecotypes, Colonization by Mycorrhizal Fungi, and Frost Tolerance

- Katrien Devos, University of Georgia, Athens

**Goal:** Develop strategies for increased frost tolerance of lowland switchgrass through (1) identifying the genetic pathway(s) that provide frost tolerance in upland switchgrass and (2) studying the potential of beneficial fungi to minimize host cold stress. This project seeks to leverage the high biomass yield of southern-adapted lowland types and the frost tolerance of northern-adapted upland types to identify candidate genes that can be exploited to enhance biomass production of switchgrass under cold stress.

#### Genetic Control of Flowering in Switchgrass

- Yiwei Jiang, Purdue University, West Lafayette, IN

**Goal:** Elucidate the genetic mechanisms and identify candidate genes controlling flowering time in switchgrass. Late-flowering genotypes yield more biomass because the growing season is extended; having a better understanding of the genes that control flowering time will help to develop a rational strategy for creating improved switchgrass lines. The knowledge generated will aid breeding programs in developing late-flowering varieties of switchgrass that fully utilize the growing season and achieve high biomass yield.

#### Accelerated Development of Optimal Pine Feedstocks for Bioenergy and Renewable Chemicals Using Genome-Wide Selection

- Matias Kirst, University of Florida, Gainesville

**Goal:** Hyper-accelerate pine breeding using genome-wide selection, generating cultivars of loblolly and slash pine tailored to produce high energy yields that are ready for deployment. Traditional genetic improvement of pines is logistically complex and expensive, and a single breeding cycle takes almost two decades to complete. Thus, the project will develop and apply new breeding strategies that accelerate development of cultivars suitable for bioenergy production.

#### Pyramiding Genes and Alleles for Improving Energy Cane Biomass Yield

- Ray Ming, University of Illinois, Urbana-Champaign

**Goal:** Accelerate energy cane breeding and maximize biomass yield by utilizing the extraordinary segregation of true F2 populations to select high biomass-yielding genotypes. Sugarcane cultivars are mostly derived from hybridization between domesticated and wild species followed by backcrossing to recover the high biomass yield and sugar content of the domestic parent while retaining stress tolerance from the wild. Because sugar content is not a limiting factor for energy cane, this project will introduce a new breeding paradigm for more efficient cultivar improvement.

#### Global Analysis of Epigenetic Regulation of Gene Expression in Response to Drought Stress in *Sorghum*

- A. S. N. Reddy, Colorado State University, Fort Collins

**Goal:** Investigate the impact of drought stress on epigenetic modifications and alternative splicing in *Sorghum*. Using recently developed high-throughput tools, this project will examine genome-wide changes in the chromatin landscape and patterns of alternative splicing in cultivars that are sensitive and tolerant to drought under normal conditions and in response to drought stress. Understanding how plants respond and adapt to drought stress at the molecular level will help in developing plants that can grow under water-limiting conditions.

#### Structural Polymorphisms as Causes of Heterosis in *Populus*

- Steven Strauss, Oregon State University, Corvallis

**Goal:** Characterize the extent of structural polymorphisms (SPs) between and within species of *Populus* that are used to produce wood and bioenergy and examine their relationship to growth, stress tolerance, and breeding efficiency. This project will study wild black cottonwoods and interspecies hybrids important in plantations in the Pacific Northwest United States and other parts of the world, with a focus on the extent to which assay of SPs could improve hybrid breeding compared to alternative approaches.



Field trial of pine feedstocks population used in genome-wide selection project. (Courtesy Matias Kirst, University of Florida, Gainesville)



## 2012 Awards

### Nine Projects Selected for Awards Totaling \$11.5 Million

#### Functional Gene Discovery and Characterization of Genes and Alleles Affecting Wood Biomass Yield and Quality in *Populus*

- Victor Busov, Michigan Technological University, Houghton

**Goal:** Discover and characterize novel genes and alleles that affect wood biomass yield and quality in *Populus*. By combining mutagenesis for functional identification of genes with next-generation sequencing technologies for identifying alleles with breeding values, these discoveries can enable knowledge-based approaches for developing specialized bioenergy poplar cultivars.

#### Identifying Differences in Abiotic Stress Gene Networks Between Lowland and Upland Ecotypes of Switchgrass

- Kevin Childs, Michigan State University, East Lansing

**Goal:** Investigate response to drought and salt stress in a diverse collection of lowland and upland switchgrass ecotypes. Comparing differential gene expression between tolerant and sensitive lines will allow a better understanding of this response, as well as the identification of genes and germplasm that can be used to improve cultivated switchgrass to better tolerate these abiotic stresses.

