

Department of Energy's Joint BioEnergy Institute is supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research, through Contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the US Department of Energy.

## Executive Summary

This Monthly Progress Report provides a summary of JBEI's activities for the month of November 2014, second month of fiscal year 2015:

Item	Count	Highlights
Publications in Print	3	"Theory, practice and prospects of X-ray and neutron scattering for lignocellulosic biomass characterization: towards understanding biomass pretreatment" "Improving Microbial Biogasoline Production in Escherichia coli Using Tolerance Engineering"
Publications in Press	6	"Expression of a bacterial 3-dehydroshikimate dehydratase reduces lignin content and improves biomass saccharification efficiency"
Media Citations	8	One story released: "Sweet Smell of Success: JBEI Researchers Boost Methyl Ketone Production in E. coli".
Invention Disclosures	3	"Metal containing ionic liquids and their applications in lignocellulosic biomass conversion to fuels and chemicals"
Presentations Given	11	Presentations were given at the "Synbio Conference" in Berkeley, CA and "AIChE Annual Meeting" in Atlanta, GA.
On-Site Seminars	8	Gregg Whited, Fellow, Dupont.
Tours & Visits	7	ABLC Next 2014 and GLBRC.
Industry Interactions	6	New interactions with SINOPEC Shanghai Research Institute of Petrochemical Technology and GLBRC.
FY15 Milestones		On track. Two milestones, despite being currently at 0%, are expected meet their deadlines: Feedstocks - "Heterologous screening of transferases to promote in planta design of polysaccharides (SB)" Fuels Synthesis - "S. cerevisiae: Consolidate xylose utilizing production strains with production pathways"

Publications
In Print
Cheng, G., Zhang, X., Simmons, B., & Singh, S. (2014). "Theory, practice and prospects of X-ray and neutron scattering for lignocellulosic biomass characterization: towards understanding biomass pretreatment". [10.1039/C4EE03147D]. <i>Energy Environ. Sci.</i> , doi, 10.1039/c4ee03147d <a href="http://dx.doi.org/10.1039/C4EE03147D">http://dx.doi.org/10.1039/C4EE03147D</a>
Foo, J. L., Jensen, H. M., Dahl, R. H., George, K., Keasling, J. D., Lee, T. S., Leong, S., & Mukhopadhyay, A. (2014). "Improving Microbial Biogasoline Production in Escherichia coli Using Tolerance Engineering". <i>MBio</i> , <b>5</b> (6), e01932-01914-e01932-01914. doi, 10.1128/mBio.01932-14 <a href="http://www.ncbi.nlm.nih.gov/pubmed/25370492">http://www.ncbi.nlm.nih.gov/pubmed/25370492</a>
McAndrew, R., Pruitt, R. N., Kamita, S. G., Pereira, J. H., Majumdar, D., Hammock, B. D., Adams, P. D., & Ronald, P. C. (2014). "Structure of the OsSERK2 leucine-rich repeat extracellular domain". <i>Acta Crystallogr D Biol Crystallogr</i> , <b>70</b> (Pt 11), 3080-3086. doi, 10.1107/S1399004714021178 <a href="http://www.ncbi.nlm.nih.gov/pubmed/25372696">http://www.ncbi.nlm.nih.gov/pubmed/25372696</a> <a href="http://journals.iucr.org/d/issues/2014/11/00/kw5096/kw5096.pdf">http://journals.iucr.org/d/issues/2014/11/00/kw5096/kw5096.pdf</a>
In Press
Alonso-Gutierrez, J., Kim, E.M., Batth, T. S., Cho, N., Hu, Q., Chan, L. J. G., Petzold, C. J., Hillson, N. J., Adams, P. D., Keasling, J. D., Garcia-Martin, H., Lee, T.S. (2015). "Principal component analysis of proteomics (PCAP) as a tool to direct metabolic engineering". <i>Metabol. Eng.</i>
George, K. W., Alonso-Gutierrez, J., Keasling, J. D., Lee, T. S. (2015). "Isoprenoid drugs, biofuels and chemicals - artemisinin, farnesene and beyond (review)". <i>Adv Biochem Eng Biotechnol.</i>
Gondolf, V. M., Stoppel R., Ebert, B., Rautengarten, C., Liwanag, A. J. M., Loqué, D., Scheller, H. V. (2014). "A gene stacking approach leads to engineered plants with highly increased galactan levels in Arabidopsis". <i>BMC Plant Biology</i> .
Eudes, A., Noppadon, S., Baidoo, E., George, A.; Liang, Y.; Yang, F.; Singh, S.; Keasling, J. D.; Simmons, B. A.; Loque, D. (2014). "Expression of a bacterial 3-dehydroshikimate dehydratase reduces lignin content and improves biomass saccharification efficiency". <i>Plant Biotechnology Journal</i> .
Furtado, A. L., Jason; Hoang, N. V.; Healey, A.; Singh, S.; Simmons, B. A.; Henry, R. (2014). "Modifying plants for biofuel and biomaterial production". <i>Plant Biotechnology Journal</i> .
Woo, H. L., Ballor, N., Hazen, T.C., Fortney, J. L., Simmons, B. A., Davenport, K. W., Goodwin, L., Ivanova, N., Kyrpides, N., Mavromatis, K., Woyke, T., Jansson, J., Kimbrel, J., and DeAngelis, K. (2014). "Complete genome sequence of the lignin-degrading bacterium <i>Klebsiella</i> sp. strain BRL6-2". <i>Standards in Genomic Sciences</i>

