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Machine learning analysis of RB-TnSeq fitness data predicts functional gene modules in Pseudomonas putida KT2440

Background/Objective

- Rapid generation and analysis of Randomly Barcoded Transposon insertion Sequencing (RB-Tn-Seq) data for functional genomics
- A large amount of RB-Tnseq data for *P. putida* is already publicly available
- Data-driven and automated elucidation of genome-scale functional modules

Approach

- Independent Component Analysis (ICA) of an existing Rb-Tn-Seq dataset collected from 179 unique experimental conditions for *P. putida*

Results

- Identification of 84 independent gene groups (fModules), recapitulating known key metabolic pathway genes and establishing hitherto unknown associations between genes

Significance/Impacts

- A reduction of the time required to make sense of and annotate gene functions relative to manual curation of RB-Tn-Seq data sets
- An integrated multi-omic analysis with iModulons from transcriptomic data to uncover control and governing expression of functional gene sets

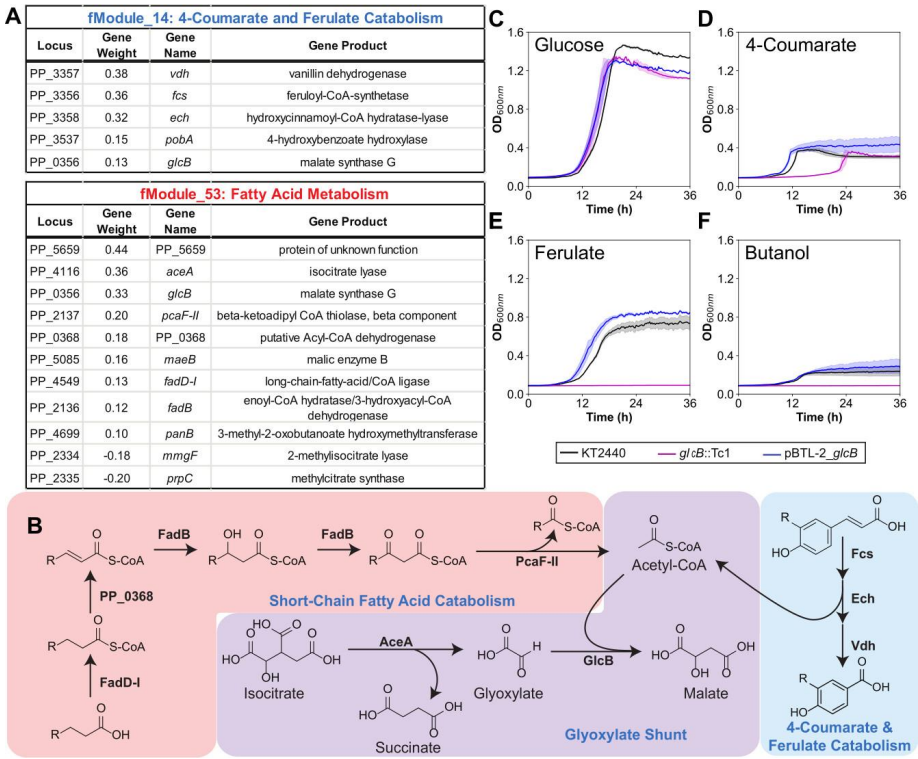

Figure 1: A - Examples of fModules and gene members. **B** – Metabolic pathways associated with fModule genes. C– Validation of the importance of *glcB*, a member of the two fModules, "4-Coumarate and Ferulate catabolism" and "Fatty Acid Metabolism" in coumarate, ferulate, butanol catabolism

Borchert A.J., et al. mSystems. doi: 10.1128/msystems.00942-23

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Foldy: An open-source web application for interactive protein structure analysis

Background/Objective

Recent advances in machine learning have led to structural bioinformatics tools with accuracy comparable to crystallization, but these tools require software experience to run. We aim to make them more accessible and thereby improve adoption.

Approach

Design an open-source web application which makes execution of AlphaFold, DiffDock, AutoDock Vina, and pfam as easy and reliable.

