Office of Science
Financial Assistance
Funding Opportunity Announcement
DE-PS02-07ER07-12

New Analytical and Imaging Technologies for Lignocellulosic Material Degradation, and for Multiplexed Screening for Plant Phenotypes

The Office of Biological and Environmental Research (BER) of the Office of Science (SC), U.S. Department of Energy (DOE), hereby announces its interest in receiving applications for research that supports the Genomics: GTL research program (www.genomicsGTL.energy.gov). In the first part of this Solicitation, applications are solicited for the development of technologies for studying lignocellulosic systems, real-time characterization of such systems in the course of processing, and other innovative techniques that could facilitate lignocellulosic material degradation. The second part of this Solicitation solicits the development of improved methods leading to high-throughput, sensitive, and selective phenotypic screening of plant feedstocks.

PREAPPLICATIONS

Potential applicants are **required** to submit a brief preapplication, referencing **Program Solicitation DE-PS02-07ER07-12 for receipt by DOE by 4:30 p.m., Eastern Time, January 4, 2007.** Preapplications will be reviewed for conformance with the guidelines presented in this Solicitation and suitability in the technical areas specified in this Solicitation. A response to the preapplications encouraging or discouraging formal applications will be communicated to the applicants by **January 12, 2007.** Applicants who have not received a response regarding the status of their preapplication by this date are responsible for contacting the program to confirm this status.

Only those preapplicants that receive notification from DOE encouraging a formal application may submit full applications. **No other formal applications will be considered.**

Potential applicants **must** submit a brief preapplication that consists of no more than three pages of narrative stating the research objectives, describing the technical approach(s), and identifying the proposed team members and their expertise. The intent in requesting a preapplication is to save the time and effort of applicants in preparing and submitting a formal project application that may be inappropriate for the program. Preapplications will be reviewed relative to the scope and research needs as outlined in the summary paragraph and in the SUPPLEMENTARY INFORMATION. The preapplication should identify, on the cover sheet, the title of the project, the institution or organization, principal investigator name, telephone number, fax number, and e-mail address. No budget information or biographical data need be included, nor is an institutional endorsement necessary.

Preapplications referencing the first aim in Program Solicitation DE-PS02-07ER07-12 should be sent as a text file without attachments or a single PDF file attachment via e-mail to: **genomicsGTL@science.doe.gov** with **"Preapplication DE-PS02-07ER07-12 Lignocellulose Lastname Institution"** as the subject. Preapplications referencing the second aim in Program Notice DE-PS02-07ER07-12 should be sent as a text file without attachments or a single PDF file attachment via e-mail to: **genomicsGTL@science.doe.gov** with **"Preapplication DE-PS02-07ER07-12 Phenotyping Lastname Institution"** as the subject. No FAX or mail submission of preapplications will be accepted.

APPLICATION DUE DATE: March 6, 2007, 8:00 pm, Eastern Time

Applications must be submitted using Grants.gov, the Funding Opportunity Announcement can be found using the CFDA Number, 81.049 or the Funding Opportunity Announcement number, DE-PS02-07ER07-12. Applicants must follow the instructions and use the forms provided on Grants.gov.

FOR FURTHER INFORMATION CONTACT:

Aim One: New Analytical and Imaging Technologies for Studying Lignocellulosic Material Degradation

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Aim Two: New Technologies for Multiplexed Screening for Plant Phenotypes

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SUPPLEMENTARY INFORMATION:

Introduction:

The conversion of lignocellulosic biomass to liquid fuels may have the promise to significantly substitute biofuels for petroleum on a domestic scale. Biofuels may successfully compete economically as well as improve energy security, reduce greenhouse gas emissions, improve balance of trade, provide jobs in rural America, and achieve other ancillary benefits. Although pilot scale biorefineries convert lignocellulosic material to ethanol today, the efficiencies are not yet as high as would be desirable for a major shift in energy use.

