



Office of Biological and Environmental Research Biological Systems Science Division Update

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Environmental Research

April 21, 2022

Update on Programmatic Activities

Completed Reviews/Activities (all virtual)

- ✓ Annual BRC Reviews (Nov, Dec, Jan, Feb)
- ✓ JGI Triennial Review (Dec 8-10)
- ✓ KBase Review February 3-4
- ✓ GSP/BSP PI Meetings (Feb 28 – Mar 1)
- ✓ Early Career Panel reviews (Mar 16-17)
- ✓ Center for Structural and Molecular Biology-ORNL (Mar 22)
- ✓ ORNL Biofuels SFA (Mar 23)

Upcoming Reviews/Activities

- Interagency review of the Protein Data Bank (PDB) – (Apr 26)
- LBNL Integrated Diffraction Analysis Technologies (IDAT) - (Apr 28)
- Panel reviews for FOAs (May 16 - Jun 8) !!
- Bioenergy Research Center reviews – (May 23-26) *In Person meeting!*
- National Microbiome Data Collaborative (NMDC) – (Jun 29-30)
- Lab Bioimaging Call panel review – (week of Jul 4)
- Three SFA reviews ORNL, ANL and LBNL (later Jul and Aug) *On Site!*

New Opportunities

DEPARTMENT OF ENERGY (DOE)
OFFICE OF SCIENCE (SC)
BIOLOGICAL AND ENVIRONMENTAL RESEARCH (BER)

SYSTEMS BIOLOGY ENABLED MICROBIOME RESEARCH TO FACILITATE PREDICTIONS OF INTERACTIONS AND BEHAVIOR IN THE ENVIRONMENT

FUNDING OPPORTUNITY ANNOUNCEMENT (FOA) NUMBER:

DEPARTMENT OF ENERGY (DOE)
OFFICE OF SCIENCE (SC)
BIOLOGICAL AND ENVIRONMENTAL RESEARCH (BER)

BIOSYSTEMS DESIGN TO ENABLE SAFE PRODUCTION OF NEXT-GENERATION BIOFUELS, BIOPRODUCTS, AND BIOMATERIALS

FUNDING OPPORTUNITY ANNOUNCEMENT (FOA) NUMBER:

DEPARTMENT OF ENERGY (DOE)
OFFICE OF SCIENCE (SC)
BIOLOGICAL AND ENVIRONMENTAL RESEARCH (BER)

GENOMICS-ENABLED PLANT BIOLOGY FOR DETERMINATION OF GENE FUNCTION

FUNDING OPPORTUNITY ANNOUNCEMENT (FOA) NUMBER:
DE-FOA-0002601

FOA TYPE: INITIAL
CFDA NUMBER: 81.049

FOA Issue Date:	December 16, 2021
Submission Deadline for Pre-Applications:	January 14, 2022 at 5:00 PM Eastern Time A Pre-Application is required
Pre-Application Response Date:	January 28, 2022 at 5:00 PM Eastern Time
Submission Deadline for Applications:	March 25, 2022 at 11:59 PM Eastern Time

DEPARTMENT OF ENERGY (DOE)
OFFICE OF SCIENCE (SC)
BIOLOGICAL AND ENVIRONMENTAL RESEARCH (BER)

QUANTUM-ENABLED BIOIMAGING AND SENSING APPROACHES FOR BIOENERGY

FUNDING OPPORTUNITY ANNOUNCEMENT (FOA) NUMBER:
DE-FOA-0002603

FOA TYPE: INITIAL
CFDA NUMBER: 81.049

FOA Issue Date:	November 12, 2021
Submission Deadline for Pre-Applications:	January 14, 2022, at 5:00 PM Eastern Time A Pre-Application is required
Pre-Application Response Date:	February 3, 2022, at 5:00 PM Eastern Time
Submission Deadline for Applications:	April 4, 2022, at 11:59 PM Eastern Time

New Funding Opportunities (FOAs) in Genomic Science and Biomolecular Characterization and Imaging Science

- Microbiome Research
- Biosystems Design Research
- Plant Genomics Research
- Bioimaging Research (with QIS concepts)

New Lab Funding Opportunities in Biomolecular Characterization and Imaging Science

- Bioimaging Science

Department of Energy
 Washington, DC 20585
 Lab Funding Opportunity

PROGRAM ANNOUNCEMENT (PA) TO DOE NATIONAL LABORATORIES
U. S. Department of Energy
Office of Science
Biological and Environmental Research

Bioimaging Research and Approaches for Bioenergy and the Environment

Issue Date: 12/17/2021

Pre-Proposal Due Date: 02/11/2022 at 5 PM Eastern Time (A Pre-Proposal is required)

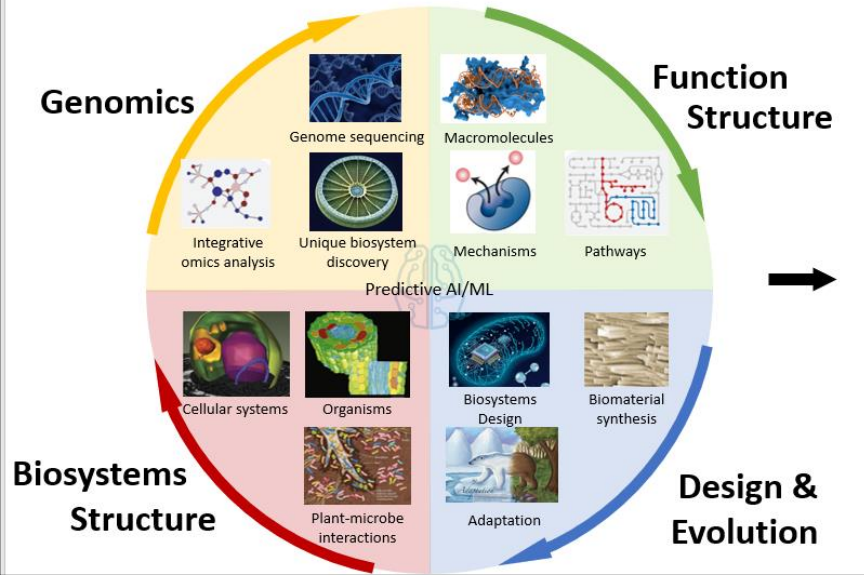
Encourage/Discontinue Date: 02/20/2022 at 5 PM Eastern Time

Proposal Due Date: 05/06/2022 at 11:59 PM Eastern Time

Technical/Scientific Program Contact:
 Dr. Paul Sammak
Paul.sammak@science.doe.gov

