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Volatile fatty acid extraction from fermentation broth using a hydrophobic ionic liquid and in situ enzymatic esterification

Background/Objective

- Volatile fatty acids (VFAs) are useful compounds that are widely used as raw materials in a variety of industries.
- However, VFAs are typically made from petroleum. In order to make VFA production more sustainable, scientists are using bioprocessing to convert carbon into VFAs.

Approach

- Extract VFAs from animal farm wastewater after anaerobic fermentation.

Results

- Researchers created a method that efficiently extracts VFAs from fermented wastewater and turns them into esters.

Significance/Impacts

- Traditional methods generate additional wastewater, raising environmental concerns. Instead, this new method uses ionic liquids as both extraction solvent and esterification media, making it a more sustainable option.

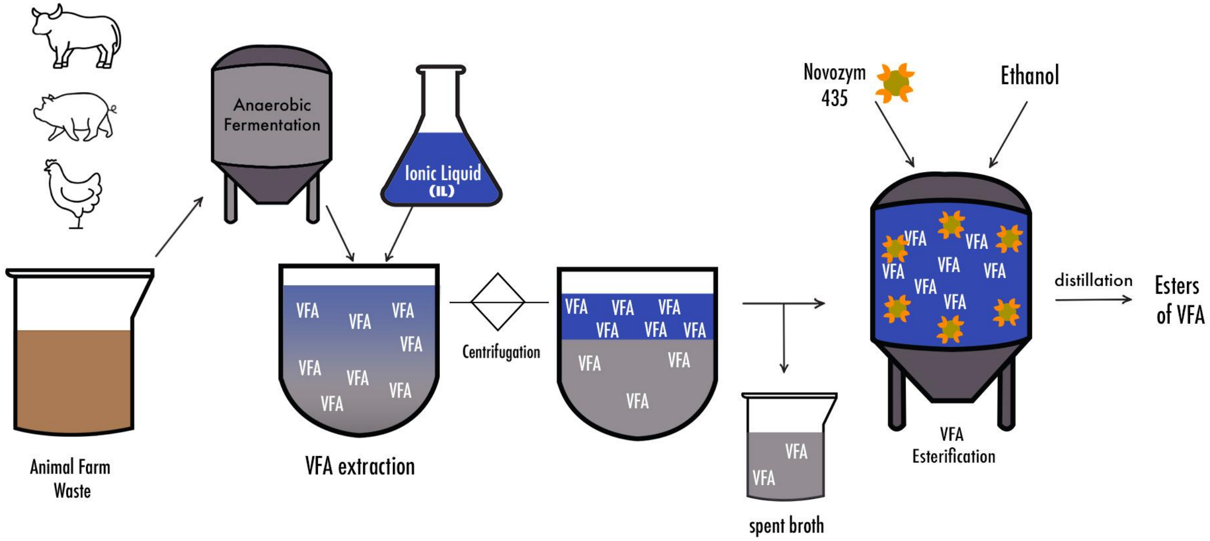

Figure: The integrated process uses ionic liquids and anaerobic fermentation to extract volatile fatty acids from wastewater and convert them into esters.

Singh R., et. al. RSC Sustainability. doi: 10.1039/D4SU00346B (JBEI #1191)

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Design and Characterization of a Transcriptional Repression Toolkit for Plants

Background/Objective

- Regulation of gene expression is essential for all life. Tools to manipulate gene expression level have therefore proven to be very valuable in efforts to engineer biological systems.