#### Poplar Interactome for Bioenergy Research

- Pankaj Jaiswal, Oregon State University, Corvallis

**Goal:** Identify genome-wide functional gene networks and subnetworks in poplar that are associated with abiotic stress tolerance and bioenergy-related traits, as well as candidate genes that interact to produce abiotic stress-resistant phenotypes. Using a combination of computational projections, gene expression analysis, and experimental validation, this project will further development of poplar varieties that can thrive under abiotic stress on marginal land unsuitable for food crops.

#### The Genetics of Biofuel Traits in *Panicum* Grasses: Developing a Model System with Diploid *Panicum hallii*

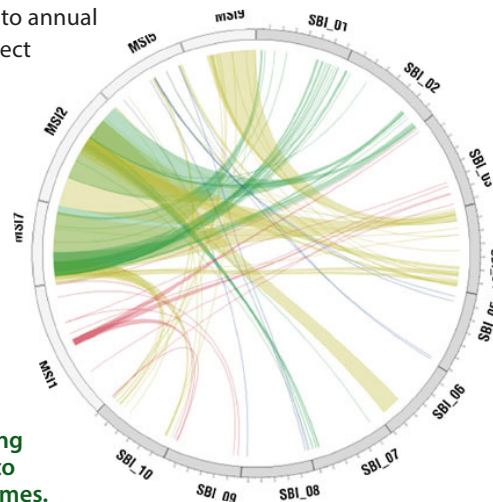
- Thomas Juenger, University of Texas, Austin

**Goal:** Utilize genetic and genomic analyses to better understand the growth and development of *Panicum* grasses, including the diploid *Panicum hallii*, and provide tools for predicting biomass- and tissue-related phenotypes from genotypes. This project will exploit natural variation to discover the genes important in biomass production, tissue quality, and stress tolerance under diverse environmental conditions, providing avenues for improving C4 perennial grasses for use as bioenergy crops.

#### Genomics of Bioenergy Grass Architecture

- Andrew Paterson, University of Georgia, Athens

**Goal:** Understand the genetic determinants of plant architecture that are important to the design of *Sorghum* genotypes optimized for biomass production in a range of environments. Optimal biomass productivity in temperate latitudes and/or under perennial production systems may require substantial changes to the architecture of plants of tropical origin that have previously been adapted to annual cultivation. This project will further enhance the value of many existing resources while also adding new dimensions to scientific research capacity.



Linkage groups of *Miscanthus sinensis* (MSI) in a circle alignment comparing MSI chromosomes to *Sorghum* chromosomes.

(Courtesy University of Georgia, Athens. Kim, C., et al. May 2012. "SSR-Based Genetic Maps of *Miscanthus sinensis* and *M. sacchariflorus*, and Their Comparison to *Sorghum*," *Theoretical Applied Genetics* 124(7) 1325–38. DOI:10.1007/s00122-012-1790-1.)

#### Deciphering Natural Allelic Variation in Switchgrass for Biomass Yield and Quality Using a Nested Association Mapping Population

- Malay Saha, Samuel Roberts Noble Foundation, Ardmore, OK

**Goal:** Understand the genetic basis of key biofeedstock traits in switchgrass by identifying genetic markers controlling important biomass traits. Most of these traits, such as biomass yield and cell wall composition, are complex and difficult to improve, but improvement can be obtained using traditional breeding augmented by marker-assisted selection. Validated markers cosegregating with bioenergy-relevant traits will be used to initiate a marker-assisted and/or genomic selection program to accelerate development of superior cultivars.

### Genetic Architecture of *Sorghum* Biomass Yield Component Traits Identified Using High-Throughput, Field-Based Phenotyping Technologies

- Patrick Schnable, Iowa State University, Ames

**Goal:** Test the hypothesis that variation in biomass growth rate can be explained by variation in photosynthetic rates and/or amounts of photo-protection. Data from a large, genetically diverse *Sorghum* collection will be collected at multiple time points during the growing season using an automated high-throughput field-based plant phenotyping system. Identifying the genetic control of biomass growth rates will allow breeders to genetically “stack” genes that control maximal growth rates, thereby paving a path to producing higher-yielding hybrids.



New field-based technology is being developed to digitize and screen for biomass yield traits. The “robot” is a converted John Deere garden tractor with a GPS-enabled auto-steer function and sensors. Before it can function on its own, researchers “train” the robot’s auto-steer function to follow the route it should take through the field. (Courtesy Patrick Schnable, Iowa State University, Ames)

### The Genomic Basis of Heterosis in High-Yielding Triploid Hybrids of Willow (*Salix* spp.) Bioenergy Crops

- Lawrence Smart, Cornell University, Ithaca NY

**Goal:** Investigate how gene expression patterns in willow hybrids are related to yield potential and other traits important for biofuels production. Yield improvement in many crops has been based on capturing hybrid vigor (i.e., heterosis), but its complex genetic basis is poorly understood. This project will investigate whether there is a bias in the expression of key genes from one parent versus the other in species hybrids and whether there is a gene dosage effect skewing gene expression patterns in triploid progeny compared with their diploid and tetraploid parents.