Future Planning: Workshops/Planning Activities

Structure Informed Discovery Cycle



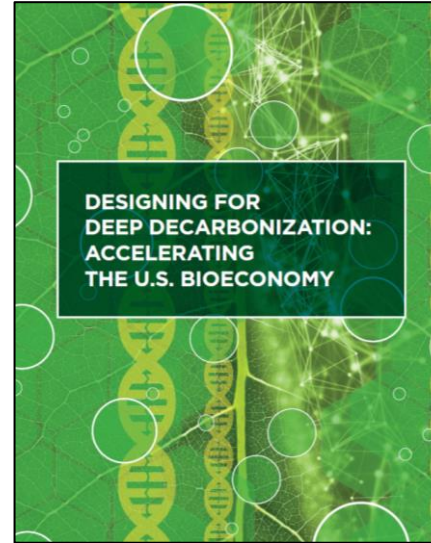
DOE Lab-led Workshop Series:

Genomes to Structure and Function

Oct 27-28, Dec 15-16, Jan 26-27 meetings

Combine unique capabilities in the BER
User facilities and Structural Biology
Resources

Joint workshops with EERE-BETO



April 30, 2021

Biotechnologies ripe for
transfer to industry:

- Transportation
- Industry
- Agriculture

***New Workshop for
FY 2022***

***Follow-up: Incorporate
automation with AI/ML
techniques into
experimentation and testing***

Chair and Co-Chairs

Huimin Zhao (UIUC)

Kristen Kleese van Dam (BNL)

James Schnable (UNL)

Nathan Hillson (LBNL- Agile Biofoundry)

Deepti Tanjore (LBNL ABPDU)

Towards a data science ecosystem for microbiome research



nmdc

National Microbiome Data Collaborative

Mission

Provide a gateway to FAIR multi-omics microbiome data leveraging best practices for data curation and processing

New Publications

2021 NMDC Annual Report

Nucleic Acids Research Eloe-Fadrosh et al., *Nucleic Acids Res* **50**, D828-D836 (2022). doi.org/10.1093/nar/gkab990

frontiers in Bioinformatics Hu et al., *Frontiers Bioinformatics* (2022). doi.org/10.3389/fbinf.2021.826370

NMDC Submission Portal: April release!

The screenshot shows the NMDC Submission Portal interface. At the top, there are navigation links: NMDC HOME, USER GUIDE, DATA SCHEMA, WORKFLOWS, and PAJAU YANGGAY. Below this is a header with tabs for Study Information, Multiomics Data, Environment Package, and Customize Metadata Export. A 'Choose spreadsheet file...' input field and a 'Jump to column...' dropdown are visible. The main area displays a table with columns for 'samp_id:default', 'MIXS:environment field', and 'emsl:default'. A tooltip is shown for the 'elevation' field, containing the following information:

- Label:** elevation
- Description:** Elevation of the sampling site is its height above a fixed reference point, most commonly the mean sea level. Elevation is mainly used when referring to points on the earth's surface, while altitude is used for points above the surface, such as an aircraft in flight or a spacecraft in orbit.
- Guidance:** Expected value: measurement value
- Examples:** 100 meter
- Null values:** default

Prototype of a coordinated metadata submission system

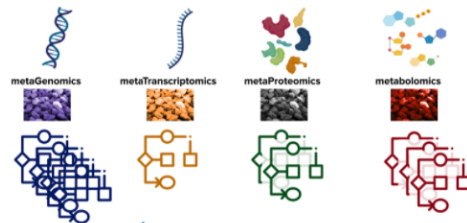
Support adoption of community-driven standards



NMDC EDGE: nmdc-edge.org

User Facility production bioinformatic pipelines are accessible to the research community

- All metagenomic workflows & NOM workflow installed
- New omics workflows in progress



NMDC Data Portal: data.microbiomedata.org

Faceted search on:

- sample metadata (EnvO, MixS)
- omics type
- study information
- functional annotations

The screenshot shows the NMDC Data Portal interface. At the top, there are navigation links: NMDC HOME, USER GUIDE, DATA SCHEMA, WORKFLOWS, and ORCID LOGIN. Below this is a search bar and a 'Found 654 results.' indicator. The main area displays a faceted search interface with a list of search criteria on the left and a bar chart on the right. The search criteria include: Study, PI Name, Function, KEGG Term, Sample, Depth, Collection date, Latitude, Longitude, Geographic Location Name, GOLD Ecosystems, GOLD classification, ENVO, and Broad-scale Environmental Context. The bar chart shows the distribution of results for 'Organic Matter' (996), 'Metagenome' (907), 'Metatranscriptome' (45), 'Metabolomics' (34), and 'Proteomics' (33). A map of the United States is also visible, showing the geographic distribution of samples. At the bottom, there is a timeline chart showing the number of samples from 2015 to 2020, and a legend for the omics types: MG (metagenomics), MT (metatranscriptomics), MP (metaproteomics), MB (metabolomics), and NM (natural organic matter).