Results

Foldy code repo defines services which can be deployed in different modes, including local execution and a more scalable cloud application.

Significance/Impacts

The LBL Foldy instance has 700+ view-only accounts, and 40+ users who submitted 6000+ folds. It has been used to predict DUFs, change enzyme substrate scope, and design chimeric PKSs.

Roberts J. B., et. al. PLoS computational biology. doi: 10.1371/journal.pcbi.1011171

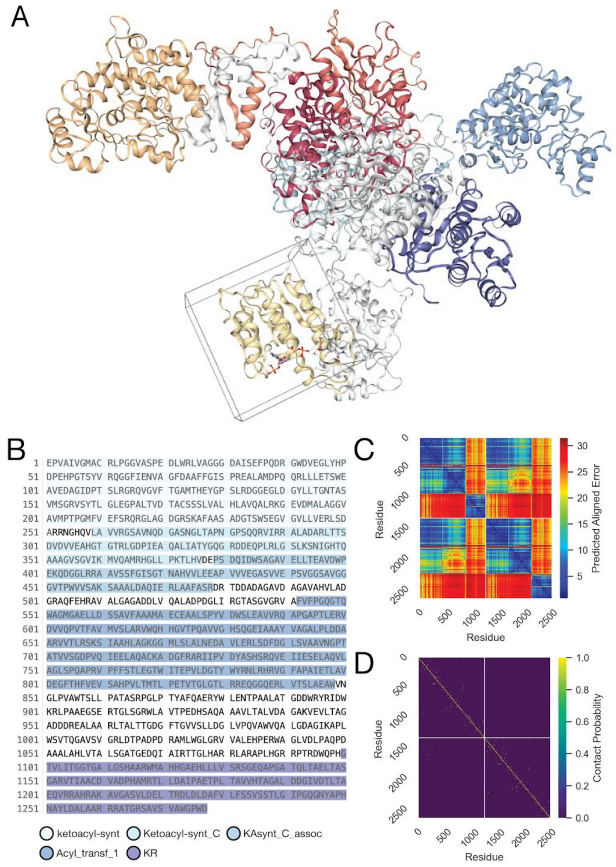

Figure 1: (A) The predicted structure of a homodimer of the polyketide synthase pik2 generated by AlphaFold, with NADPH docked into the AT domain with AutoDock Vina (B) The pik2 sequence with pfam annotations overlaid (C and D) the residue-residue predicted alignment error and residue contact probability.

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Genome-scale and pathway engineering for the sustainable aviation fuel precursor isoprenol production in Pseudomonas putida

Background/Objective

- Sustainable aviation fuel (SAF) will significantly impact global warming, and isoprenol is a precursor for a promising SAF compound DMCO.
- *Pseudomonas putida* can utilize carbon sources from inexpensive plant biomass and is a promising host for isoprenol bioproduction.

Approach

- We engineered metabolically versatile host *P. putida* for isoprenol production.
- We employed computational modeling approaches to predict gene knockout targets and optimize the pathway in *P. putida* to maximize isoprenol production

Results

- Two computational modeling approaches predicted gene knockout targets, and we prioritized them and reduced the total number of targets to engineer.
- The synergistic application of GSMM-guided gene knockouts and rational pathway optimization led to the highest titer of isoprenol in *P. putida* at 1.1 g/L; a 10-fold improvement vs. the starting strain.

Significance/Impacts

- We engineered *P. putida* strains that can produce the SAF precursor isoprenol from plant-derived carbon sources at much improved level by simultaneously engineering based on rational and GSMM-based computational approaches.