News Citations		
Date	Article	Source
Nov 5	Tolerance engineering boosts production of biogasoline in bacteria	mBiosphere
Nov 5	Engineered for Tolerance, Bacteria Pump Out Higher Quantity of Renewable Gasoline	Science Newsline Biology
Nov 5	Engineered for tolerance, bacteria pump out higher quantity of renewable gasoline	Science Daily
Nov 6	Bacteria Boosted to Produce More Isopentenol for Gasoline	Before it's news
Nov 6	Bacteria Boosted to Produce More Isopentenol for Gasoline	Newenergyandfuel.com
Nov 6	LBNL startup Afingen uses precision method to enhance plants	Ethanol Producer Magazine
Nov 7	Bioengineers Boost Ability of Bacteria to Produce Biofuels	AZoCleantech
Nov 9	Researchers Take Major Step To Effective 'Bio-gasoline'	Oil price

Inventions Disclosed	Inventor(s)
2015-016 - "Lignin for Photovoltaic (PV) Technologies"	Mark Allendorf, Parthasarathi Ramakrishnan, Blake A. Simmons and Seema Singh.
2015-027 - "Metal containing ionic liquids and their applications in lignocellulosic biomass conversion to fuels and chemicals"	Tanmoy Dutta, Parthasarathi Ramakrishnan, Jian Shi, Blake A. Simmons and Seema Singh.
2015-029 - "New method to depolymerize lignin using a chelator-mediated Fenton reaction"	Michael Kent.