Objective

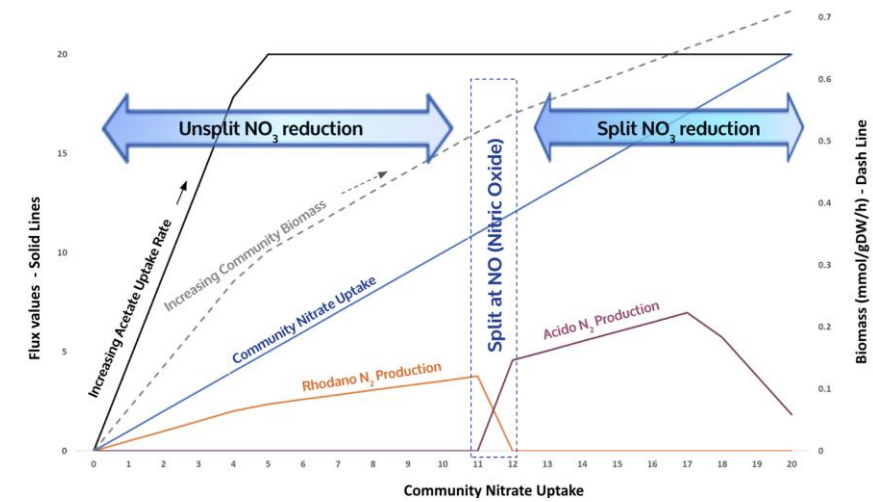
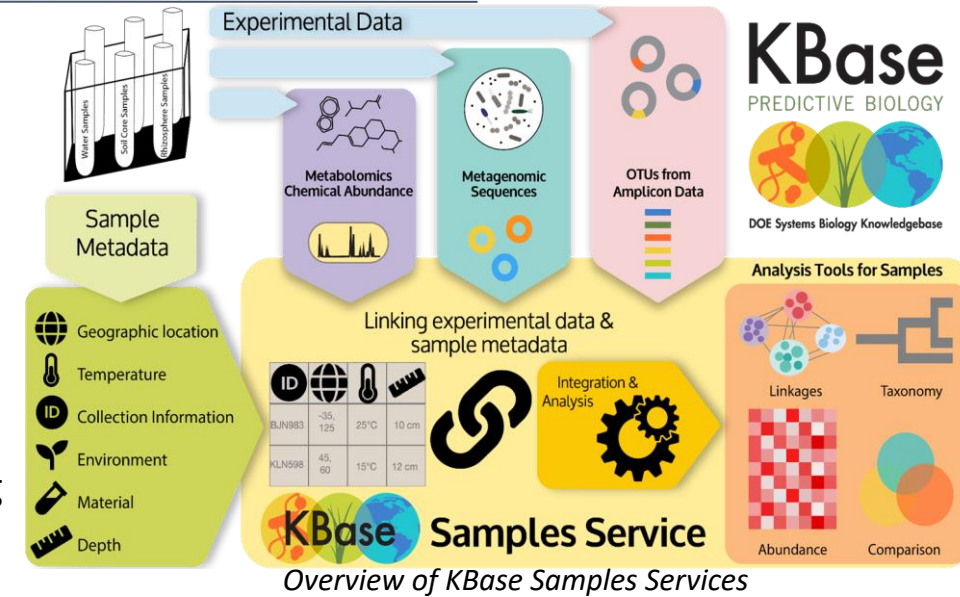
Create services for large-scale research projects to organize sample data and related measurements in KBase. Allows researchers to link amplicons, metagenomes, genomes, and chemical abundance data together and visualize sample summaries, map views and linked measurements.

Approach

Partnered with the ENIGMA SFA to design **Samples** services, then uploaded campaign-specific sample sets alongside validated and ontologically labeled sample metadata. Using Samples services, organized multiple collection campaigns from the ENIGMA project and analyzed the linked amplicons, metagenomes, and chemical abundances to better understand N cycling in subsurface communities.

Results/Impacts

- KBase was able to identify novel *Acidovorax* and *Rhodanobacter* genomes and model community interactions in nitrate reduction
- KBase can perform cross-correlation across samples; model relationships between biological factors, physical and geochemistry measurements; and draw inferences about relationship between biological factors and geochemistry.
- Researchers with diverse interests can utilize the same sets of consistently curated samples, build custom sample sets for analysis.



Analysis of *Rhodanobacter*/*Acidovorax* community interactions in nitrate reduction

Isolating Valuable Compounds from Complex Mixtures of Lignin Products

Objective

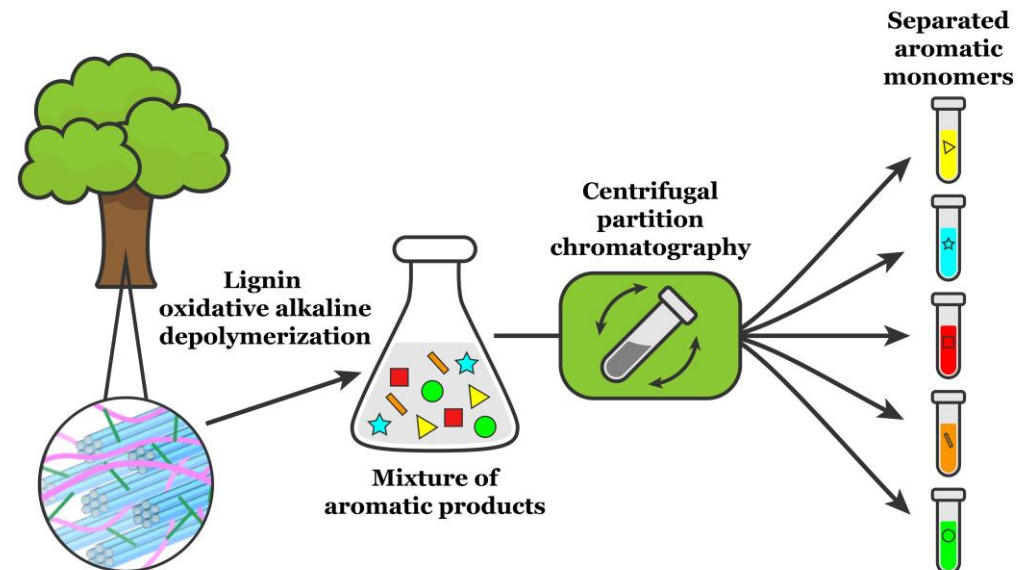
Isolate individual compounds from complex product mixtures that result from lignin processing

Approach

- Digest poplar lignin in the presence of oxygen to create a mixture of oxygenated aromatic compounds
- Apply centrifugal partition chromatography to separate individual monomers based on their solubility in two non-mixing solvents

Result/Impacts

- Centrifugal partition chromatography successfully isolated vanillin, syringic acid, syringaldehyde, vanillic acid, and *p*-hydroxybenzoic acid in two stages of extraction.
- The method requires only a few steps, uses no expensive added chemicals, and can be adapted to large-scale use, suggesting that it can provide a scalable way to isolate valuable industrial chemicals from lignin and other biomass-derived feedstocks.



Centrifugal partition chromatography provides an effective strategy to separate valuable aromatic products obtained from lignin depolymerization mixtures.