One of the pivotal issues for successful economic utilization of biomass for biofuels is developing an effective strategy to overcome the defenses that plants have evolved against the degradation of lignocellulose - the major component of plant cell walls. Plant cell walls are made up of celluloses, hemicelluloses, pectins, lignins, proteoglycans, and other organic molecules. Each plays a distinct role in both the structural integrity of the plant as well as in the recalcitrance of conversion and the ultimate energy content of the energy products.

Fundamental scientific understanding of the biology of cell wall degradation by bacteria, fungi, microbial communities, or symbiotic associations between microbes and animals, as well as processes that alter plant cell wall composition and morphology, will promote effective options to modify and/or decompose the complicated mixture of recalcitrant materials. This knowledge of the underlying mechanisms of cell wall deconstruction to sugars and other energy-containing substrates will enable development of conversion processes with increased yields and reaction rates and decreased creation and/or impact of inhibitory byproducts.

New and innovative applications of technologies are needed to understand the properties of these materials and to relate these properties to the behavior of the materials under a large variety of treatment conditions. The scientific community will require these technologies to develop new strategies for handling and pretreating the feedstock, and converting it into products suitable for fermentation or other downstream processes. Research using the new technologies will enable better understanding of how feedstock material is altered during different treatment options with consequent changes in the accessibility of the material to microbes or enzymes used for processing into fuels. For example, dehydrating biomass as it is stored may inhibit later saccharification. Certain chemical or heat pretreatments may cause the cellulose or lignin to re-anneal over the lignocellulosic material and reduce subsequent enzymatic access. A deeper understanding at a molecular scale of the external surface properties of the lignocellulosic material may enable better treatment options that facilitate hydration and enzymatic effectiveness.

Research Topics:

Research and technology development is invited that will result in new capabilities for characterizing the lignocellulosic material in plants, the changes undergone by lignocellulosic material during the stages of pretreatment, the effects of enzymes and chemical reagents that deconstruct the lignocellulosic material into saccharides and other products, and the cell wall degradative strategies of microbes, fungi, etc. Techniques for characterizing these materials either non-invasively or with minimal requirements for sampling are of particular interest. Techniques for characterizing and/or imaging treatment processes in real time are also of high interest.

Proposed research should seek resolution in space and time sufficient to understand the materials and processes under study at the molecular level. For instance, the morphological fate of the lignin or cellulose portions that are altered through the addition of heat, acid, base, or pressure may hinder further accessibility by downstream processes.

Development of computational techniques for analyzing data produced using these new technologies is also of interest. Applications may include objectives that focus on the computational tools needed to make optimal use of the new analytical technology that is the main focus of the proposed project. Computational research should support the proposed research into characterization and imaging technologies.

A comprehensive description of the major research and development needs for converting lignocellulosic materials to fuels is given in "Breaking the Biological Barriers to Cellulosic

Ethanol", http://genomicsgtl.energy.gov/biofuels/b2bworkshop.shtml. The Genomics: GTL Roadmap includes discussions of the basic research goals of the program, http://genomicsgtl.energy.gov/roadmap/index.shtml.

A. Analytical and Imaging Technologies for Lignocellulosic Material Degradation

Research funded through this component of the solicitation will support the development of technologies for studying lignocellulosic systems, real-time characterization of such systems in the course of processing, and other innovative techniques that could enable progress in the biomass research program described above.

Potential approaches include but are not limited to:

- Development of novel techniques for characterizing the degradation of plant cell walls on a cellular or a subcellular/molecular basis. These techniques could involve any part of the electromagnetic spectrum from ultrasound to x-rays and gamma rays or beams of particles such as neutrons or electrons.
- Development of novel techniques to visualize intracellular processes and/or to functionally characterize lignocellulosic materials in the course of processing using spectroscopic or electrochemical or other approaches.
- Visualization and functional characterization of microbial populations and communities with respect to their potential roles in the processing of lignocellulosic materials.
- Development of selective, non-perturbative probes to enable determination of the spatial and temporal concentration profiles of nutrients, metabolites, signaling molecules, extracellular matrices and other biomolecules during lignocellulosic degradation and associated supporting processes. Such research should enable, for example, dynamic measurements of chemical states and energy transfer kinetics. Probes and methodologies developed must be adaptable by, and available to, the wider scientific community.
- Development of innovative instrumentation that will visualize and quantitate dynamic aspects of lignocellulosic degradation over a wide range of dimensions and time scales; enable simultaneous co-localization of different intra-cellular processes with high spatial resolution; and/or permit visualization of bacterial community composition and functions in the laboratory.
- Development of computational methods may be proposed as a component of technological research projects (such as the examples above) for rapidly processing, storing, reconstructing, and modeling of large data sets obtained from the analysis of the degradative processing of lignocellulosic materials by imaging or other technologies. Computational systems are needed to integrate data sets derived from different instruments and technologies.

B. Improved technologies for multiplexed screening for plant phenotypes

Another prerequisite for a competitive biofuel industry is the development of plant feedstocks that exhibit both desirable cell-wall traits and high biomass productivity under sustainable low-input conditions. Full leveraging of plant genome sequence information against advances in conventional breeding requires robust functional genomic tools and high throughput phenotyping

platforms for identification and characterization of improved plant feedstock species. Such optimization might include decreasing the lignin content or changing the lignin cross-linked structure in plant feedstocks, to facilitate access of degradative enzymes to cellulosic substrates. Similarly, improved microscreens or high-throughput screens for specific alterations in defined cellular targets or molecular metabolites may result in more rapid identification of potentially interesting plant mutants exhibiting desired changes in metabolic reactions resulting from introduced genetic modifications. This information may contribute to testable models of the genes and regulatory mechanisms that control plant growth, increase net photosynthetic CO2 fixation and direct carbon flux to cell wall polysaccharides and storage polymers. The resulting fundamental knowledge will lead to the accelerated development of a suite of new crops and new varieties of existing crops specifically bred for biofuels and adapted to a range of different soil types and climatic conditions.

In this second component of this solicitation, applications are sought for the development of improved methods leading to high-throughput, sensitive, and selective phenotypic screening of plant feedstocks. These screening methodologies will be used to identify quantitative and qualitative differences in plant cell wall phenotypes of major cell wall biopolymers (lignin, cellulose, hemicellulose, and others), net photosynthetic efficiency and CO2 fixation, and morphometric traits. Applicants must describe how their proposed method will result in a significant improvement with respect to accuracy, dynamic range, or throughput over existing phenotyping methods. The proposed method must permit recovery of the individual from the screening population subsequent to analysis, either through low materials-input requirements that do not consume the entire test sample or using nondestructive sampling techniques.

Examples of current analytical technologies that may be optimized for high throughput screening platforms include:

- spectroscopic methods for cell wall compositional and structural characterization (e.g, birefringence, NIR reflectance, analytical pyrolysis, FTIR, Raman, MALDI-TOF MS)
- methods for plant metabolic fingerprinting and profiling
- microanalytical methods for single cell (or small groups of cells) sampling.
- fluorescence-based methods for net photosynthetic efficiency and CO2 assimilation
- innovative use of promoter reporter-gene fusions (GFP, GUS) to interrogate plant metabolic status with respect to spatial and temporal expression patterns.
- Microscopies and imaging methods for morphometric trait analysis

Data and results that are generated through these investigations that are appropriate to share with the broader community should be provided in timely, open, and machine-readable format where possible. Funded investigators are expected to contribute to and participate with the GTL working group on data management, and to adhere to the group's consensus on data sharing.

The Genomics:GTL program supports a combination of large, well integrated, multidisciplinary research teams and smaller, focused research projects. This solicitation will support smaller, focused research projects to develop new technologies, research strategies, or research resources needed by the Genomics:GTL program.

Information on the research projects currently funded by the Genomics: GTL program and a description of project goals and overall program organization can be found at: http://genomicsgtl.energy.gov/).

Posted on the Office of Science Grants and Contracts Web Site
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