



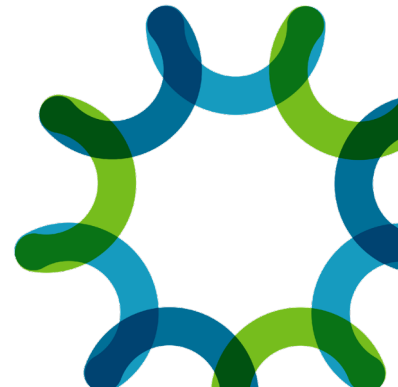
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DOE BioEnergy Technologies Office (BETO)  
2023 Project Peer Review

# ABF – Introduction and Overview

April 3, 2023  
Conversion Technologies

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Berkeley Lab





# Project Overview

# Project History

- **The ABF has operated as a BETO-supported project since 2016**
  - In 2016, the ABF began as a \$3M / 18-month pilot project that included four National Labs
- **In 2017 the ABF expanded to a \$20M/year BETO-consortium**
  - The ABF now includes seven National Labs
- **In October 2022, the ABF began a strategic planning process**
  - Under BETO-guidance to reimagine and redirect its activities
- **This presentation will focus on the ABF prior to October 2022**
  - Subsequent presentations (tomorrow) will cover the ABF after October 2022

# Project Goal and BETO Relevance

- **ABF goal:** Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry to productionize synthetic biology
- **Relevance to BETO's goals:** The ABF empowers BETO to achieve its goals in decreasing development times for industrially-relevant microorganisms, and in advancing bioprocess development
- **Specific research question:** How can a public biofoundry develop and demonstrate capabilities that enable commercially-relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts?



# 1 – Approach

# Technical Approach

- **Develop and demonstrate the effective use of ABF capabilities**
  - Engineering Biology Design / Build / Test / Learn (DBTL) infrastructure
  - Demonstration projects and the establishment of metabolic beachheads
- **Employ Techno-Economic Analysis and Life-Cycle Assessment**
  - Set relevant bioprocess performance metric goals
  - Prioritize efforts (e.g. between Titer / Rate / Yield, which strategic metabolic beachheads to pursue)
- **Onboard and further develop industry-relevant host organisms**
  - Support a wide range of bioproducts and bioprocess conditions
- **Integrate bioprocess scale-up as a core Test activity**
  - Increase commercial relevance through ABF technology de-risking
- **Industry engagement and outreach**
  - Industry Advisory Board, customer discovery, communications, industry listening days, funding opportunities

# Changes made after 2021 Peer Review

- **Better support in ABF software for tracking cycle metrics**
  - Software platforms (DIVA and EDD) were further developed to better track engineering cycle metrics
- **Better dissemination of ABF capabilities, beachheads, hosts**
  - Capabilities section of ABF website was updated, and ABF began providing capabilities webinars
  - ABF metabolic map depicting beachheads was added to the website
  - ABF's Host Onboarding Tool (HOBT) now publicly provides information about onboarded host organisms
- **Conducted an industry gaps and priorities analysis**
  - Surveyed industry collaborators to determine key priorities and challenges for commercialization
- **ABF metabolic beachheads used in collaboration projects**
  - Beachhead development / selection was informed by industry and one example is a collaboration project that leverages acetyl-CoA/malonyl-CoA beachheads

# Top Potential Challenges

- **Leverage past collaboration learnings with future collaborators**
  - Only portions of past collaborative data or learning methods that do not reveal the underlying primary data may be available
- **Predictive scale-up, and method transferability/reproducibility**
  - Our lack of ability to predict how a process will scale, or how well a method can be transferred across facilities, may limit the impact of our research and development efforts
- **Intellectual framing of strategic beachhead work**
  - It may be difficult to quickly and convincingly convey innovative TEA/LCA approaches for evaluating beachheads and metrics (e.g. flux vs accumulation, metabolic space coverage) to gauge progress in establishing and strengthening beachheads
- **Quantifying overall bioprocess development time improvements**
  - It will be challenging to develop leading indicators (timelines extending years beyond ABF involvement), and control experiments (development in parallel without the ABF) will rarely if ever be pursued