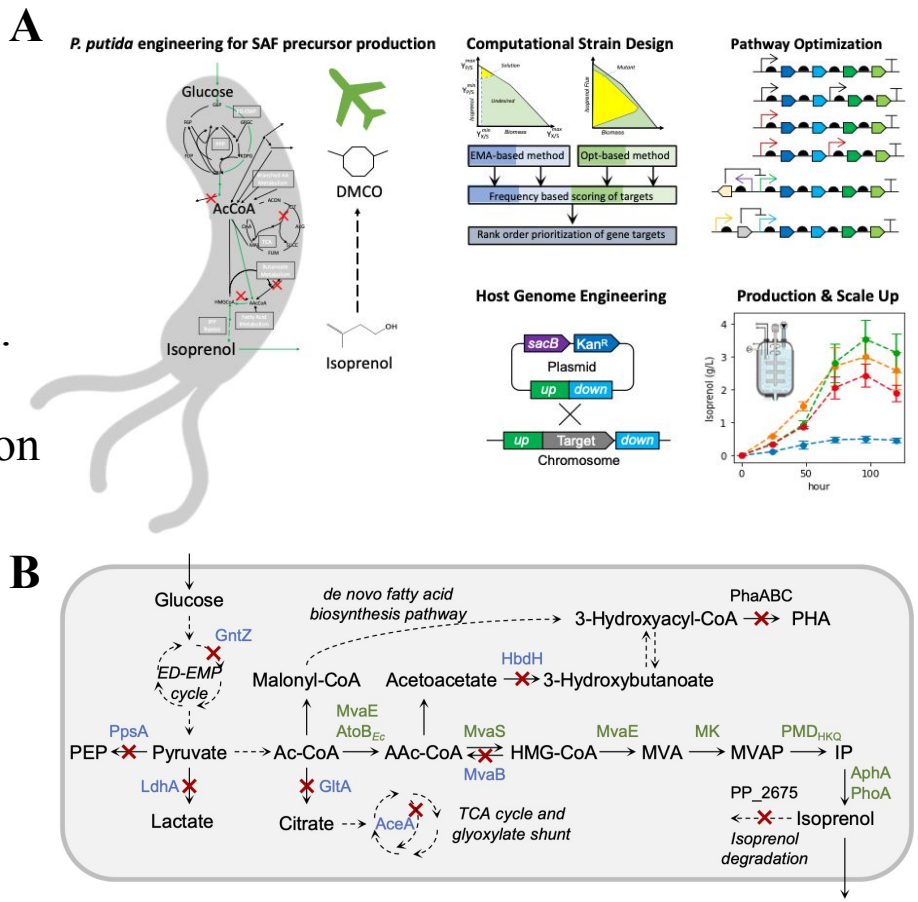

Figure 1. (A) Genome-scale metabolic and pathway engineering for production of the precursor of sustainable aviation fuel DMCO (1,4-dimethylcyclooctane), isoprenol, in Pseudomonas putida. (B) Metabolic pathways for isoprenol production and the gene knockout targets

Banerjee D., et. al. Metabolic Engineering. doi: 10.1016/j.ymben.2024.02.004

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Green horizons: how plant synthetic biology can enable space exploration and drive on Earth sustainability

Background/Objective

- As humanity looks towards expanding activity from low Earth orbit to the Moon and beyond, resource use efficiency and self-sustainability will be critical to ensuring success in the long term.
- Furthermore, solutions developed for the stringent requirements of space will be equally valuable in meeting sustainability goals here on Earth.
- Advances in synthetic biology allow us to harness the complex metabolism of life to produce the materials we need *in situ*.

Approach

Here, we focused our review on how plants could support provision of three key classes: biofuels, bioplastics and pharmaceuticals