### The Dual Effect of Tubulin Manipulation on *Populus* Wood Formation and Drought Tolerance

- Chung-Jui Tsai, University of Georgia, Athens

**Goal:** Determine how tubulin levels and/or tubulin protein modifications affect wood development and water use in *Populus*. Tubulin proteins form microtubule scaffolds that participate in cell wall biogenesis as well as regulate stomatal guard cell movements for photosynthesis and transpiration. This project will allow dissection of the contribution of tubulins to two interdependent processes, water utilization and the development of lignocellulosic biomass, both of which are relevant to bioenergy crop improvement.

## 2011 Awards

### Ten Projects Selected for Awards Totaling \$12.2 Million

#### Association Mapping of Cell Wall Synthesis Regulatory Genes and Cell Wall Quality in Switchgrass

- Laura E. Bartley, University of Oklahoma, Norman

**Goal:** Identify natural genetic variation in switchgrass that correlates with lignocellulose-to-biofuel conversion qualities. Most plant dry matter is composed of lignocellulose, and because switchgrass yields high amounts of this material and tolerates drought and other stresses it is an attractive candidate for development into a biofuel crop. This project should enhance understanding of the qualities that critically impact the conversion efficiency of lignocellulose into biofuels.

#### Functional Interactomics: Determining the Roles Played by Members of the Poplar Biomass Protein-Protein Interactome

- Eric Beers, Virginia Polytechnic and State University, Blacksburg

**Goal:** Identify key interactions between proteins associated with wood formation in poplar, a woody biomass crop. Wood characteristics result from the coordinated actions of enzymes and structural proteins in the cells, which typically interact with other proteins to perform their roles. This project will uncover the potential of the biomass protein-protein interactome to contribute to the development of poplar trees with superior biomass feedstock potential.

#### Functional Genomics of Sugar Content in Sweet *Sorghum* Stems

- David M. Braun, University of Missouri, Columbia

**Goal:** Improve sucrose accumulation in sweet *Sorghum* through investigating the mechanisms regulating carbon allocation to stems. A rapidly growing, widely adaptable crop, sweet *Sorghum* accumulates in the stem high concentrations of sucrose that can be efficiently converted to ethanol, making this a valuable candidate bioenergy feedstock. This research will use a combination of approaches to identify bioenergy-relevant genes and to understand their functions in carbon partitioning in sweet *Sorghum*.

#### Creation and High-Precision Characterization of Novel *Populus* Biomass Germplasm

- Luca Comai, University of California, Davis

**Goal:** Provide new genomic tools for poplar breeders to identify germplasm with unique genotypes and increased biomass yields and develop techniques for creating poplar hybrids with unique combinations of chromosomal regions. Because such properties can confer faster growth, this project addresses a challenge posed by the long generation time of trees through fast and cost-effective nontransgenic genetic manipulation.

#### Genomic and Breeding Foundations for Bioenergy *Sorghum* Hybrids

- Stephen Kresovich, University of South Carolina, Columbia

**Goal:** Build the germplasm, breeding, genetic, and genomic foundations necessary to optimize cellulosic *Sorghum* as a bioenergy feedstock. This project will facilitate breeding *Sorghum* lines optimized for energy production and selected to maximize energy accumulation per unit time, land area, and/or production input.

#### An Integrated Approach to Improving Plant Biomass Production

- Jan Leach, Colorado State University, Fort Collins

**Goal:** Expedite discovery of genes controlling biomass productivity in switchgrass by leveraging results from rice, a well-studied model grass. Switchgrass and other perennial grasses are promising candidates for bioenergy feedstocks; however, the genetic resources necessary to develop these species are currently limited. This work will greatly expand the research tool box for switchgrass and advance its improvement as an energy crop.

#### Modulation of Phytochrome Signaling Networks for Improved Biomass Accumulation Using a Bioenergy Crop Model

- Todd C. Mockler, Donald Danforth Plant Science Center, St. Louis

**Goal:** Identify genes involved in light perception and signaling in the model grass *Brachypodium distachyon* to increase yield and improve the composition of bioenergy grasses. Plant growth and development, including biomass accumulation, are affected by the light environment. Finding key genes involved in modulating light perception could be useful in targeted breeding or engineering efforts for improved bioenergy grass crops.