Approach

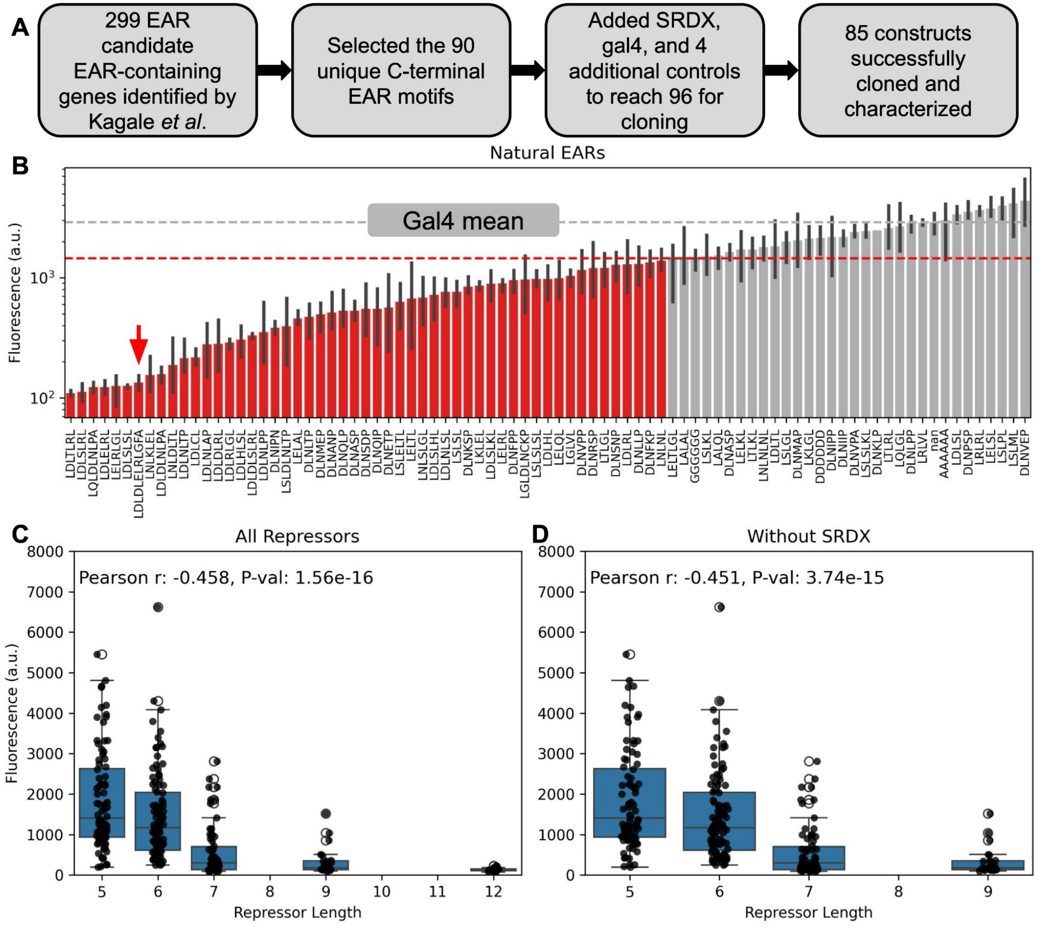
- We characterized the repression activity of a library consisting of repression motifs from approximately 25% of the members of the largest known family of repressors.

Results

- Combining sequence information with our trans-regulatory function data, we next generated a library of synthetic transcriptional repression motifs, with function predicted in advance.

Significance/Impacts

- Our study represents the largest plant repressor motif library experimentally characterized to date, providing unique opportunities for tuning transcription in plants.



A

299 EAR candidate EAR-containing genes identified by Kagale *et al.*

Selected the 90 unique C-terminal EAR motifs

Added SRDX, gal4, and 4 additional controls to reach 96 for cloning

85 constructs successfully cloned and characterized

Markel K., et. al. ACS Synthetic Biology. doi: 10.1021/acssynbio.4c00404 (JBEI #1192)

Impact of Drought Stress on Sorghum bicolor Yield, Deconstruction, and Microbial Conversion Determined in a Feedstocks-to-Fuels Pipeline

Background/Objective

- Determine response of different sorghum types and varieties to drought stress and location.
- First application of the Feedstocks-to-Fuels pipeline.

Approach

- 6 types of sorghum were grown at two location in California with pre- and post-flowering drought treatment.
- Biomass was harvested and analyzed for conversion to bisabolene using the F2F pipeline.

Results

- Drought had significant impact on biomass yield per area
- Surprisingly, biomass from drought stressed plants was more efficiently converted into bisabolene.

Significance/Impacts

- Drought stress often limits bioenergy crop growth and has been reported to sometimes cause inhibition of conversion into fuels. The opposite was found in this study. The study demonstrates the useful application of the F2F pipeline.