Alherech, M., Omolabake, S., Holland, C.M., Klinger, G.E., Hegg, E.L., Stahl, S.S., "From lignin to valuable aromatic chemicals: lignin depolymerization and monomer separation via centrifugal partition chromatography." *ACS Central Science* (2021) [DOI: [10.1021/acscentsci.1c00729](https://doi.org/10.1021/acscentsci.1c00729)]

Responsiveness of Miscanthus and Switchgrass Yields to Stand Age and Nitrogen Fertilization: A Meta-Regression Analysis

Objective

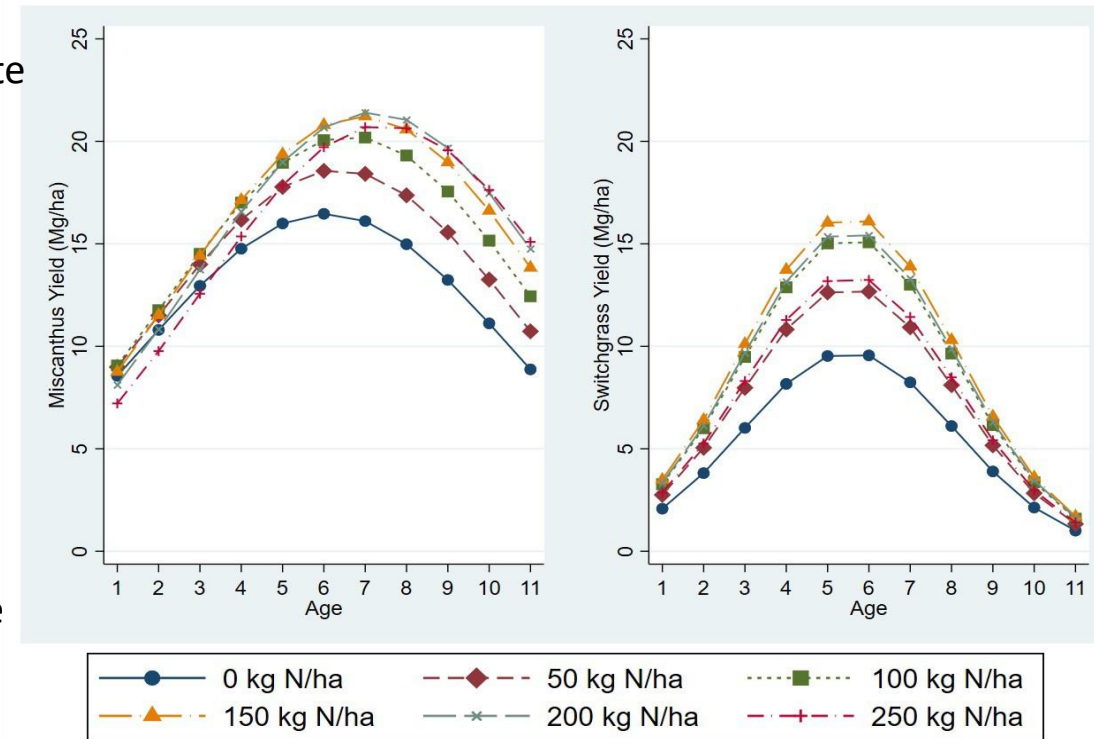
A multi-level-mixed-effects (MLME) meta-regression model is used for multivariate regression of yield response to N and stand age while controlling for climate, location, and other factors related to study design.

Approach

Applied an MLME meta-regression model to 1,403 and 2,811 yield observations for miscanthus and switchgrass, from experiments conducted between 2002 and 2019 across the U.S. rainfed region.

Results/Impacts

- An additional year of maturity increased miscanthus and switchgrass yield but at a decreasing rate. Yields peaked in the 7th and 6th years, respectively, for the observed range of applied N rates.
- Increasing N results in a small increase in yield but at a declining rate which varies with crop age.
- N-impact is largest on older miscanthus stands in contrast to middle-aged switchgrass



Mean predicted deterministic yield across maturity stages at various N-application levels for miscanthus (left) and switchgrass (right).

Provides a basis for developing N-application recommendations and optimal rotation age for miscanthus and switchgrass. Shows that growth of these crops on low-productivity land can equal that on high-productivity land.

Sharma et al. 2022. “Responsiveness of Miscanthus and Switchgrass Yields to Stand Age and Nitrogen Fertilization: A Meta-Regression Analysis.” *GCB Bioenergy*. DOI:10.1111/gcbb.12929.

*Lepidopteran mevalonate pathway optimization in *Escherichia coli* efficiently produces isoprenol analogs for next-generation biofuels*

Objective

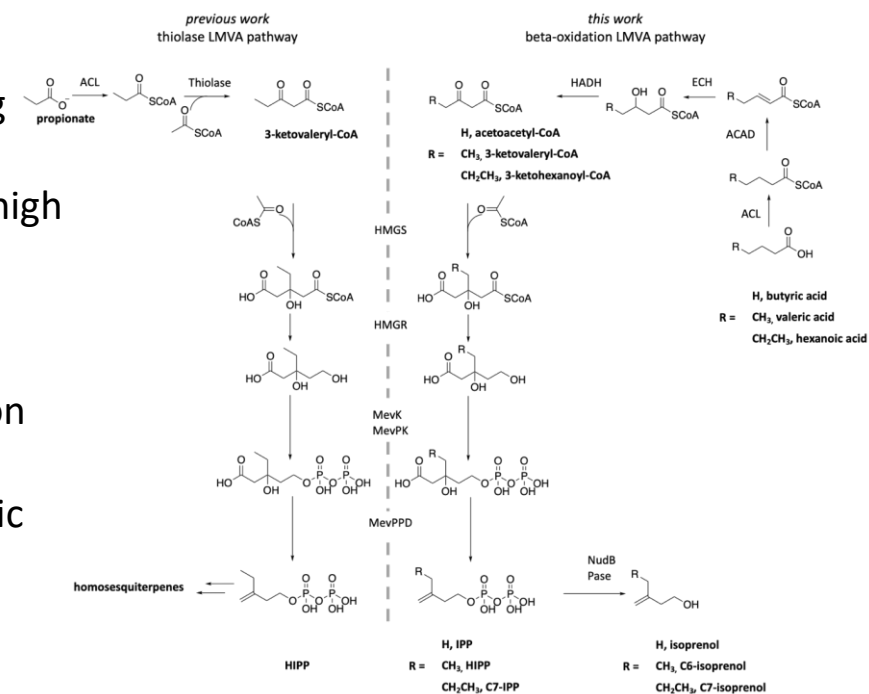
The lepidopteran mevalonate (LMVA) pathway produces six-carbon analogs of terpene building blocks. The hydrolyzed products of these building block analogs are potential next-generation biofuels. This study optimizes the lepidopteran mevalonate (LMVA) pathway in *E. coli* towards high productivity.

Approach

- The LMVA pathway was linked to NudB, a promiscuous phosphatase, to produce a six-carbon analog of isoprenol (C6-isoprenol) for GC analysis
- The LMVA pathway is redirected to start from β -oxidation (BOX) in *E. coli* to transform valeric acid into C6-isoprenol
- Substrate promiscuity of the BOX-LMVA was studied

Results/Impacts

- With NudB, the BOX-LMVA pathway transforms valeric acid into 390 mg/L C6-isoprenol
- The BOX-LMVA-NudB pathway also converts butanoic acid and hexanoic acid into isoprenol and C7-isoprenol, suggesting the BOX-LMVA pathway produces IPP and C7-IPP from the corresponding fatty acids
- The longer chain isoprenol analogs have lower water solubilities, similar or higher energy densities, and comparable research octane number (RON) boosting effects to isopentenols



The natural lepidopteran mevalonate (LMVA) pathway depends on a thiolase to produce the key intermediate, 3-ketovaleryl-CoA, while the pathway here employs beta-oxidation enzymes to afford this intermediate and the analogs thereof, to increase the productivity of the whole pathway.