#### Quantifying Phenotypic and Genetic Diversity of *Miscanthus sinensis* as a Resource for Knowledge-Based Improvement of *M. × giganteus* (*M. sinensis* × *M. sacchariflorus*)

- Erik J. Sacks, University of Illinois, Urbana-Champaign

**Goal:** Facilitate development of *Miscanthus* as a bioenergy crop by acquisition of fundamental information about genetic diversity and environmental adaptation. *Miscanthus* is among the most promising cellulosic biofuel crops, but its improvement as a feedstock will require a broader genetic base. Identification of molecular markers associated with traits of interest will improve *Miscanthus* breeding efforts.

#### Discovering the Desirable Alleles Contributing to the Lignocellulosic Biomass Traits in *Saccharum* Germplasm Collections for Energy Cane Improvement

- Jianping Wang, University of Florida, Gainesville

**Goal:** Improve energy cane by identifying the genetic components contributing to biomass production. Energy cane (*Saccharum* complex hybrids) holds great potential as a bioenergy feedstock in the southern United States. This project will produce foundational genetic resources for energy cane breeders to efficiently develop cultivars with increased biomass production and reduced input requirements.

#### *Sorghum* Biomass Genomics and Phenomics

- Jianming Yu, Kansas State University, Manhattan

**Goal:** Integrate key genomics-assisted approaches into biomass *Sorghum* research and combine with high-throughput and traditional field-based phenotyping methods to enable advanced breeding strategies. Exploiting genetic diversity and understanding the genotype-phenotype relationship can lead to development of predictive approaches for efficient and cost-effective breeding.



## 2010 Awards

### Nine Projects Selected for Awards Totaling \$9 Million

#### Genome-Wide Analysis of miRNA Targets in *Brachypodium* and Biomass Energy Crops

- Pamela J. Green, University of Delaware, Newark

**Goal:** Identify the targets of MicroRNAs (miRNAs) in different organs and under adverse environmental conditions in the model grass *Brachypodium* and in the energy crops switchgrass, *Miscanthus*, and *Sorghum*. miRNAs are important regulatory molecules that repress selected “target” genes to enable normal development, stress responses, and other processes. This project should enhance understanding of regulatory networks and may suggest new strategies for improving biomass energy crops.

#### Organ and Tissue-Specific Sucrose Transporters: Important Hubs in Gene and Metabolite Networks Regulating Carbon Use in Wood-Forming Tissues of *Populus*

- Scott A. Harding, University of Georgia, Athens

**Goal:** Investigate how sucrose transporter proteins (SUTs) function to facilitate the distribution of sucrose for transient storage and biosynthetic use among different pathways in the developing wood matrix. Wood for lignocellulosic feedstocks is synthesized from sucrose that is exported from leaves and then processed in the wood-forming organs. SUTs mediate the export and efficient movement of sucrose from source leaves to sink organs in all plant species.

#### The Role of Small RNA in Biomass Deposition and Perenniality in Andropogoneae Feedstocks

- Matthew E. Hudson, Energy Bioscience Institute, University of Illinois, Urbana-Champaign

**Goal:** Investigate the role of small RNA molecules in biomass production and their importance in the regulation of cellulose and lignin biosynthesis. The tissues and organs of next-generation biofuel crops that provide biomass for energy production are primarily composed of lignin and cellulose. This research will focus on *Miscanthus* species as well as other biomass crops including switchgrass and prairie cordgrass.

#### Development of a Low-Input and Sustainable Switchgrass Feedstock Production System Utilizing Beneficial Bacterial Endophytes

- Chuansheng Mei, The Institute for Advanced Learning and Research, Danville, VA

**Goal:** Understand the molecular and physiological mechanisms by which interaction with bacterial endophytes promotes growth in the promising bioenergy crop switchgrass. The use of naturally occurring beneficial bacterial endophytes with switchgrass represents a practical and feasible way to develop a low-input and sustainable feedstock production system.

#### Functional Analysis of Regulatory Networks Linking Shoot Maturation, Stem Carbon Partitioning, and Nutrient Utilization in *Sorghum*

- Stephen Moose, University of Illinois, Urbana-Champaign

**Goals:** Determine whether changes in the Glossy15 gene system of *Sorghum* might contribute to current physiological differences among grain and sweet and biomass *Sorghums* and whether this gene can be used to convert superior *Sorghum* grain hybrids to cultivars enhanced for bioenergy production.

#### Genomics of Energy *Sorghum* Biomass Accumulation

- John Mullet, Texas A&M University, College Station

**Goal:** Identify the genetic and biochemical basis for increasing yield and improving the composition of high-biomass cellulosic energy *Sorghum*. Select genotypes will be analyzed for stem biomass yield, structure, and composition. The resources developed will enable analysis of the genes that modulate these traits and facilitate improvement of energy *Sorghum* and other bioenergy grasses.