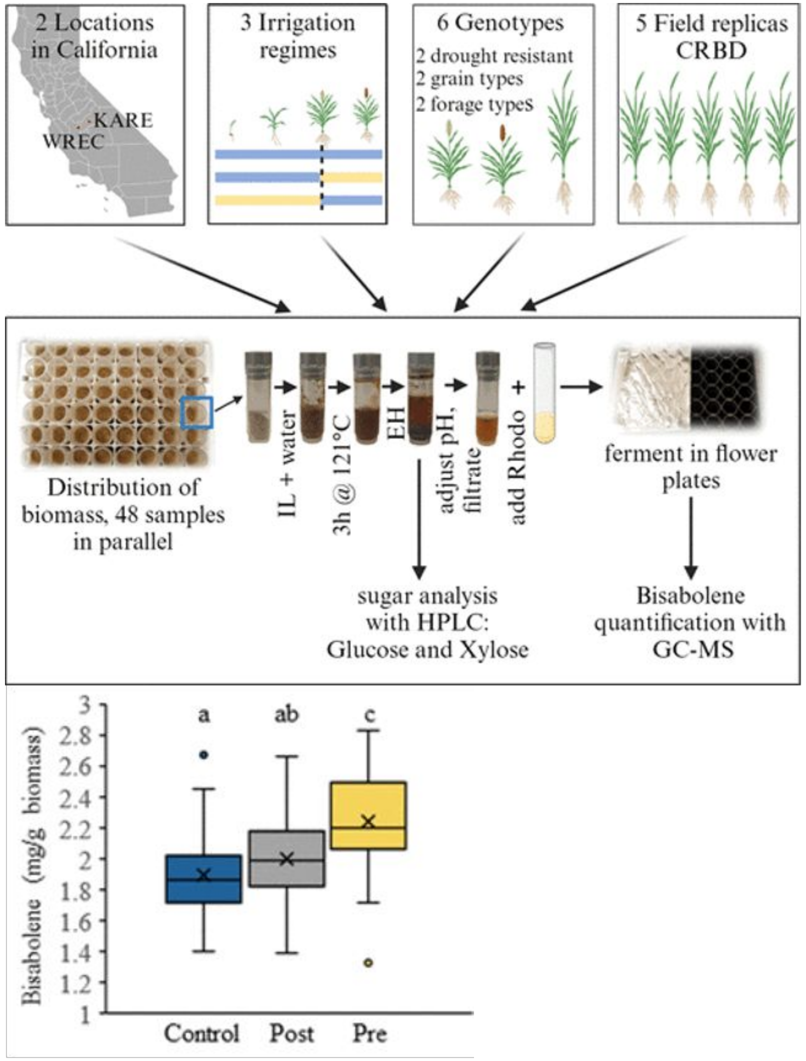


Figure: Top panels show schematic of the study. The lower panel shows the higher yield of bisabolene per unit of drought stressed biomass

Dalton J., et. al. ACS Sustainable Chem. Eng. doi: 10.1021/acssuschemeng.4c05826 (JBEI #1193)

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Carbon accounting for carbon dioxide removal

Background/Objective

- Biofuel and bio-based chemical production systems offer options for carbon removal through CO_2 waste gas capture or other stable products.

Approach

- We distinguished between carbon accounting for carbon removal and life-cycle assessment.
- Established broadly applicable equations for the community to use.

Results

- Carbon accounting for CDR requires larger system boundaries
- Many projects claiming to be CDR are achieving mitigation, but not removal.

Significance/Impacts

- Establishes expert consensus across 3 national labs and path forward for integrating carbon accounting in bioenergy systems.

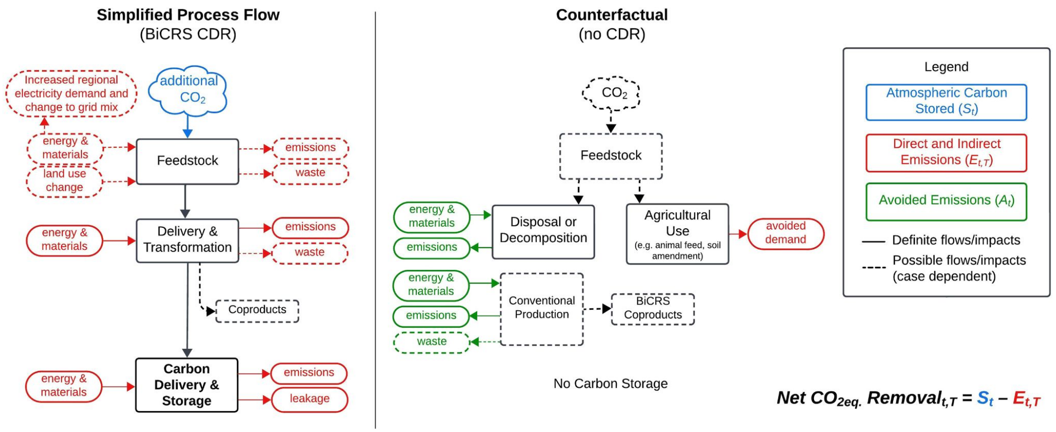

Figure 1: System boundaries and counterfactual for biomass carbon removal and storage