# Go/No-Go Decision Point

- **Date:** March 31, 2021
- **Goal:** demonstrate transferability of ABF technologies and ability to accelerate bioprocess development
- **Description:** 5 target molecules or tools transferred between host organisms that are able to at least achieve 1 g/L or higher in the first host. Successful target molecule transfers will have product titers greater than 1 g/L. For 3 of 5 of these, 2X biological engineering cycle efficiency gains demonstrated over attempts made in prior host organisms
- **Result:** Go

## Transfers of target molecule pathways between hosts and realized efficiency gains

Target molecule	First host (titer g/L)	New host (titer g/L)	Wall time	Clock time	Resources	Overall
3HP		<i>A. niger</i> (>1)	4X	6X	2X	
3HP						

## Transfers of tools between hosts and realized efficiency gains

Tool	First host	New host	Wall time	Clock time	Resources	Overall
Muconate biosensor		<i>C. glutamicum</i>	1.5X	3X	2X	
Microfluidics screening		<i>C. glutamicum</i>				2X
Microfluidics screening		<i>Rhodobacter</i>	2X		1X	2X
Genomic integration		<i>C. necator</i>	>10X	>10X	1X	10X
Fungal transporters						

# Economic and Technical Metrics

- **DBTL and tool/target transfer efficiency**
  - Efficiency: samples (or equivalents), per time (wall), per resource (reagent/human/instrument)
  - Platonic DBTL cycle: efficiency estimated from underlying unit operations
- **Beachhead (including host/process) coverage/flux**
  - Beachheads chosen strategically across metabolic space are subject to stage-gating based on TRY metrics achieved with exemplar targets
- **Host Onboarding and Development Tier System**
  - Tier 1: minimum set of tools/knowledge that a host needs to be used constructively in the DBTL cycle - all must be met to be considered a Tier organism
  - Tiers 2 – 4: minimum 70% of Tier criteria met to proceed to next Tier, and sum of percentage criteria met in the current and higher Tiers must  $\geq 100\%$
- **TEA/LCA (including beachheads/exemplars)**
  - Evaluate best case for TRY for given beachhead/exemplar up to 100% of theoretical
  - TEA metrics: Process yields, minimum selling price (MSP, \$/kg)
  - LCA metrics: Greenhouse gas emissions (CO<sub>2</sub>e/kg), water consumption (L/kg)

# Risks and Mitigations

Risk	Severity	Description	Mitigation Plan
Distributed model inefficiencies	Low	Important to consider the effects a distributed model has on the ABF's goals	Monitor and minimize DBTL cycle delays or other inefficiencies due to distributed operations
Insufficient data to fully leverage Learn	Medium	Multi-omics datasets may not be of the quality, quantity, or consistency needed for statistical analysis to identify engineering targets that lead to gains in titers, rates, and yields	Explicitly include the Learn team during the Design process to ensure suitability of generated data
Infrastructure operating costs and value	Low	Costs of infrastructure (both hardware and software) maintenance and asset depreciation becomes unsustainable	Offload maintenance to more cost-effective and sustainable off-the-shelf vendor-supported solutions where possible
Lack of target/host transferability	Medium	Not able to leverage learnings from one demonstration project/ beachhead in work for another	Further Learn the extents/likelihood of transferability
Designs do not work in selected host	Medium	Promoters/enzymes/pathways do not function as intended in the selected host	Further Test and Learn from lack of function, and suggest Design changes to restore function

# Related BETO projects and IAB

- **Other BETO consortia**

- Continue to integrate TEA/LCA support across consortia
- ChemCatBio: catalytically convert ABF molecules into value-added compounds
- SepCon: secreted hydrophobic, acid, and intracellular products recovery
- FCIC: understanding the effect of feedstock variability on strain robustness
- Performance-Advantaged BioProducts: ABF molecules could be used
- CCPC (BPMS): Bayesian inference of metabolic kinetics collaborations

- **BETO State Of Technology (SOT)**

- Improve genetic tools for SOT organisms to accelerate & increase DBTL cycle efficiency

- **Industry Advisory Board**

- Contributes to the ABF maintaining industrial relevance and understanding industry pain points and innovations
- Acts as a sounding board for feedback on ABF progress and plans