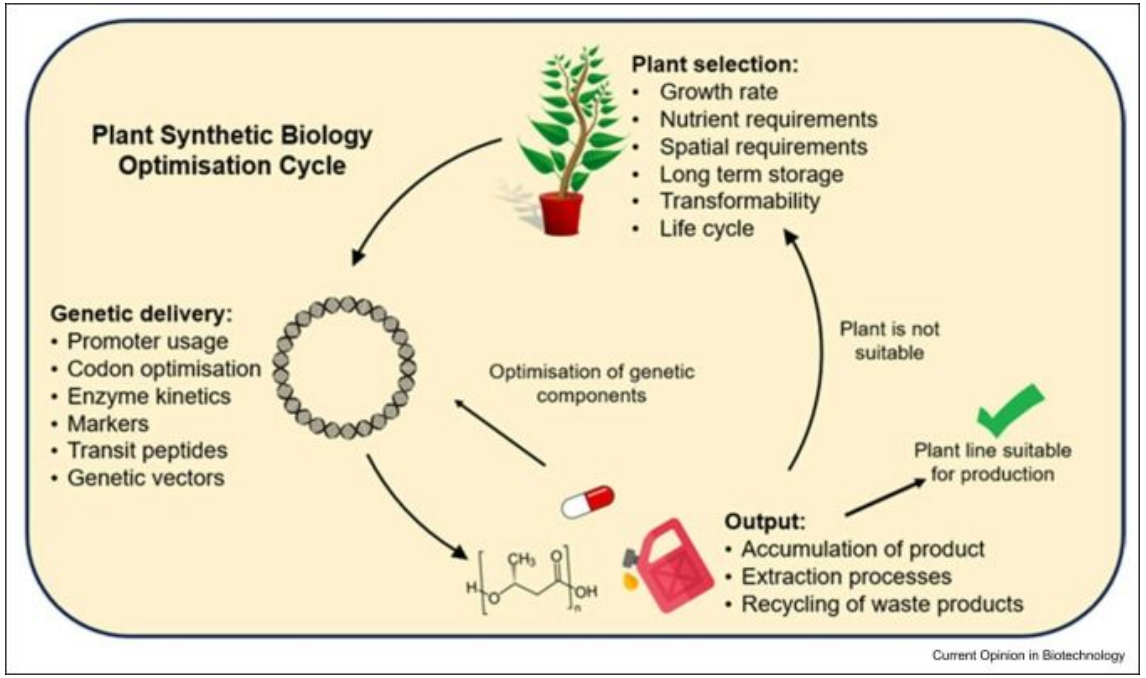

Figure 1. When planning and optimising the production of valuable biomolecules via metabolic engineering, various components of the expression and production system can be tuned, depending upon compound and purpose.

Significance/Impacts

- Translating lessons learned from microbial systems to more carbon-efficient photosynthetic organisms is an area of growing interest.
- Significant research bottlenecks will need to be addressed to enable to support widespread implementation, but much recent progress has been made.

Morgan M. F., et. al. Current opinion in biotechnology. doi: 10.1016/j.copbio.2024.103069

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Pathway Evolution Through a Bottlenecking-Debottlenecking Strategy and Machine Learning-Aided Flux Balancing

Background/Objective

- The evolution of pathway enzymes enhances the biosynthesis of high-value chemicals.
- Unpredictable evolutionary landscapes of pathway genes often hinder successful evolution.

Approach

- A biofoundry-assisted strategy was developed for pathway bottlenecking and debottlenecking, enabling the parallel evolution of all pathway enzymes along a predictable evolutionary trajectory in six weeks.
- This study then utilized a machine learning model, ProEnsemble, to further balance the pathway by optimizing the transcription of individual genes.

Results

- An *Escherichia coli* chassis with evolved and balanced pathway genes produced 3.65 g L−1 naringenin.

Significance/Impacts

- This approach can be readily adapted for any given number of enzymes in the specific metabolic pathway, paving the way for automated chassis construction in contemporary biofoundries.