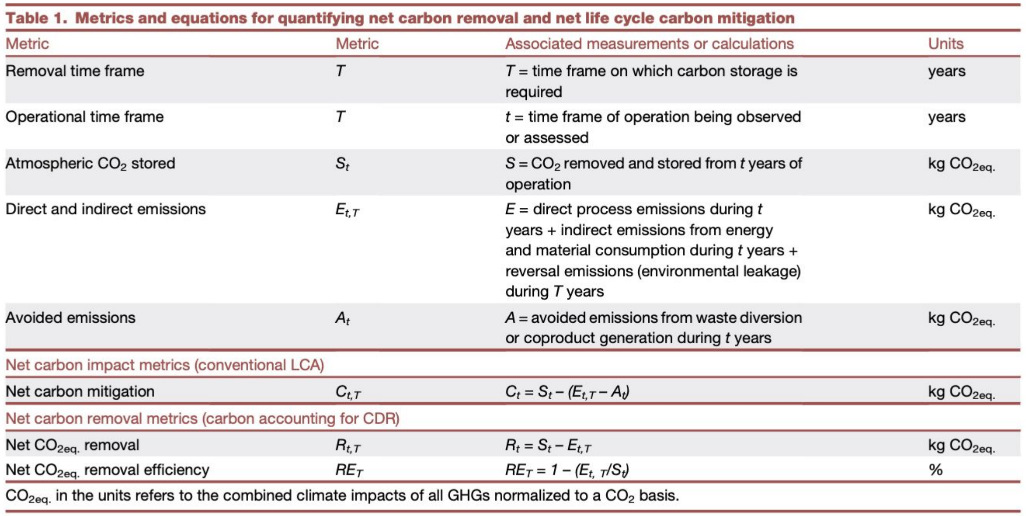

Table 1. Metrics and equations for quantifying net carbon removal and net life cycle carbon mitigation

Metric	Metric	Associated measurements or calculations	Units
Removal time frame	T	T = time frame on which carbon storage is required	years
Operational time frame	T	t = time frame of operation being observed or assessed	years
Atmospheric CO_2 stored	S_t	S = CO_2 removed and stored from t years of operation	kg $CO_{2eq.}$
Direct and indirect emissions	$E_{t,T}$	E = direct process emissions during t years + indirect emissions from energy and material consumption during t years + reversal emissions (environmental leakage) during T years	kg $CO_{2eq.}$
Avoided emissions	A_t	A = avoided emissions from waste diversion or coproduct generation during t years	kg $CO_{2eq.}$
Net carbon impact metrics (conventional LCA)			
Net carbon mitigation	$C_{t,T}$	$C_t = S_t - (E_{t,T} - A_t)$	kg $CO_{2eq.}$
Net carbon removal metrics (carbon accounting for CDR)			
Net $CO_{2eq.}$ removal	$R_{t,T}$	$R_t = S_t - E_{t,T}$	kg $CO_{2eq.}$
Net $CO_{2eq.}$ removal efficiency	RE_T	$RE_T = 1 - (E_{t,T}/S_t)$	%

$CO_{2eq.}$ in the units refers to the combined climate impacts of all GHGs normalized to a CO_2 basis.

Nordahl S.L., et. al. One Earth. doi: 10.1016/j.oneear.2024.08.012 (JBEI #1194)

A miniaturized Feedstocks-to-Fuels pipeline for screening the efficiency of deconstruction and microbial conversion of lignocellulosic biomass

Background/Objective

- Efficient conversion of lignocellulosic biomass into biofuels and bioproducts often requires extensive testing of all steps in the process.

Approach

- We developed a high-throughput feedstocks-to-fuels screening platform to address these challenges.

Results

- A miniaturized semi-automated platform that leverages the capabilities of a solid handling robot, a liquid handling robot, analytical instruments, and a centralized data repository, adapted to operate as an ionic-liquid-based biomass conversion pipeline.