Presentations				
Date	Division	Presenter(s)	Title	Presentation Location
Nov 4	Fuels Synthesis	Simirenko, L., Harmon-Smith, M., Visel, A., M. Rubin, E.M. and Hillson, N.J.	The Joint Genome Institute's Synthetic Biology Internal Review Process	Workshop on Research Agendas in the Societal Aspects of Synthetic Biology, Tempe, AZ
Nov 10-12	Deconstruction	Simmons, B.A.	Driving the Future: Advanced Biomass Conversion Technologies at JBEI	ABLC Next 2014, San Francisco, CA
Nov 10	Fuels Synthesis	Keasling, J.D.	Engineering Microbes for Chemicals and Fuels	Synbio Conference, Berkeley, CA
Nov 10-12	Feedstocks	Loque, D.	Gene Stacking Approach to Build and Control Metabolic Pathways in Plants	SynBio Conference, Berkeley, CA
Nov 10-12	Feedstocks	Hernandez-Garcia, C.M., Shih, P., Chiu, T.-Y., Vuu, K., Manalansan, B., Ayad, L., Loque, D.	Developing Synthetic Biology Tools to Improve Nutrient Acquisition of Energy Crops	SynBio Conference, Berkeley, CA
Nov 16-21	Deconstruction	Konda, N.V.S.N., Simmons, B.A., and Klein-Marcuschamer, D.	Economics of a Macroalgae Biorefinery: (How) Can It be Viable?	AIChE Annual Meeting, Atlanta, GA
Nov 16-21	Deconstruction	Konda, N.V.S.N.	Towards a Sustainable Energy Future: MODEST – Model aided Optimization and Design of Energy Systems' Toolkit	AIChE Annual Meeting, Atlanta, GA
Nov 16-21	Deconstruction	Nemser, S., Campos, D., Campos, P.R., Bowser, J.,	Perfluorinated Membranes for the Dehydration of Ionic Liquids for	AIChE Annual Meeting, Atlanta, GA

		Majumdar, S., Rogers, R.D., Gurau, G., Simmons, B.A., and Singh, S.	Processing Biomass	
Nov 16-21	Deconstruction	Shi, J., Pattathil, S., Dutta, T., Venkatachalam, S., Hahn, M.G., Simmons, B.A., and Singh, S.	Unveiling the Ionic Liquid Deconstruction of Lignocellulosic Biomass Using GlycomeProfiling	AIChE Annual Meeting, Atlanta, GA
Nov 24	Fuels Synthesis	Keasling, J.D.	Advanced Plants to Advanced Fuels	Synbio Conference, Berkeley, CA
Nov 24	Fuels Synthesis	Keasling, J.D.	Engineering Microbes for Chemicals and Fuels	Mexican Petroleum Institute, Mexico, D.F., Mexico

Onsite Seminars				
Date	Type	Presenter	Title	Affiliation
Nov 5	General Seminar Synberc Guest	Gregg Whited	Large Scale Industrial Biotechnology at Dupont	Dupont
Nov 6	General JBEI Seminar	Murthy Konda	The 16 Billion Gallon Puzzle: A Techno-economic perspective on the cellulosic bio-refineries	Deconstruction Division
Nov 12	General Seminar Synberc Guest	Brandon Chen	Science and Business – A Tale of Two Perspectives	Genomatica
Nov 12	Group Seminar	Dan Arlow	Single-molecule DNA construction: towards de novo synthesis of plasmid-sized DNA	Fuels Synthesis Division
Nov 12	Group Seminar	Charles Denby	Incorporating an ATP: Citrate lyase-mediated metabolic cycle into <i>Saccharomyces cerevisiae</i> for efficient conversion of fermentable sugars to Ac-CoA-derived products	Fuels Synthesis Division
Nov 19	Group Seminar	Rachel Li	Terpenoid production and terpene synthase characterization in <i>S.</i> <i>cerevisiae</i>	Fuels Synthesis Division
Nov 19	Group Seminar	Jacquelyn Blake-Hedges		Fuels Synthesis Division
Nov 19	General Seminar Jay Keasling Guest	Prof. Aurore Richel	Biorefining in Wallonia: towards a regional strategy for a biobased economy	University of Liege - Gembloux Agro-Bio Tech Unit of Biological & Industrial Chemistry