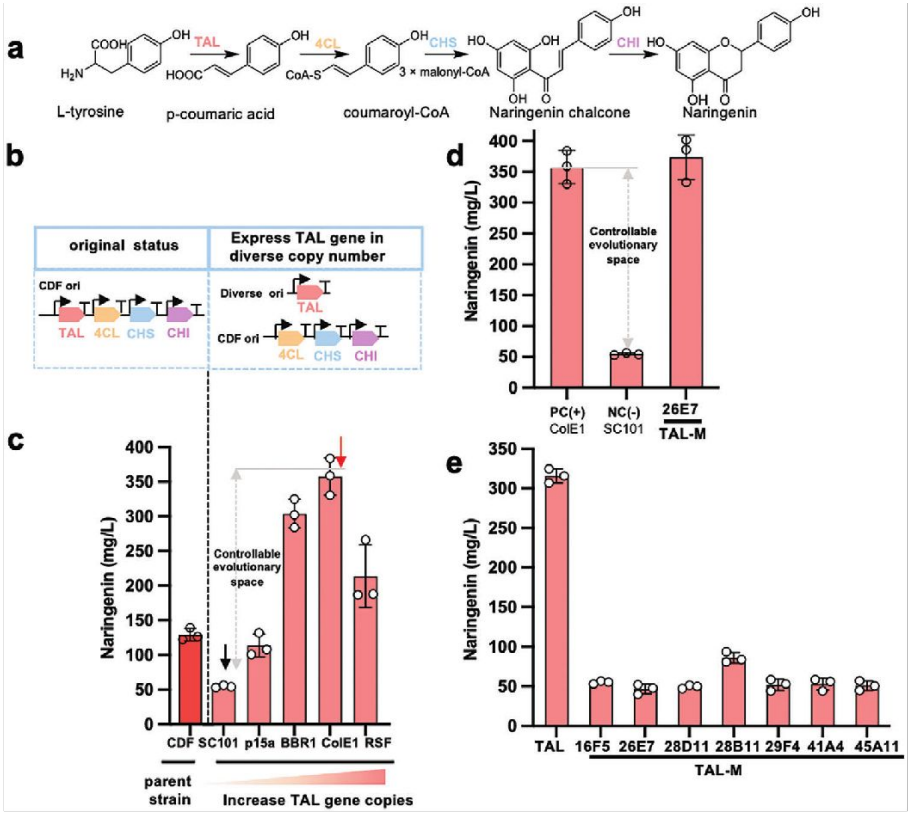

Figure 1. Directed evolution of TAL as an example to evaluate potential epistasis of the heterologous naringenin biosynthetic pathway a) Naringenin biosynthesis pathway. b) Various constructs were tested for narinengin production. c) Influence of different plasmid copy numbers on naringenin production. d) TAL, when present in low copy number plasmid, was successfully evolved to produce the same amount of naringenin as the positive control. e) Investigating the potential epistasis of the TAL gene.

Deng H., et. al. Advanced science. doi: 10.1002/advs.202306935

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Measuring the economic efficiency of laboratory automation in biotechnology

Background/Objective

- Laboratory automation with robot- assisted processes enhances synthetic biology, but its economic impact on projects is uncertain.

Approach

- We have proposed an experiment price index (EPI) for a quantitative comparison of factors in time, cost, and sample numbers, helping measure the efficiency of laboratory automation in synthetic biology and biomolecular engineering.

Results

- Automated EPI calculation facilitates integration into laboratory automation processes, aiming to monitor EPIs in the active project.

Significance/Impacts

- This provides valuable insights for optimizing economically efficient processes across a range of biotechnological endeavors.
- Furthermore, the EPI will be utilized to analyze the service business aspect of laboratory automation, encompassing considerations for the installation, maintenance, and expansion of laboratory automation machinery.