Pang et al. (2021) *Metabolic Engineering*, doi:10.1016/j.ymben.2021.10.007

Alteration of *KNAT7* transcription factor expression in poplar changes cell wall characteristics and increases saccharification efficiency

Objective

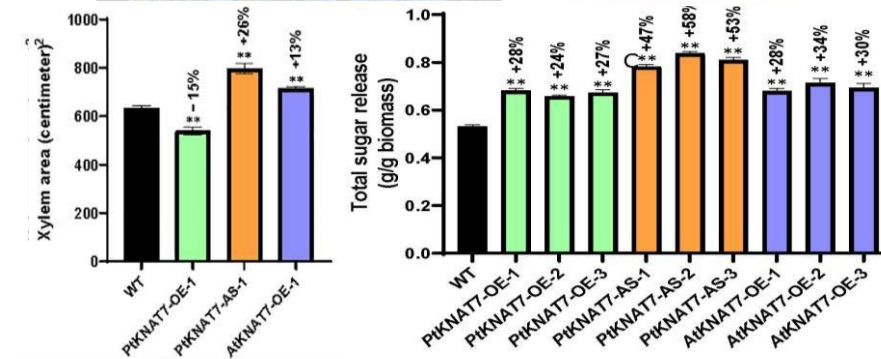
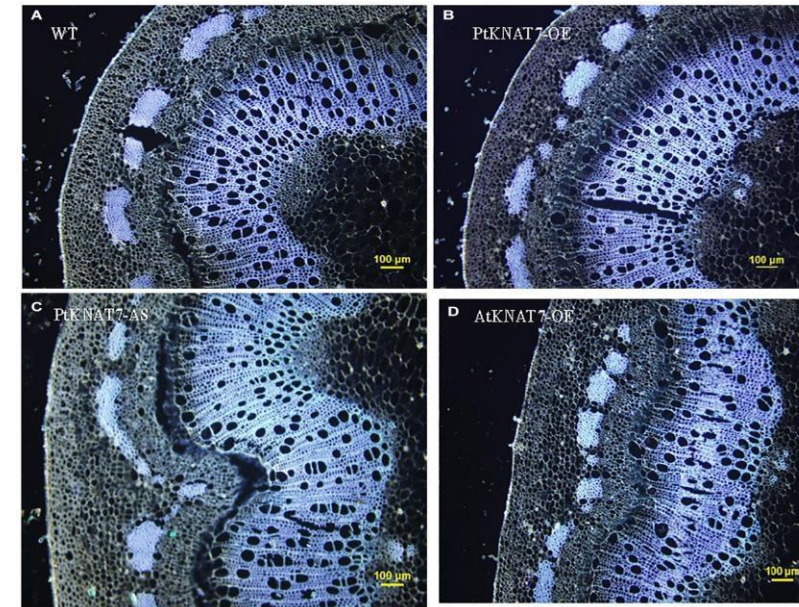
Expression of *KNAT7* may impact cell wall physiology and composition in poplar biomass, which may improve properties for production of bio-derived products

Approach

- Overexpression (OE) transgenic lines were generated using *Agrobacterium* in *Populus tremula* x *P. alba* 717-1 B4 to investigate role of *PtKNAT7* genes in (SCW)
- Heterologous overexpression of *AtKNAT7* in transgenic poplar was also studied
- Gene expression, microscopy, growth measurements, lignin analysis, and saccharification studies were used to monitor affects of *KNAT7* expression

Results/Impact

- Expression of *KNAT7* transgenes driven by developing xylem-specific promoter DX15; many downstream SCW biosynthesis genes highly expressed
- No changes in lignin content were observed in OE lines, although all transgenic lines had higher lignin S/G than WT.
- Saccharification efficiency was significantly higher in all *KNAT7* transgenics
- Modification of *KNAT7* expression represents a strategy to improve ethanol production through altered lignification and improved saccharification efficiency of poplar without sacrificing biomass growth and yield potential



Autofluorescence cross-section images show differences in xylem area and improved sugar release are observed in transgenic poplar in comparison to wild type (WT).

Ahlawat, Y. K. et al. "Genetic Modification of *KNAT7* Transcription Factor Expression Enhances Saccharification and Reduces Recalcitrance of Wood Biomass in Poplars". *Frontiers in Plant Science* 2021, 12:762067, doi:10.3389/fpls.2021.762067.

Carbon-negative bioproduction of industrial chemicals

Objective

Develop a novel carbon-negative route for production of commodity chemicals acetone and isopropanol, providing a blueprint for accelerated bioprocessing.