# Approach to Diversity, Equity, Inclusion

Task / Process Description	Status
<p>DEI speakers at all-hands meeting</p> <p>Lead DEI brainstorming discussion at all-hands meeting</p> <p>Conduct DEI poll</p> <p>Develop DEI plan</p> <p>Improve documentation and reporting of community outreach and STEM activities by adding a section to the ABF quarterly report</p> <p>Develop DEI vision statement</p> <p>Quantify current demographics across ABF with voluntary survey</p> <p>Participate in student internship programs promoting diversity</p> <p>Distribute ABF funding opportunities to diverse communities</p> <p>Make online tools ADA compliant</p> <p>Give presentations at MSIs</p> <p>Organize IAB panel on opportunities in biomanufacturing careers for ABF post-docs</p>	



## 2 – Progress and Outcomes

# Progress made towards project goal

- **Acceleration of biomanufacturing commercialization**

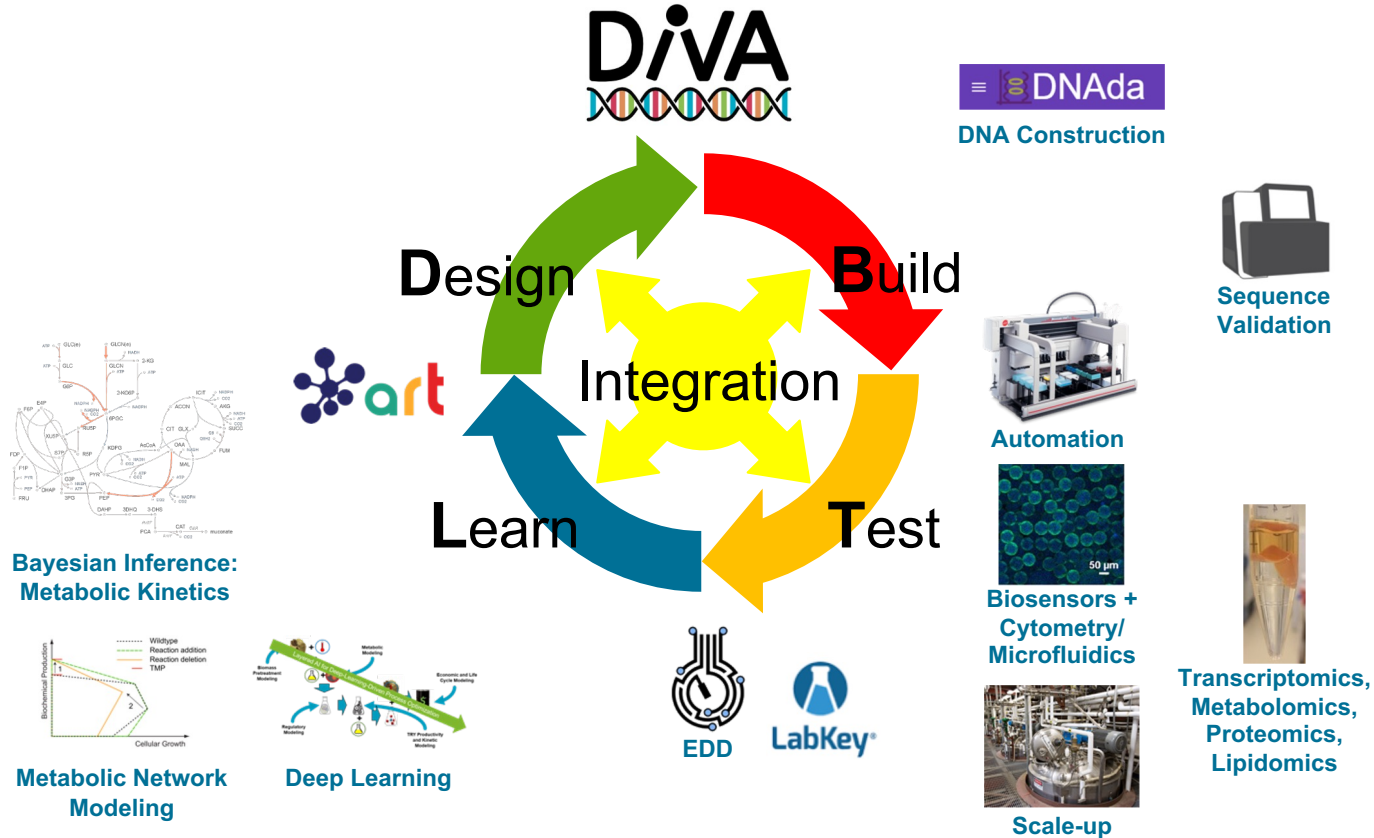
- Collaboration: Industrial and academic collaboration projects will be presented over the next two days. Through the outcomes of these collaborations over time, the ABF endeavors to definitively establish end-to-end impacts on time from bioprocess conception to scale-up and commercialization
- Internal: The ABF, as assessed through target/tool transfer and DBTL efficiency metrics, along with established beachheads and hosts, is itself making good progress towards this goal