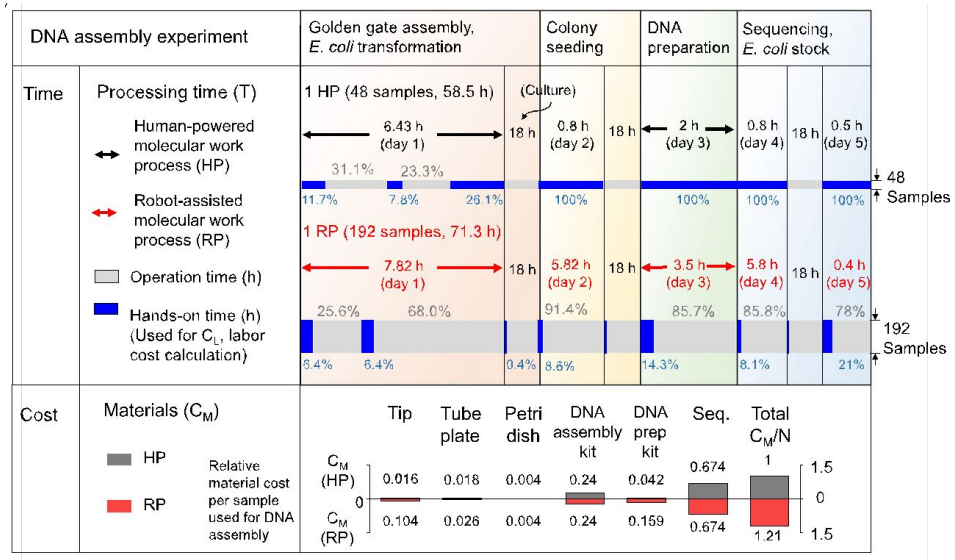

Figure 1: Detailed workflow of either a human-powered molecular work process (HP) or a robot-assisted molecular work process (RP) for the DNA assembly.

Woo H. M., et. al. Trends in biotechnology. doi: 10.1016/j.tibtech.2024.02.001

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Engineering Brassica Crops to Optimize Delivery of Bioactive Products Postcooking

Background/Objective

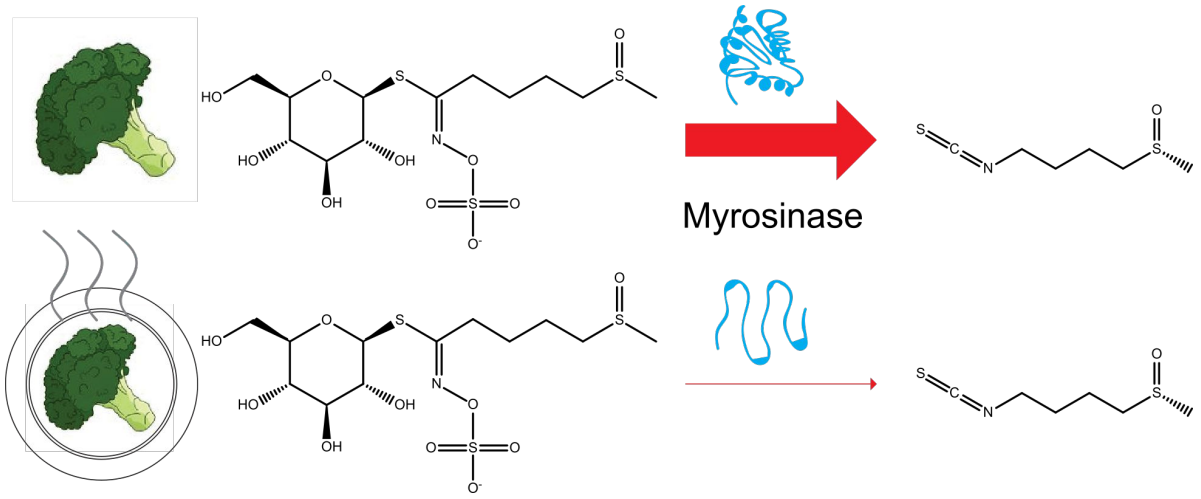
Glucosinolates are plant-specialized metabolites that can be hydrolyzed by glycosyl hydrolases called myrosinases, creating a variety of hydrolysis products that benefit human health. While cruciferous vegetables are a rich source of glucosinolates, they are often cooked before consumption, limiting the conversion of glucosinolates to hydrolysis products due to the denaturation of myrosinases.

Approach

Here, we screen a panel of glycosyl hydrolases for high thermostability and engineer the *Brassica* crop, broccoli (*Brassica oleracea* L.), for the improved conversion of glucosinolates to chemopreventive hydrolysis products.

Results

Our transgenic broccoli lines enabled glucosinolate hydrolysis to occur at higher cooking temperatures, 20°C higher than in wildtype broccoli.



Significance/Impacts

Our findings demonstrate the promise of leveraging genetic engineering to tailor crops with novel traits that cannot be achieved through conventional breeding and improve the nutritional properties of the plants we consume.

Barnum C. R., et. al. ACS synthetic biology. doi: 10.1021/acssynbio.3c00676