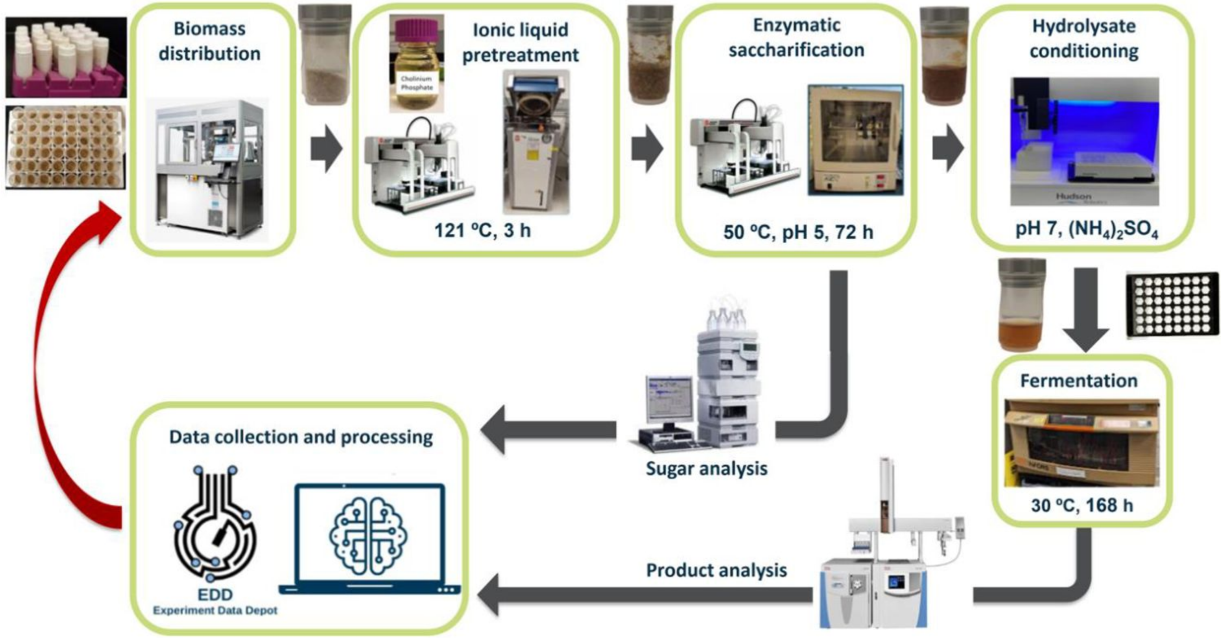

Figure: Overview of the feedstocks-to-fuels pipeline. The workflow begins with biomass weighing and distribution (top left) and ends with data collection and processing (bottom left). The processed data can be used in future campaigns to inform process parameter selection (depicted by the red arrow).

Significance/Impacts

- This Feedstocks-to-Fuels pipeline will become an effective tool to screen thousands of bioenergy crop and feedstock samples and assist process optimization efforts and the development of predictive deconstruction approaches.

Pidatala V.R., et. al. PLoS One. doi: 10.1371/journal.pone.0305336 (JBEI #1195)

The short- and long-run environmental value of waste conversion

Background/Objective

- LCA is being used for prioritization of early-stage R&D, vetting technologies for commercialization, and determining GHG-intensities for policy.
- The answers depend on what qualifies as a waste.

Approach

- The article provides a systematic exploration of how feedstocks/products are classified as wastes in LCA.

Results

- Move to short- and long-term counterfactuals for wastes rather than a single.
- Track market values over time to determine when an input to a bioenergy process is no longer a waste product.

Significance/Impacts

- Biofuels are heavily reliant on GHG-focused incentives and classifications of inputs as wastes is a major determining factor.

World view

https://doi.org/10.1038/s44286-024-00060-2

The short- and long-run environmental value of waste conversion

By Corinne D. Scown

Check for updates

Wastes can be leveraged for decarbonization, provided we know how to think about them, argues Corinne D. Scown.

rather than shifting limited resources from one useful application to another. A common pitfall among existing studies is the failure to acknowledge current uses of materials deemed wastes or residues. Some crop resi-

generators and emission rates on the grid. For waste, many of the same considerations apply. Loads of solid waste can be redirected in a matter of hours or days, but the lead time for utilizing wastes that require new sorting or

Figure: In life-cycle assessment, wastes do not carry the environmental burden of the process that produced them. This has huge implications for how bioenergy and bioproducts are analyzed. For example, corn stover is classified as a waste. Purpose-grown sorghum is not a waste, but sorghum stubble from a grain sorghum crop is. Lignin can be considered, for now, a waste product from biorefineries but this could change as technologies develop to cost-effectively convert it.

Scown, C.D. et. al. Nat Chem Eng. doi: 10.1038/s44286-024-00060-2 (JBEI #1196)

ENABLED PUBLICATIONS

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Comparative genomic analysis of thermophilic fungi reveals convergent evolutionary adaptations and gene losses

Background/Objective

- Thermophily is a strait scattered around the fungal tree of life but is prevent in certain families.
- Determine how mesophilic and thermophilic fungi are differentiated using genome analysis.

Approach

- Compare genomes of 37 thermophilic and thermotolerant species and 42 mesophilic species for genes that separate mesophiles and thermophiles.

Results

- Thermophily results in a reduction in genome size.
- Machine learning identified a subset of PFAM domains whose sequences can distinguish between mesophiles and thermophiles.

Significance/Impacts

- Thermophily is the ancestral state of families with high prevalence of thermophilic fungi.
- Thermophilic fungi may be excellent model organisms to study stress tolerance.