#### Identification and Genetic Characterization of Maize Cell Wall Variation for Improved Biorefinery Feedstock Characteristics

- Markus Pauly, University of California, Berkeley

**Goal:** Identify and characterize maize lines with enhanced biorefinery feedstock characteristics, particularly those containing higher yields of fermentable sugars. Stover, the material from the corn plant that remains after removal of the grain, consists primarily of cellulose, hemicellulose, and lignin. Because corn stover is generated by U.S. agriculture in significant amounts, this lignocellulosic residue is desirable to use as a biofuel source.

#### Systems View of Root Hair Response to Abiotic Stress

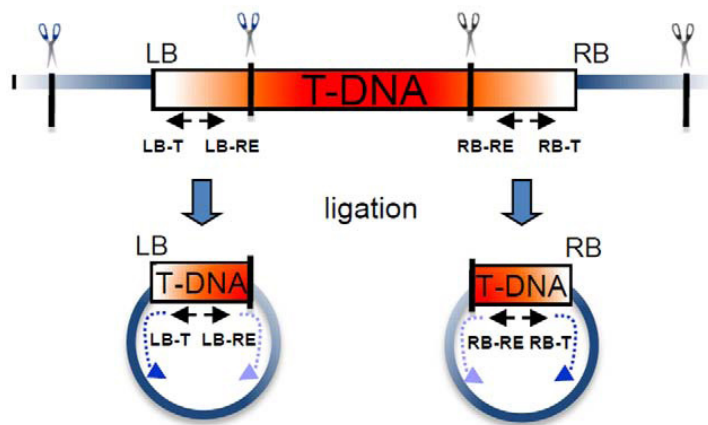
- Gary Stacey, University of Missouri, Columbia

**Goal:** Gain insight into the impacts of variations in temperature and water availability on nutrient uptake by root cells. Root hair cells function to increase root surface area and to mediate water and nutrient uptake. The data obtained in this project should provide a better understanding of the impacts of climate change (heat and water limitation) on plant root physiology.

#### Insertional Mutagenesis of *Brachypodium distachyon*

- John Vogel, USDA Agricultural Research Service, Albany, CA

**Goals:** Generate 30,000 additional insertional mutants in the model grass *Brachypodium distachyon* and sequence DNA flanking the insertion sites. Insertional mutants are powerful research tools that allow researchers to rapidly determine the function of specific genes. Mutants from outside collaborators will be integrated into this collection and made available through a public database.



**Inverse PCR strategy for obtaining T-DNA flanking sequences within *Brachypodium* genome sequence.** (Courtesy USDA Agricultural Research Service, Albany, CA. Bragg, J. N., et al. 2012. “Generation and Characterization of the Western Regional Research Center,” *PLoS ONE* 7(9), e41916. DOI:10.1371/journal.pone.0041916.g004.)

## 2009 Awards

### Seven Projects Selected for Awards Totaling \$6.3 Million

#### Improving Alfalfa as a Biofuel Feedstock

- E. Charles Brummer, University of Georgia, Athens

**Goal:** Develop in the long term the biofuel-ready alfalfa cultivars that have improved yield and quality. Maximizing the production of energy with biofuel crops requires a high yield of biomass with optimum fuel quality. This project will identify molecular markers in alfalfa that are associated with optimal biofuel characteristics and directly integrate them into traditional field-oriented alfalfa breeding programs.

#### A Systems Biology Approach to Elucidate Regulation of Root Development in *Populus*

- Victor Busov, Michigan Technological University, Houghton

**Goal:** Identify key regulators of root architecture in relation to nitrogen and water use in the bioenergy crop *Populus* using an integrated systems biology approach. This research will generate resources and innovations that can enable robust biomass productivity under marginal conditions for sustainable lignocellulosic biomass production.



**Populus samples.** Processed *Populus deltooides* ground samples ready for bioprocessing, unprocessed segments, and live *Populus* seedlings in the background. [Image courtesy Oak Ridge National Laboratory]

#### Characterization of Nitrogen Use Efficiency in Sweet *Sorghum*

- Ismail Dweikat, University of Nebraska, Lincoln

**Goal:** Enhance the ability of sweet *Sorghum* to utilize nitrogen, increasing its potential as a leading and cost-effective bioenergy crop. This project will identify novel nitrogen use efficiency alleles in wild *Sorghum* germplasm that can be used to improve sweet *Sorghum*.

#### Mechanism of Carbon Partitioning Regulation by *cpg13* in the Bioenergy Woody Crop Poplar

- Matias Kirst, University of Florida, Gainesville

**Goal:** Characterize genes that regulate the balance of carbon going to cellulose or lignin, leading to the development of plant materials that are more suitable for biofuel production.