Tours and VIP Visits at JBEI		
Date	Company	Attendees
Nov 3	SINOPEC Shanghai Research Institute	Messrs. Ruan, Yuan, Teng and Dr. Frank Chen
Nov 11	Agilent Technologies	Dr. Steven Mueller, Steve Royce
Nov 12	ABLC Next 2014	ADM, Air and Specialty Sheet Metal, Air Liquide, ALS Bioenergy, Biorefining Conversions Network, University of Alberta, Cadev, Ceres Inc., Colorado State University, Deinove, Dodds & Associates LLC, DSM, DuPont, GLBRC, Government of Alberta – Canada, Great Falls Montana Development Authority, HKS Energy Consulting/Sveaskog, Husch Blackwell LLP, Industrial Microbes, InVironmental Integrity, Inc., Kincannon & Reed, Muradel, NextGen Climate America, NREL, Rentech, Roeslein Alternative Energy, Sapphire Energy, Inc., Sustainable Conversion Ventures, Sylvatex, Inc., The Linde Group, Treasure Coast Research Park, U.California, U.S. Dept of Commerce, USDA Rural Development, USDA/Rural Development, Verdezyne, Vistaara Marketing, Westar Trade Resources, Wm. Wrigley Jr. Company
Nov 13	University of Wisconsin - Madison, Great Lakes Bioenergy Research Center	Tina Nielsen, GLBRC, Jennifer Gotwald, Wisconsin Alumni Research Foundation
Nov 13	Invest in Champagne Ardenne, Invest in France	Franck Mode and Morgan Jacquat
Nov 14	Stanford University	Ivy Clift
Nov 17	Pfizer-Rinat	Arvind Rajpal

Industry Interactions	
Date	Company / Contact
Nov 1	SINOPEC Shanghai Research Institute of Petrochemical Technology / Dr. Weiming Yang, Vice President and Chief Engineer
Nov 11	Agilent / Dr. Steffen Mueller and Steve Royce
Nov 13	ALS Bio / Ariel Scaparro and Expedito Parente
Nov 14	University of Wisconsin-Madison - Great Lakes Bioenergy Research Center (GLBRC) / Tina Nielsen and Jennifer Gotwald
Nov 17	Pfizer / Arvind Rajpal
Nov 18	Futuragene / Dan Siegel



<b>Milestones</b>			
<b>Feedstocks Division</b>			
<b>Area Initiatives</b>	<b>Milestone/Deliverables</b>	<b>Due</b>	<b>% Complete</b>
Plant Cell Wall Biosynthesis and Its Regulation	Novel acetyl and feruloyltransferase activities characterized (CWB)	Sept. 2015	18
	Genetic characterization of mutant candidates from rice stem saccharification screen (GG)	Sept. 2015	16
	Correlation between cell wall modification and environmental interactions investigated (GG)	Sept. 2015	16
	Characterization of novel nucleotide sugar transporters (SB)	Sept. 2015	15
	Characterization of novel GT activities (SB)	Sept. 2015	20
Lignin and Hemicellulose Modification for Fuels Production	Evaluation of engineered plants with new lignin traits (including analysis with the Deconstruction and Technology Divisions) (CWE)	Sept. 2015	20
	Transgenic plants with dominant repressors of hemicellulose biosynthesis characterized (CWB)	Sept. 2015	17
	Nucleotide sugar transporters used for cell wall engineering (CWB)	Sept. 2015	18
	Optimization of translation control technologies to improve tissue-specific expression (CWE)	Sept. 2015	23
	Analysis of stacked traits in Arabidopsis (CWE)	Sept. 2015	12
	Heterologous screening of transferases to promote in planta design of polysaccharides (SB).	Sept. 2015	0

<b>Milestones</b>			
Feedstocks Division (continued)			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Engineering of Modifications in Different Plant Species	Cell wall related genes in switchgrass functionally annotated, integrated into phylogenomics databases, and integrated with Kbase (GG)	Sept. 2015	16
	Resequencing of Kitaake mutants (with JGI) and a database set up for hi throughput in silico screening (GG).	Sept. 2015	16
	Translation of best technologies developed in Arabidopsis to other plant species (CWE)	Sept. 2015	18