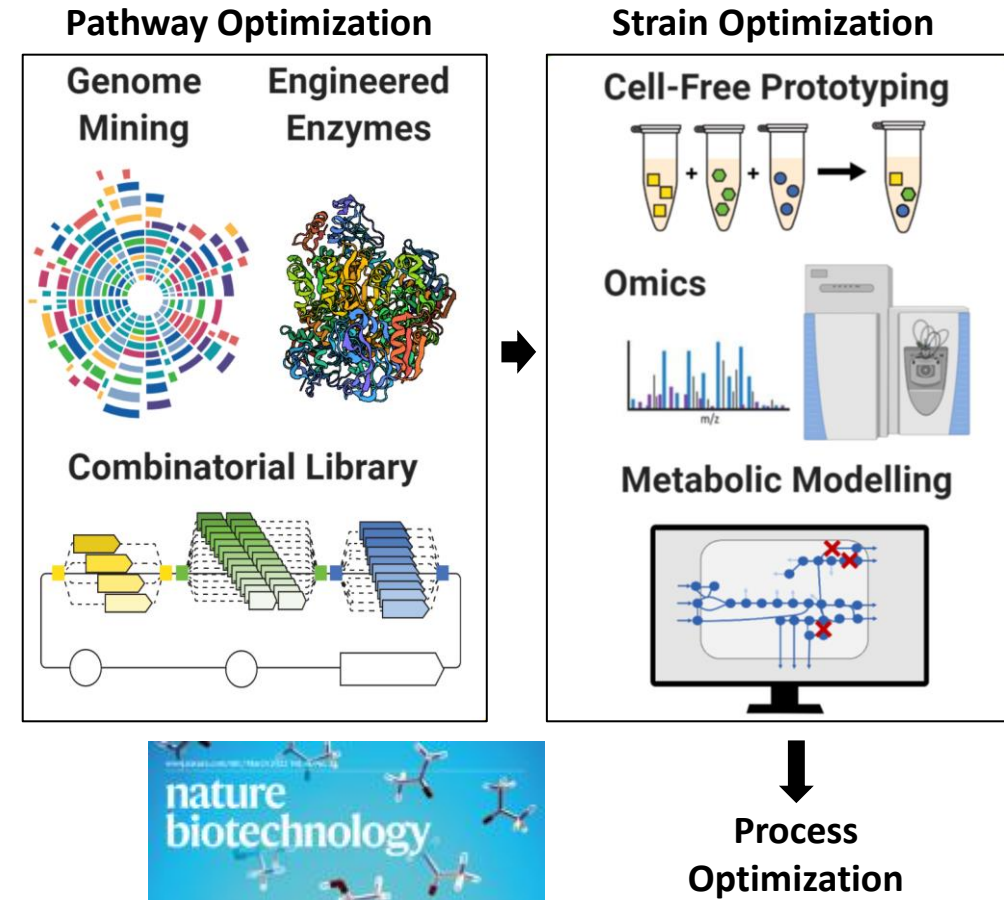
Approach

A combination of genomic analyses, computational modeling, and cell-free pathway optimization was conducted to engineer *Clostridium autoethanogenum* strains to maximize production of acetone and isopropanol from industrial emissions and syngas.

Result/Impacts

- A carbon-negative bioprocess was pioneered to convert industrial and agricultural waste gases into valuable chemicals.
- The research provides a path for faster development of efficient cell-based production platforms with low environmental impacts.
- This new approach will enable the production of plastics, fuels, and other large-scale chemicals more sustainably.

Liew et al., Nature Biotech (2022) 40:335



Fungal Recyclers: Fungi Reuse Fire-Altered Organic Matter

Objective

Understand the ability to utilize and the genetic basis for growth on pyrogenic organic matter (PyOM) by the fungus *Pyronema domesticum*, which often quickly grows after fire to strongly dominate post-fire soil communities.

Approach

- Demonstrate growth of *P. domesticum* on lab-generated PyOM
- Conduct RNAseq to identify PyOM regulated pathways
- Utilize ^{13}C -Labeled PyOM in respiration experiment to demonstrate direct utilization of substrate



Images courtesy of Monika Fischer, University of California, Berkeley

Result/Impacts

- *P. domesticum* exhibits robust growth on lab-generated PyOM and mineralizes ^{13}C -labeled substrate to $^{13}\text{CO}_2$
- Transcriptomics indicated that upregulation of pathways funneling aromatic compounds into central metabolism via cytochrome P450 and FAD monooxygenases. This is consistent with PyOM enrichment in aromatic and polyaromatic carbon compounds.
- The recycling of PyOM by fungi may be an important route by which carbon is recycled back into the food web after a fire. Fungi thus may have broad impacts on post-fire succession and recovery in soil microbial communities.

M. S. Fischer, *et al.*, "Pyrolyzed Substrates Induce Aromatic Compound Metabolism in the Post-fire Fungus, *Pyronema domesticum*." *Frontiers in Microbiology* 12, 728289 (2021). DOI: <https://doi.org/10.3389/fmicb.2021.729289>

Two new technologies for editing specific species and genes within complex laboratory bacterial communities

Objective

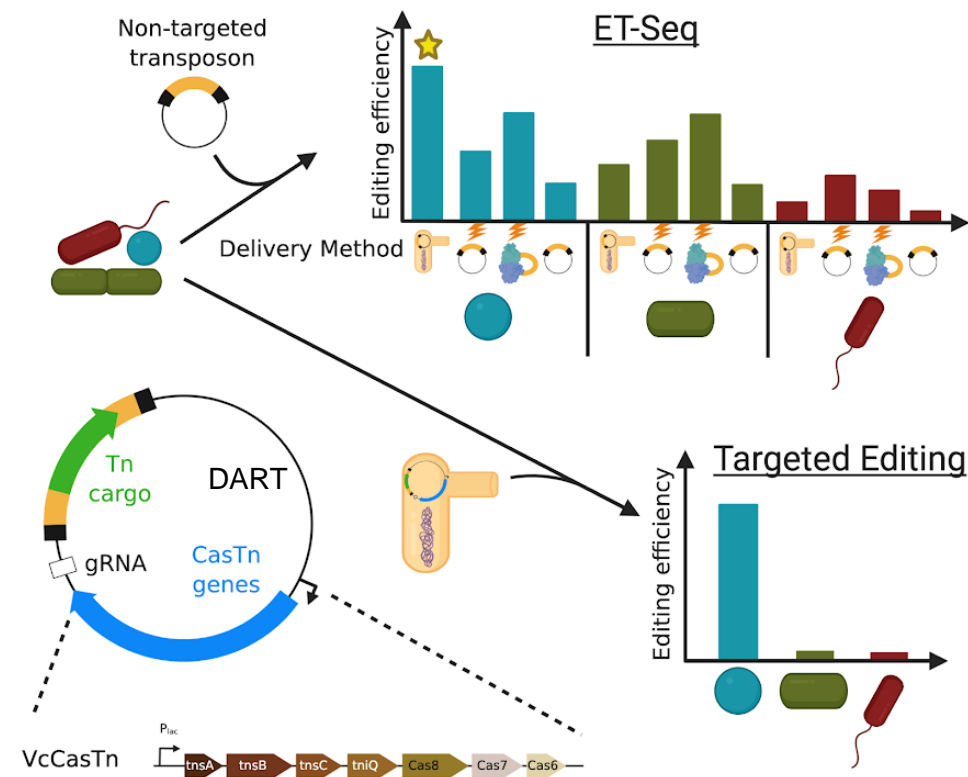
Develop tools for *in situ* genetic manipulation of bacteria within their communities

Approach

Environmental transformation sequencing (ET-seq) identifies genetically tractable species within a consortium, by delivering a transposon into the community and subsequent metagenome sequencing. DNA-editing all-in-one RNA-guided CRISPR-Cas transposase (DART) allows manipulation of specific genes in selected bacterial species identified by ET-seq.

Result/Impacts

- The combination of both techniques allowed the enrichment of targeted bacterial species, introduction of novel metabolic traits, and measurement of gene fitness of bacteria within a community context.
- ET-seq and DART technologies decrease the need for strain isolation for genetic analysis and allow *in situ* bacterial genetics.