#### Accelerating the Domestication of *Miscanthus* for Biofuel Production

- Andrew H. Paterson, University of Georgia, Athens

**Goal:** Provide genomic tools and resources for a promising cellulosic biofuel crop, *Miscanthus*, that will (a) foster innovative strategies for its improvement and (b) develop comparative and bioinformatic approaches to enhance fundamental knowledge of *Miscanthus* genome structure, function, and organization.

#### The Hunt for Green Every April: Factors Affecting Fitness in Switchgrass

- Gautam Sarath, USDA Agricultural Research Service, Lincoln, NE

**Goal:** Investigate winter survival in switchgrass populations and individual plants specifically selected for greater yields and with known differences in winter survival. Molecular events occurring in the crowns and rhizomes will be studied over two growing seasons and winters. The project will make a significant and lasting contribution to the future improvement of switchgrass as a bioenergy crop. It also will directly benefit researchers working on developing other perennial grasses into biomass energy crops.

#### Phenomic Analysis of Natural and Induced Variation in *Brachypodium distachyon*

- John Vogel, USDA Agricultural Research Service, Albany, CA

**Goal:** Conduct high-throughput phenotypic analysis (phenomics) of homozygous T-DNA mutants and natural accessions of the model grass *Brachypodium distachyon* to accelerate the understanding of the basic underlying biological traits that control the utility of grasses as energy crops.



## 2008 Awards

### Ten Projects Selected for Awards Totaling \$10.8 Million

#### Development of Genomic and Genetic Tools for Foxtail Millet, and Use of These Tools in the Improvement of Biomass Production for Bioenergy Crops

- Jeff Bennetzen, University of Georgia, Athens

**Goal:** Generate a variety of genomic and genetic tools for foxtail millet, including SNPs, BAC libraries, optimized foxtail millet transformation technology, and a high-density QTL and genetic map of foxtail millet for significant biomass traits. These resources will complement the DOE Joint Genome Institute whole-genome sequencing of foxtail millet, enhancing its value as a functional genomic model for second-generation bioenergy crops such as switchgrass.

#### Identifying Genes Controlling Ferulate Cross-Link Formation in Grass Cell Walls

- Marcia Maria de Oliveira Buanaflina, Pennsylvania State University, University Park

**Goal:** Investigate the regulation of ferulic acid cross-linking in the cell walls of *Brachypodium distachyon* and generate a saturated EMS mutant population for forward genetic studies in this model bioenergy crop.

#### Computational Resources for Biofuel Feedstock Species

- C. Robin Buell, Michigan State University, East Lansing

**Goal:** Provide computational tools and resources for data-mining of genome sequence, genome annotation, and large-scale functional genomic datasets available for biofuel feedstock species. Such species include candidates within the Poaceae, Pinaceae, and Salicaceae families, for which a diversity of genome sequence resources currently exist, ranging from whole-genome sequences to modest EST transcriptome datasets.

#### Translational Genomics for the Improvement of Switchgrass

- Nick Carpita, Purdue University, West Lafayette, IN

**Goal:** Study the cell walls of grass species, performing bioinformatics analyses on cell wall biosynthetic genes in maize, and annotate switchgrass orthologs. The project also will generate mutants in selected candidate cell wall-related genes, with direct analysis of saccharification of maize and switchgrass cell wall mutants.

#### Identification of Genes That Regulate Phosphate Acquisition and Plant Performance During Arbuscular Mycorrhizal Symbiosis in *Medicago truncatula* and *Brachypodium distachyon*

- Maria Harrison, Boyce Thompson Institute for Plant Research, Ithaca, NY

**Goal:** Identify genes controlling arbuscular mycorrhizal symbiosis, as well as key factors regulating gene function and the acquisition of key nutrients such as phosphate. The results will provide mechanistic and molecular-level understanding of plant-fungal partnerships in natural ecosystems and their role in maintaining a terrestrial soil environment for sustainable biofuel production.

#### Systems-Level Engineering of Plant Cell Wall Biosynthesis to Improve Biofuel Feedstock Quality

- Samuel Hazen, University of Massachusetts, Amherst

**Goal:** Identify and characterize cell wall biosynthetic regulatory genomic binding sites using reverse and forward genetic approaches with candidate transcription factors in *Brachypodium* and *Arabidopsis*, two model plant systems. The results will contribute to the understanding of key tissue-specific and developmental regulators of plant cell wall biosynthesis in monocot and dicot bioenergy crops.

#### Genomics of Wood Formation and Cellulosic Biomass Traits in Sunflower

- Steven Knapp, University of Georgia, Athens

**Goal:** Develop genomic resources for woody biomass trait identification in hybrid sunflower, a species that is extremely drought tolerant. This fundamental knowledge will complement the existing body of work on this species with respect to oilseed production.