Milestones			
Deconstruction Division			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Biomass Pretreatment	Predict and develop task specific ILs for selective lignin or cellulose dissolution and low temperature pretreatment	May 2015	25
	Complete characterization of lignin streams from various IL pretreatment process configurations	July 2015	15
	Design, synthesis and assessment of lignin and hemicellulose derived IL mixtures for mixed and engineered feedstock pretreatment	Aug. 2015	20
	Use multi-scale simulations strategies (from first principle quantum mechanics to force field based) to understand interactions between biomass derived ILs and lignocellulosic biomass	Sept. 2015	15
Enzyme Optimization	Develop an assay for screening lignin degrading enzymes on insoluble lignin and begin populating a lignin degrading enzyme database (LigDB).	Feb. 2015	45
	Expand our database of ionic liquid tolerant enzymes, particularly to include enzymes from the GH3, GH5, GH6, GH9, GH10, AA9 and AA10 families.	May 2015	25
	Improve the overall sugar yields of our existing thermo and ionic liquid tolerant multi-component enzyme mixture by including enzymes for hydrolysis of both cellulose and hemicellulose and by including LPMOs.	July 2015	18
	Demonstrate using directed evolution to engineer enhanced stability and activity of a $\beta$ -glucosidase in up to 20% [C2mim][OAc].	Sept. 2015	15

Milestones			
Deconstruction Division (continued)			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Microbial Communities	Complete characterization of active component of JTherm	March 2015	40
	Establish complementary thermophilic cellulase cocktail focusing on crystalline substrates	July 2015	25
	Identify five bacterial strains capable of metabolizing mixtures of aromatics from pretreatment-derived lignin streams	Sept. 2015	20
Fungal Biotechnology	Design new expression systems based on recent “omics” discoveries in regard to promoters and assess heterologous GH expression	Feb. 2015	40
	Initiate ChIP-Seq analysis of histone modifications in <i>A. niger</i> aimed at identifying epigenetic determinants of high productivity	April 2015	30
	Optimize cultivation conditions for heterologous enzyme production, including temperature and pH, and initiate ‘omic analysis of optimal vs. baseline conditions	July 2015	25
	Validate candidate genes for hyperproduction phenotypes through gene deletion and/or overexpression	Sept. 2015	25

Milestones			
Fuels Synthesis Division			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Discovery of Novel Hydrocarbon Biochemistries	In vitro, anaerobic testing of toluene biosynthesis candidates	Sept. 2015	16
	In vitro, anaerobic testing of ladderane biosynthesis candidates	Sept. 2015	16
Optimization of Hydrocarbon Biosynthetic Pathways	Improve methyl ketone production in E. coli with NIMS-aided HT screening and metabolic modeling	Sept. 2015	16
	E. coli pathway engineering for isopentenol production both in aerobic and anaerobic conditions	Sept. 2015	17
	Genes encoding isoprenoid pathway integrated into E. coli chromosome	Sept. 2015	17
	Three enzymes in S. cerevisiae glycolytic pathway replaced and impact on fatty acid-derived fuel tested	Sept. 2015	16
	Type 1 fatty acid synthases from several organisms expressed in E. coli and production of fatty acid-based fuels tested	March 2015	100
	Develop analytical method for LC-MS of MEV and DXP intermediates	April 2015	40
Host Engineering	S. cerevisiae: Consolidate xylose utilizing production strains with production pathways.	Sept. 2015	0
	E. coli membrane editing: With JGI, generate 10-20 single and double mutants and test improvement in expression of beneficial tolerance pump and corresponding tolerance to toxic fuels.	Sept. 2015	35

Milestones			
Fuels Synthesis Division (continued)			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Synthetic Biology Chance to Synbio Infor. But leave it here.	Codon optimization tool (GeneDesign) integrated into the DIVA platform.	Sept. 2015	16
	Clonal sequence validation (Sanger and/or MiSeq) pipeline implemented and integrated with DIVA/ICE	Sept. 2015	16
Quantitative Metabolic Modeling for Host and Pathway Engineering	Usage of EDD, MvT and 2S-13C MFA to improve production of fatty acids in <i>S. cerevisiae</i> .	Sept. 2015	40
	Perform flux analysis of <i>S. cerevisiae</i> $\Delta sip1$ strain	June 2015	70
	Implement interactive metabolic engineering on MvT	Sept. 2015	15