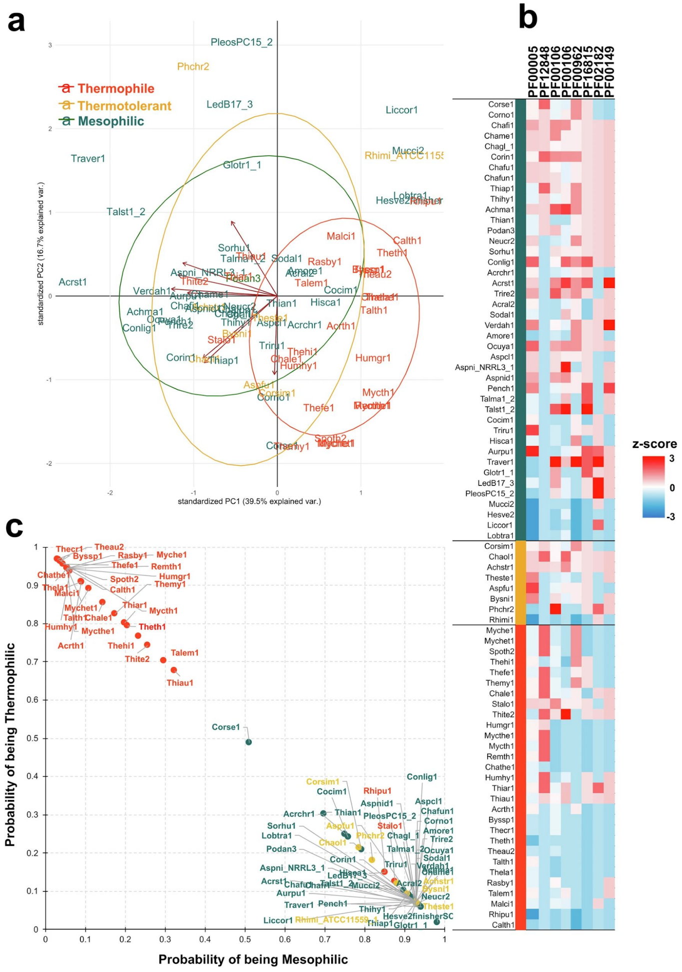

Figure: **a** Biplot PCA using the eight most informative clusters showing the separation between thermophiles and mesophiles. **b** Heatmap of the eight most informative HOGs showing prevalent gene loss in thermophiles. **c** Scatterplot of probabilities of a genome being a thermophile or mesophile based on these eight gene clusters

Steindorff AS, et. al. Commun Biol. doi: 10.1038/s42003-024-06681-w (JBEI #99)

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DRMY1 promotes robust morphogenesis in Arabidopsis by sustaining the translation of cytokinin-signaling inhibitor proteins

Background/Objective

- Development is highly reproducible. For example, flowers have the same number of sepals of a similar size. It is unclear how biological systems can achieve this level of robustness. A mutant with variable development, *drmy1*, is used to study the molecular basis of developmental reproducibility.

Approach

- Genetics, quantitative proteomics, ribosome profiling and TOR inhibitors revealed that the *drmy1* mutant has a lower translation rate because of lower levels of TOR signaling.
- Live imaging of labile proteins is limited when the protein lifetime is comparable to the maturation time of fluorescent proteins. LlamaTags bypass this maturation limit and allowed imaging short lived proteins.