Rubin et al., *Nature Microbiology* (2022) 7:34–47

Mapping carbon hotspots in the rhizosphere

Objective

Create a synthetic soil habitat that enables dynamic imaging and spatial chemical sampling of plant roots.

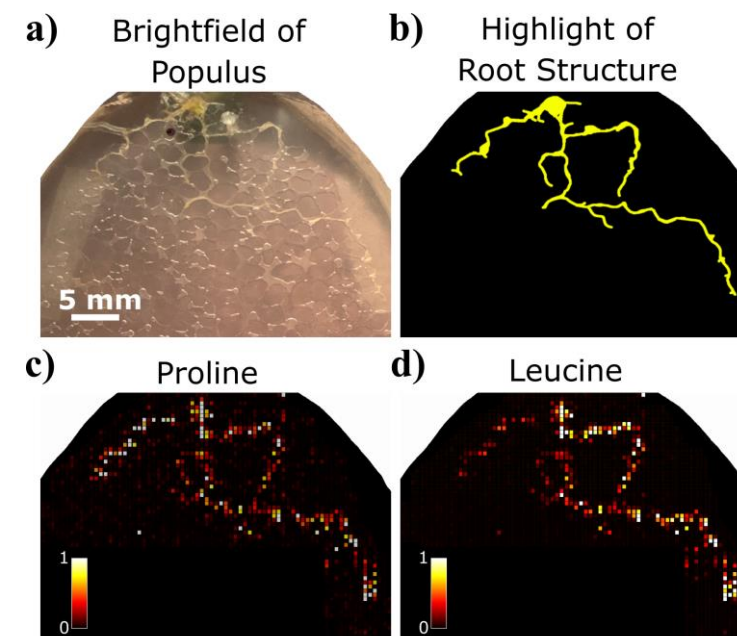
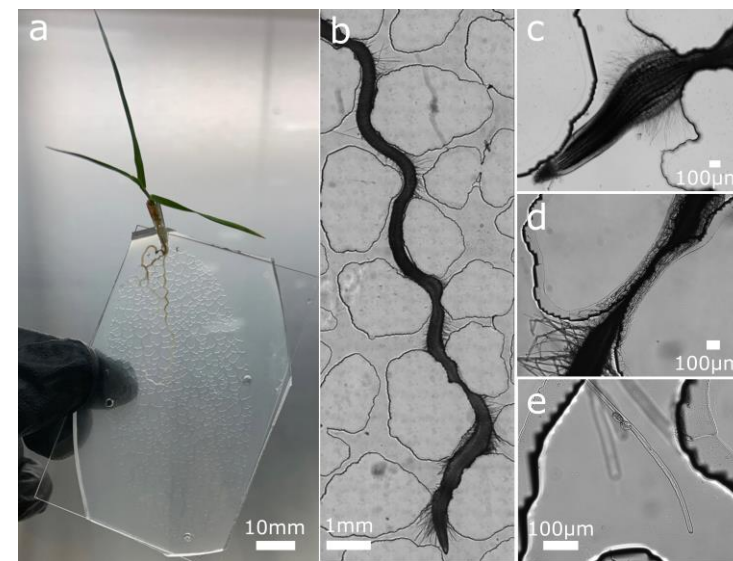
Approach

- *Brachypodium distachyon*, a model grass species, was grown within the rhizosphere-on-a-chip for one month.
- Computational simulations of root exudate diffusion predicted that the interaction of roots with the synthetic soil would create concentrated hotspots of labile carbon.
- The existence of root exudate hotspots in the rhizosphere-on-a-chip was experimentally validated using liquid micro-junction surface sampling probe mass spectrometry.
- The spatial distributions of the detected amino acids were nonuniform and varied by amino acid type.

Result/Impacts

Development of the rhizosphere-on-a-chip provides a tractable approach for studying carbon hotspot formation and plant-microbe interactions in soils.

Aufrecht JA, Khalid M, Walton CL, Tate K, Cahill JF and Retterer ST. **Lab on a Chip**. DOI: 10.1039/D1LC00705J .



Overlay of *in situ*-LE-MS chemical image of Proline and Leucine



Inter-subunit coupling enables the fastest CO₂ fixation by reductive carboxylase enzymes

Objective

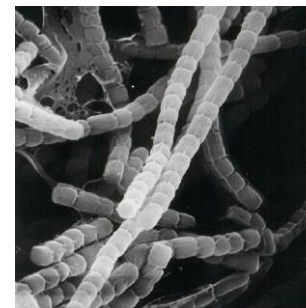
Understand the molecular mechanisms of the most efficient CO₂ fixing enzyme, enoyl-CoA reductase/carboxylase (ECR), from bafilomycin producing actinobacteria for improving C-cycling energy efficiency and production of high value bioproducts

Approach

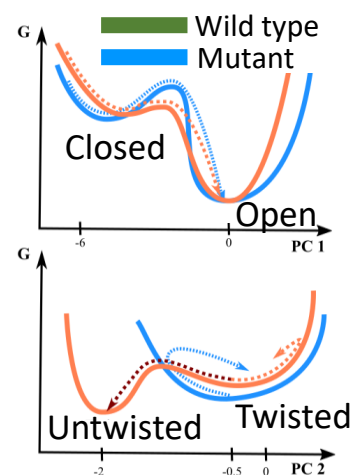
Elucidate the CO₂ fixation using ambient temperature XFEL and cryogenic synchrotron protein crystallography, quantum mechanical / molecular mechanism (QM/MM) simulations, principal component analysis, structure-based mutagenesis