#### Identification of Genes that Control Biomass Production Using Rice

- Jan Leach, Colorado State University, Fort Collins

**Goal:** Provide an integrated breeding and genomics platform to identify biomass traits in rice, for translation to second-generation bioenergy grasses such as switchgrass and *Miscanthus*.

#### A Universal Genome Array and Transcriptome Atlas for *Brachypodium distachyon*

- Todd Mockler, Oregon State University, Corvallis

**Goal:** Develop an Affymetrix genome tiling array based on the DOE Joint Genome Institute sequence of *Brachypodium distachyon*, and make the array available for broad community use. Investigators will use the array to generate an expression atlas representing major developmental stages or stress responses in *Brachypodium*, a model species for polyploid, perennial grasses with complex genomes, such as wheat and switchgrass.

#### Epigenomics of Development in *Populus*

- Steven Strauss, Oregon State University, Corvallis

**Goal:** Construct a study of the role of chromatin modification (epigenetics) in the regulation of development and dormancy induction in poplar and other woody species. The investigators will characterize changes in DNA methylation patterns on specific tissues during dormancy induction and poplar development.



Rice. (Courtesy USDA Agricultural Research Service)

## 2007 Awards

### Eleven Projects Selected for Awards Totaling \$8.3 Million

#### **Towards a Map of the *Populus* Biomass Protein-Protein Interaction Network**

- Eric Beers, Virginia Polytechnic and State University, Blacksburg

**Goal:** Map protein-protein interactions relevant to biomass production by focusing on proteins coexpressed in poplar xylem, site of the majority of lignocellulose synthesis and hence biomass accumulation in poplar.

#### **Strategies for Using Molecular Markers to Simultaneously Improve Corn Grain Yield and Stover Quality for Ethanol Production**

- Rex Bernardo, University of Minnesota, Minneapolis-St. Paul

**Goal:** Optimize the use of DNA markers to simultaneously breed for high corn grain yield (for nonenergy and energy uses) and high stover quality for ethanol production.

#### **Developing Association Mapping in Polyploid Perennial Biofuel Grasses**

- Ed Buckler, USDA Agricultural Research Service, Ithaca, NY

**Goal:** Undertake an association-mapping study of two important biofuel grasses, switchgrass and reed canarygrass, to identify molecular markers tightly linked to biomass-related trait loci. This will enable marker-assisted selection and greatly accelerate breeding programs for enhanced biomass production.

#### **Resource Development in Switchgrass, an Important Bioenergy Crop for the U.S.A.**

- Katrien Devos, University of Georgia, Athens

**Goal:** Construct a detailed genetic map of switchgrass based on simple sequence repeats and align it with maps produced in rice, maize, and *Sorghum*. This comparison will allow the exploitation of resources and sequence information generated for these well-studied cereals. The genetic maps also will serve as a framework for locating genes that control bioenergy traits.

#### **Development of Genomic Tools to Improve Prairie Cordgrass (*Spartina pectinata*), a Highly Productive Bioenergy Feedstock Crop**

- Jose Gonzalez, South Dakota State University, Brookings

**Goal:** Develop PCR markers for this species and construct an initial linkage map for prairie cordgrass, a native perennial high-biomass yielding grass.

#### **Analysis of Small RNAs and mRNAs Associated with Abiotic Stress Responses in *Brachypodium distachyon***

- Pam Green, University of Delaware, Newark

**Goal:** Identify small RNAs related to stresses such as drought, temperature, and nutrient deprivation and relate them to the emerging genome sequence of *Brachypodium distachyon*, thus enhancing its value as a functional genomic model for energy crops and temperate grasses.

#### **Identification of Cell Wall Synthesis Regulatory Genes Controlling Biomass Characteristics and Yield in Rice (*Oryza sativa*)**

- Zhaohua Peng, Mississippi State University, Mississippi State

**Goal:** Examine cell wall synthesis in rice, a model grass bioenergy species and the source of rice stover residues, using reverse genetic and functional genomic and proteomic approaches.

#### **Linkage Analysis Appropriate for Comparative Genome Analysis and Trait Selection in Switchgrass**

- Christian Tobias, USDA Agricultural Research Service, Albany, CA

**Goal:** Create a comprehensive marker set for switchgrass based principally on simple sequence repeats and initiate development of a linkage map.

#### **A Functional Genomics Approach to Altering Crown Architecture in *Populus*: Maximizing Carbon Capture in Trees Grown in Dense Plantings**

- Jerry Tuskan, Oak Ridge National Laboratory, Oak Ridge, TN

**Goal:** Gain a molecular understanding of phytochrome-mediated responses to competition in *Populus* and then use that knowledge to maximize carbon capture per unit of land area for increased biomass production.