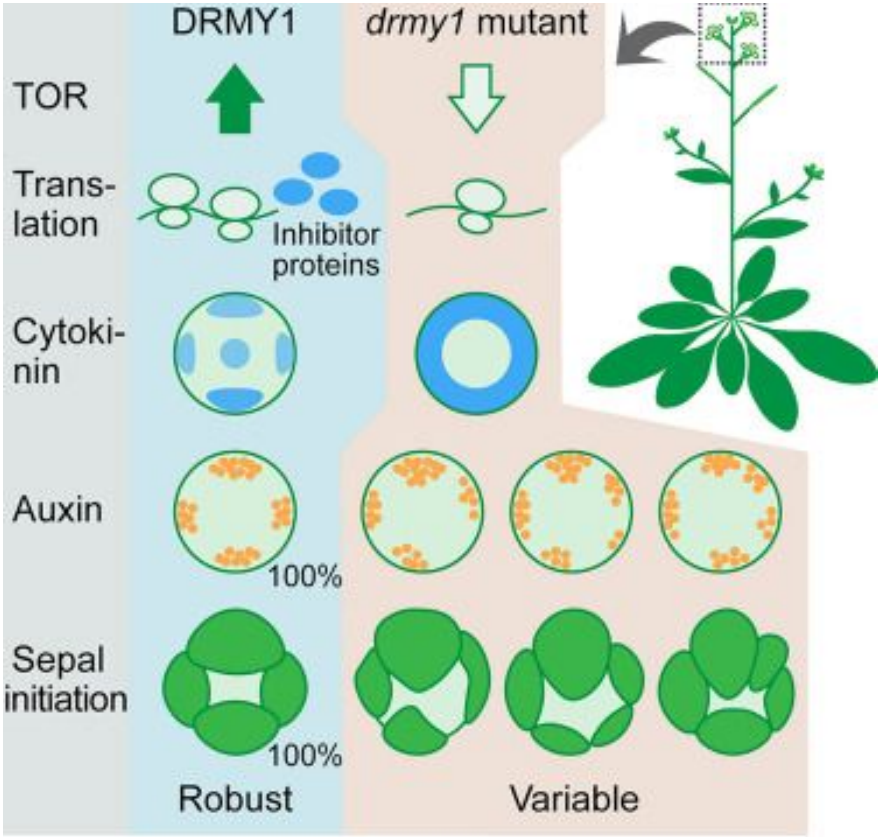
Results

- LlamaTags were used for the first time in plants, revealing that DRMY promotes the translation of cytokinin signaling inhibitors.

Significance/Impacts

- Maintaining the translation rate of developmental regulators as a new mechanism to ensure developmental reproducibility in plants.

Kong, S., et. al. Developmental cell. doi: 10.1016/j.devcel.2024.08.010 (JBEI #100)



DRMY1, Development Related MYB-like 1; TOR, Target Of Rapamycin

Figure: The DRMY protein acts through TOR signaling to promote the translation of cytokinin inhibitor proteins in the development of the Arabidopsis flower. This ensures a reproducible number of floral organs, all of a similar size.

The elite haplotype OsGATA8-H coordinates nitrogen uptake and productive tiller formation in rice

Background/Objective

- Excessive nitrogen promotes the formation of non-productive tillers in rice, which decreases nitrogen use efficiency (NUE).

Results

- The transcription factor OsGATA8 as a key coordinator of nitrogen uptake and tiller formation in rice. OsGATA8 negatively regulates nitrogen uptake by repressing transcription of the ammonium transporter gene OsAMT3.2.2.
- It also promotes tiller formation by repressing transcription of OsTCP19, a negative modulator of tillering. We identify OsGATA8-H as a high-NUE haplotype with enhanced nitrogen uptake and higher proportion of productive tillers.

Significance/Impact

- This study provides molecular and evolutionary insights into the regulation of NUE, and may facilitate the breeding of sorghum for higher NUE.

Wu, W., et. al. Nature genetics. doi: 10.1038/s41588-024-01795-7 (JBEI #101)