Milestones			
Technology Division			
Area Initiatives	Milestone/Deliverables	Due	% Complete
High Throughput Screening	Support the optimization of T and IL tolerant cellulose/hemicellulase cocktail using HT-NIMS	Sept. 2015	18
	Apply HT NIMS screen for fatty acids based fuel molecules (e.g. FAMK) in collaboration with the Fuels Division.	Sept. 2015	22
	Implement software to automate unit operations (e.g., droplet dispensing, routing, merger, and sorting) on an integrated droplet chip	Sept. 2015	16
	Demonstrate microfluidic droplet platform for integration of DNA assembly, transformation of cells, and culturing	July 2015	20
Proteomics	Develop rapid untargeted proteomics methods for relative quantification of >800 E. coli and S. cerevisiae proteins	Sept. 2014	15
	Develop absolute quantification targeted proteomic assays for 150 S. cerevisiae proteins	Sept. 2015	25
	Implement membrane proteomics assays to enable E. coli membrane editing with Fuels Synthesis Division	Sept. 2015	30

<b>Milestones</b>			
Technology Division (continued)			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Physical Characterization	SEM and TEM analysis of genetically altered feedstock biomass (including lignin and protein mapping) with Feedstocks and Deconstruction Divisions	Sept. 2015	17
	Electron tomographic analysis of plant cell walls of engineered feedstocks, and CAD model generation for mechanical properties simulation.	Sept. 2015	17
Structural Biology	Test expression of multiple GT clones in insect cell platform to produce quantities of protein for crystallization trials	Sept. 2015	35
	Characterize lignin degrading enzymes in collaboration with GLBRC	Sept. 2015	30
Informatics For dec rename Synbio infor. Reword this	Implemented an updated, refactored version of the EDD to support JBEI's future data needs	Sept. 2015	20
	Integrate EDD with data and workflows for characterizing biological parts, with Fuels Synthesis	Sept. 2015	10
	Integrate EDD with data and workflows for characterizing enzymes, with Deconstruction Division	Sept. 2015	15



Milestones			
Operations Management Division			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Intellectual Property Management	Generate >10 inventions	Sept. 2015	69
	Educate researchers about the tech transfer process	Sept. 2015	19
Licensing	Increase number of patent applications or inventions licensed or optioned and/or licensing agreements executed relative to the average of years 1 - 7.	Sept. 2015	18
Start-up Company Creation	Identify any IP or portfolios of IP that could lead to a start-up company and create an internal commercialization team to develop an initial go-to-market plan around that IP.	Sept. 2015	17
	Maintain regular JBEI on-site office hours for the Berkeley Lab entrepreneur adviser.	Sept. 2015	17
Business Development Outreach	Promote each JBEI invention to at least 700 contacts in the biofuels industry.	Sept. 2015	18
	Conduct meetings with at least fifteen companies each year, at least one-third of which are new to JBEI	Sept. 2015	19
Industry Advisory Committee	Hold an annual meeting of the IAC to inform JBEI research of industry concerns and technology bottlenecks.	Sept. 2015	10
Industry Partnership Program	Strengthen relationships with current partners and pursue collaborations with new industry partners in areas of complementary research.	Sept. 2015	18

<b>Milestones</b>			
Operations Management Division (continued)			
Area Initiatives	Milestone/Deliverables	Due	% Complete
Annual Scientific Advisory Meeting	Hold the annual meeting and report to DOE on the findings of the committee	Sept. 2015	17
Annual JBEI Conference	Annual JBEI Retreat	Sept. 2015	22
Human Resources	Staffing for year 8 complete	Sept. 2015	17