#### **Development of Brown Midrib Sweet *Sorghum* as a Dual-Source Feedstock for Ethanol Production**

- Wilfred Vermerris, University of Florida, Gainesville

**Goal:** Maximize the amount of fermentable sugar in the whole *Sorghum* plant by identifying and isolating genes that control the high stalk juice sugar trait and a decreased stalk lignin trait, with the aim of eventually combining both traits in a single germplasm.

#### **Insertional Mutagenesis of *Brachypodium distachyon***

- John Vogel, USDA Agricultural Research Service, Albany, CA

**Goal:** Create a collection of insertional mutants in *Brachypodium distachyon*. This resource collection then can be used to identify mutations in genes predicted to affect biomass quality and agronomic characteristics of other perennial grass energy crops.



*Brachypodium distachyon*. (Courtesy Oregon State University)



## 2006 Awards

### Nine Projects Selected for Awards Totaling \$5.7 Million

#### Using Association Mapping to Identify Markers for Cell Wall Constituents and Biomass Yield in Alfalfa

- Charles Brummer, University of Georgia, Athens

**Goal:** Use genomics approaches to identify chromosomal regions and, ultimately, genes controlling the two most important bioenergy traits, biomass yield and composition, and develop genetic markers that can be used directly in applied plant breeding programs to improve the bioenergy qualities of alfalfa.

#### Manipulation of Lignin Biosynthesis to Maximize Ethanol Production from *Populus* Feedstocks

- Clint Chapple, Purdue University, West Lafayette, IN

**Goal:** Generate transgenic poplar up- or down-regulated for four enzymes known to impact lignin quantity and quality; develop metabolic profiling methods for poplar and their application to greenhouse- and field-grown wild-type and transgenic plants; conduct morphometric analysis of transgenic lines grown in field plots; and analyze cell wall deconstruction for wild-type and lignin-modified transgenic lines.

#### Genomic Knowledgebase for Facilitating the Use of Woody Biomass for Fuel Ethanol Production

- Vincent Chiang, North Carolina State University, Raleigh

**Goal:** Establish a knowledgebase about the possible genes and transcription factor genes involved in lignocellulosic formation and those genes that may enable effective manipulation of lignocellulosic traits to facilitate ethanol production.

#### Systematic Modification of Monolignol Pathway Gene Expression for Improved Lignocellulose Utilization

- Richard Dixon, The Samuel Roberts Noble Foundation, Ardmore, OK

**Goal:** Determine which features of the lignocellulosic material (lignin content, lignin composition, or other factors) are most detrimental to the fermentation of biomass to ethanol and develop the crop plant alfalfa (*Medicago sativa*) as a model system for genomic studies on biomass utilization.

#### Genetic Dissection of the Lignocellulosic Pathway of Wheat to Improve Biomass Quality of Grasses as a Feedstock for Biofuels

- Bikram Gill, Kansas State University, Manhattan

**Goal:** Investigate the expression of ~80 candidate genes for lignin biosynthesis, their enzymatic activities, and lignin content and composition in different organs at different stages of diploid wheat plant; silence these 80 genes individually by VIGS; identify knockout mutants of these genes using TILLING; and characterize the silenced tissues and knockout mutants by metabolite profiling.

#### Biochemical Genomics of Wood Formation: O-Acyltransferases for Alteration of Lignocellulosic Property and Enhancement of Carbon Deposition in Poplar

- Chang-Jun Liu, Brookhaven National Laboratory, Upton, NY

**Goal:** Identify genome-wide acyl-CoA dependent acyltransferase genes from poplar genomics database; systemically explore the tissue-specific and stress-responsible expression patterns of O-acyltransferase genes to identify the enzymes specifically involved in lignocellulosic biosynthesis; and systemically characterize the biochemical functions of acyltransferases responsible for polysaccharide acetylation, lignol biosynthesis, and phenolic compound modification.

#### Streamlined Method for Biomass Whole Cell Wall Structural Profiling

- John Ralph, USDA Agricultural Research Service, Madison, WI

**Goal:** Provide the plant cell wall and biomass research communities with improved methods for polysaccharide and lignin structural profiling, based on complete cell wall solubilization and NMR. The aim is to develop and streamline procedures to allow 20 to 30 samples per day to be profiled.

#### *Sorghum* Biomass/Feedstock Genomics Research for Bioenergy

- William Rooney, Texas A&M University, College Station

**Goal:** Annotate genes, pathways, and regulatory networks identified in the *Sorghum* genome sequence that are important for biomass generation and identify, map, and clarify the function of trait loci that modulate accumulation and quality of biomass in *Sorghum*.

#### Development of a Proteoglycan Chip for Plant Glycomics

- Chris Somerville, Carnegie Institute of Washington, Stanford, CA

**Goal:** Develop high-throughput methods and reagents that will facilitate the assignment of function to large numbers of glycosyltransferases and other glycan-modifying enzymes.