Results/Impacts

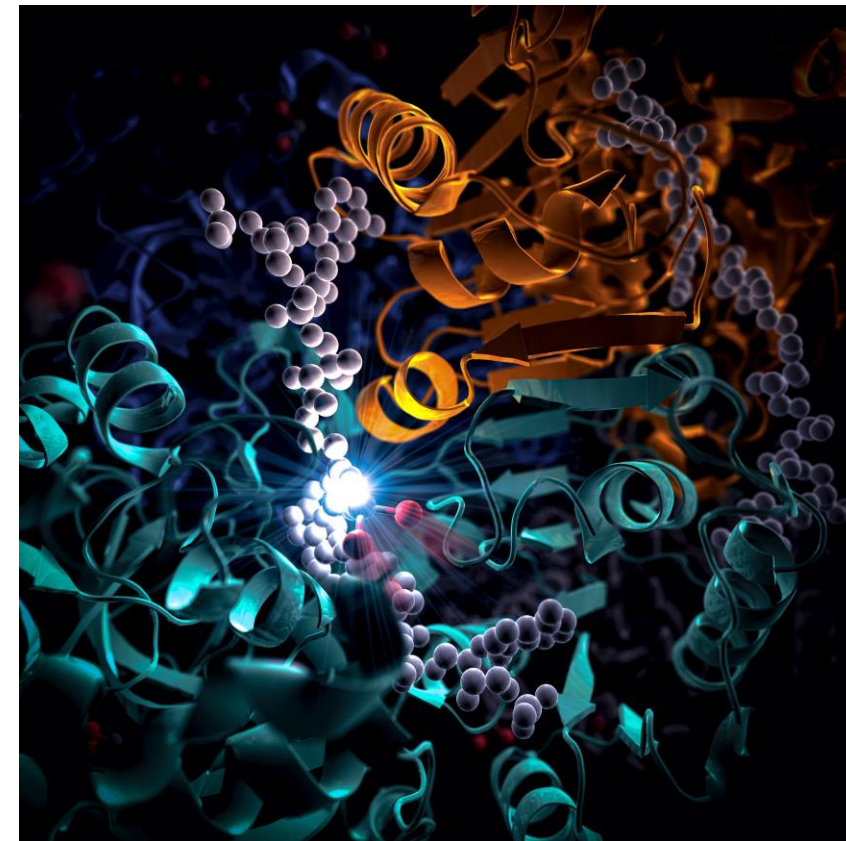
- Shown swing (open-close) and twist motions of coupled catalytic domains enable fast CO₂ fixation
- Provided basis for engineering a large class of CO₂ fixing enzymes for improving their rates and promiscuity/specificity by introducing molecular glue between subunits



Kitasatospora setae



Energy landscapes of swing and twist motions



CO₂ capture into the active site for fast catalysis

H. DeMirici et al., " Inter-subunit coupling enables fast CO₂-fixation by reductive carboxylases", **ACE Central Science**, 2022, *in press*; DeMirici et al., bioRxiv 607101; doi: <https://doi.org/10.1101/607101>

Pennycress (Thlaspi arvense) genome sequence – Resources for Brassicaceae research

Objective

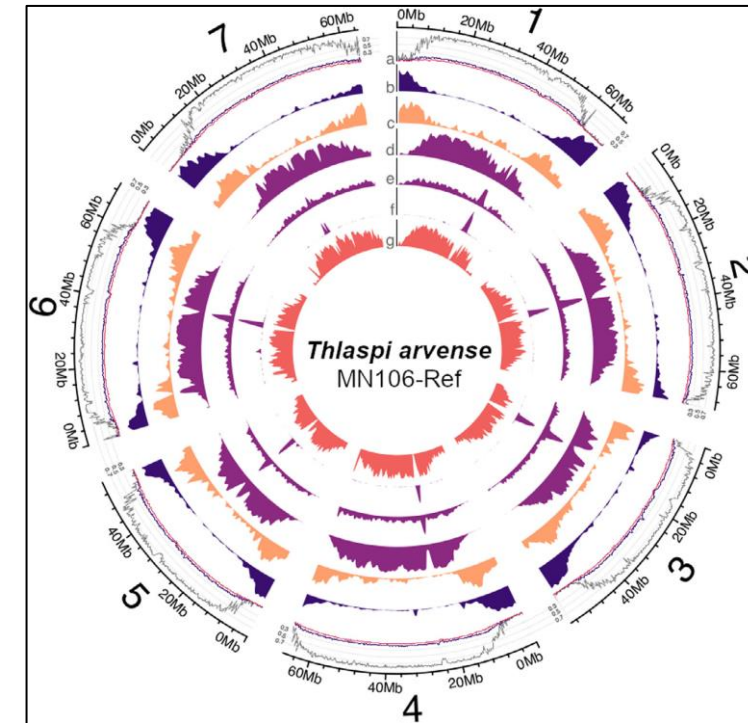
Develop genomic resources in field pennycress for investigation of genetic diversity, gene expression, and genetic structure to and facilitate crop improvement.

Approach

- Generate high-quality, chromosome-level reference genome assembly
- Enhanced annotations including protein-coding and non-coding genes, predicted pseudogenes, transposable element families
- Construct gene expression atlas of tissue-specific expression patterns
- Analyze genome-wide DNA methylation profiles for investigation of gene regulation, epigenetic mechanisms
- Perform whole-genome resequencing and analysis of 40 wild accessions to characterize population structure and genetic variation.

Result/Impacts

These new genomic resources will facilitate exploration of pennycress' untapped genetic potential as well as translational research across the Brassicaceae through insight into the regulatory landscape, gene localization and genetic variation, and the discovery of genes affecting important agronomic traits.



Nunn, A., et al. (2022). **Plant Biotechnol J.**, <https://doi.org/10.1111/pbi.13775>.

Streamlining A Tool for Building Genome-Wide Site Maps at Scale

Objective

A technology known as DNA affinity purification sequencing (DAP-seq) allows researchers to study the transcription factors that control how genes are turned on in organisms. Building on this technology, JGI researchers developed two streamlined approaches that are now available to the JGI user community.

Approach

- Biotin DAP-seq is a quick protein purification approach that reduces the workflow from months to days and halves the reagent cost.
- MultiDAP allows researchers to conduct comparative analyses across the genomes of multiple species in a single experiment.

Results/Impact

- Combining Biotin DAP-seq and multiDAP allows for ultra high throughput discovery of transcription factor binding sites across many different genomes.
- DAP-seq at scale is a unique capability offered by the JGI to users through current proposal calls.
- Biotin DAP-seq technology is now available for licensing or collaborative research through the Berkeley Lab Intellectual Property Office.



*Collage capturing a diverse set of bacteria and functions that can be better understood using DAP-seq technology.
(Eduardo de Ugarte, Berkeley Lab Creative Services Office)*

Video at <http://bit.ly/jgiDAPseq>

Baumgart LA and Lee JE et al. [Persistence and plasticity in bacterial gene regulation](#). **Nature Methods**. 2021 Nov 25. doi: 10.1038/s41592-021-01312-2.

The background features a large, light blue circular seal of the U.S. Department of Energy. The seal contains an eagle at the top, a shield with various energy symbols (sun, atom, lightning, oil rig, wind turbine, and motor), and the text "U.S. DEPARTMENT OF ENERGY" and "UNITED STATES OF AMERICA" around the perimeter.

Thank you

<https://science.osti.gov/ber>

<https://www.energy.gov/science/ber/biological-and-environmental-research>