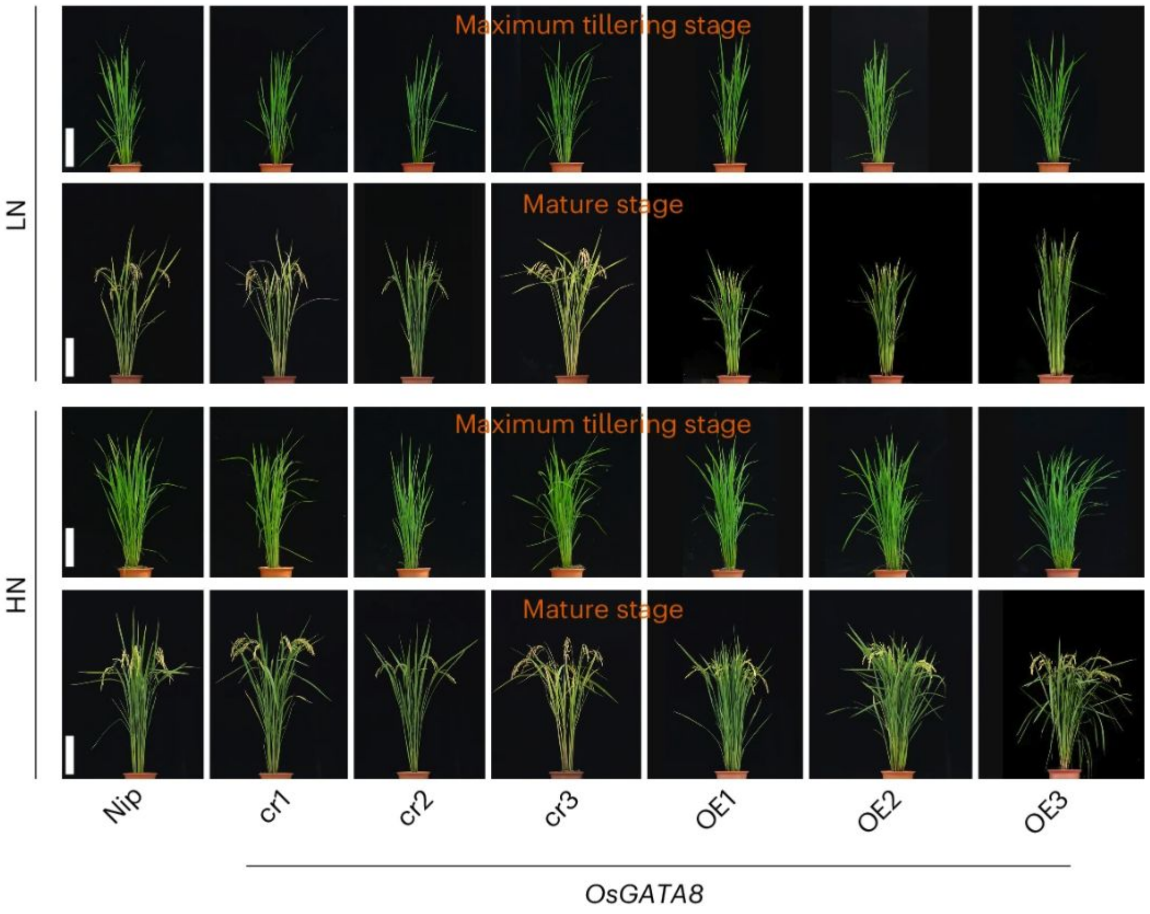


Figure: Compared with the wild-type (Nip) plants, both PTNR and the proportion of productive tillers were higher in the OsGATA8 knockout plants These results indicate that OsGATA8 is a negative regulator of PTNR and the proportion of productive tillers under low nitrogen conditions.

Measuring the economic efficiency of laboratory automation in biotechnology

Background/Objective

- Reducing experimental expenditure is crucial for achieving the goals of synthetic biology applications involving high-throughput (HT) experiments.

Approach

- We developed an experiment price index (EPI) to compare factors in time, cost, and sample numbers, helping measure the efficiency of laboratory automation.

Results

- EPI principles guide economic benefits and a higher project success rate.
- Automated EPI calculation facilitates integration into laboratory automation processes.

Significance/Impacts

- This provides valuable insights for optimizing economically efficient processes across a range of biotechnological endeavors.
- The EPI will be utilized to analyze the service business aspect of laboratory automation.

Woo H.M., et. al. Trends in Biotechnology. doi: 10.1016/j.tibtech.2024.02.001 (JBEI #102)

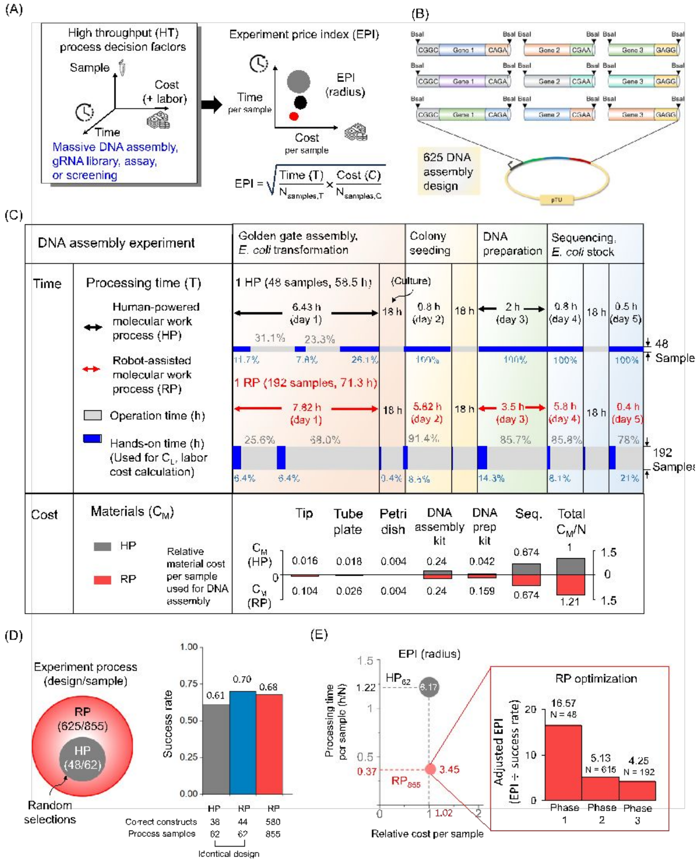


Figure: The experiment price index (EPI) to economically evaluate molecular work processes. (A) Schematic of process decision factors. (B) DNA assembly designs for synthetic biology and metabolic engineering. C) Detailed workflow of either a human-powered molecular work process (HP) or a robot-assisted molecular work process (RP) for the DNA assembly. (D) Comparison of HP and RP for sample numbers and success rate. (E) Bubble plot of EPIs of either HP62 or RP855 for either cost per sample or time per sample.