- **Subsequent presentations will detail our progress**

- DBTL Infrastructure, Demonstration Projects, and Beachheads
- Industry Engagement and Outreach, and Management
- Host Onboarding and Development
- Process Integration and Scale-Up
- TechnoEconomic Analysis and Life Cycle Assessment

- **The following slides will offer concise highlights thereof**

# Highlights - DBTL Infrastructure

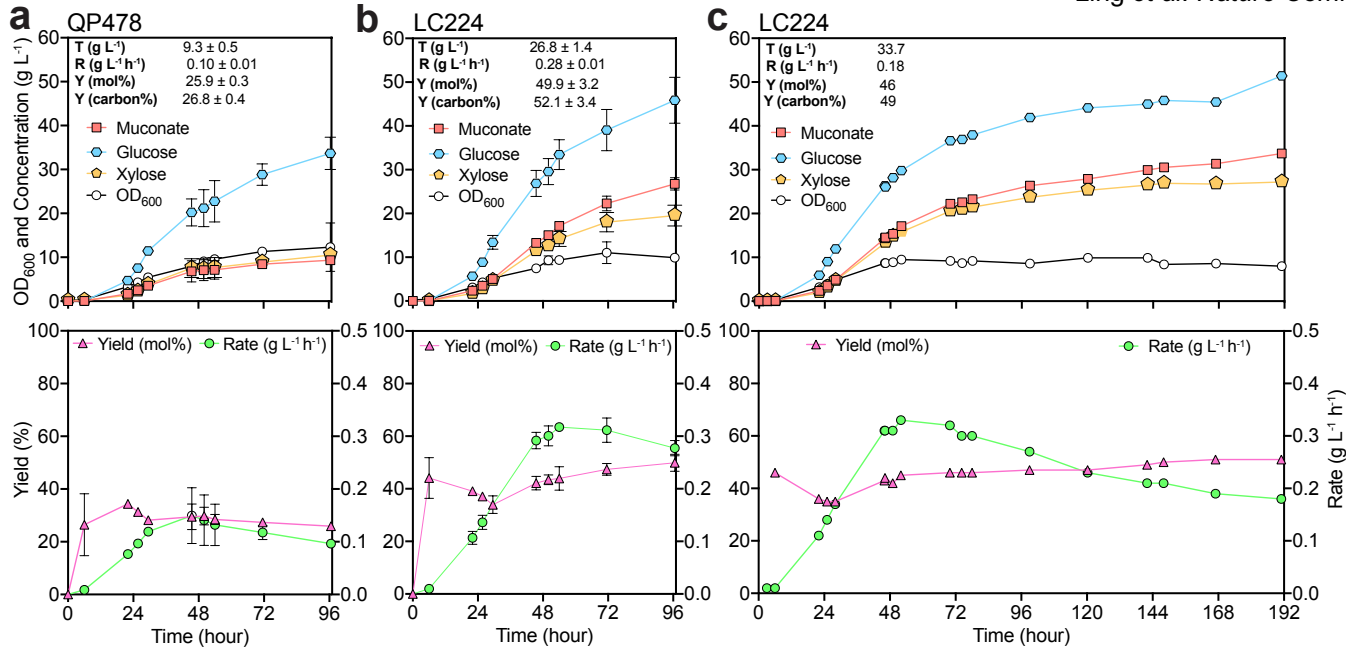




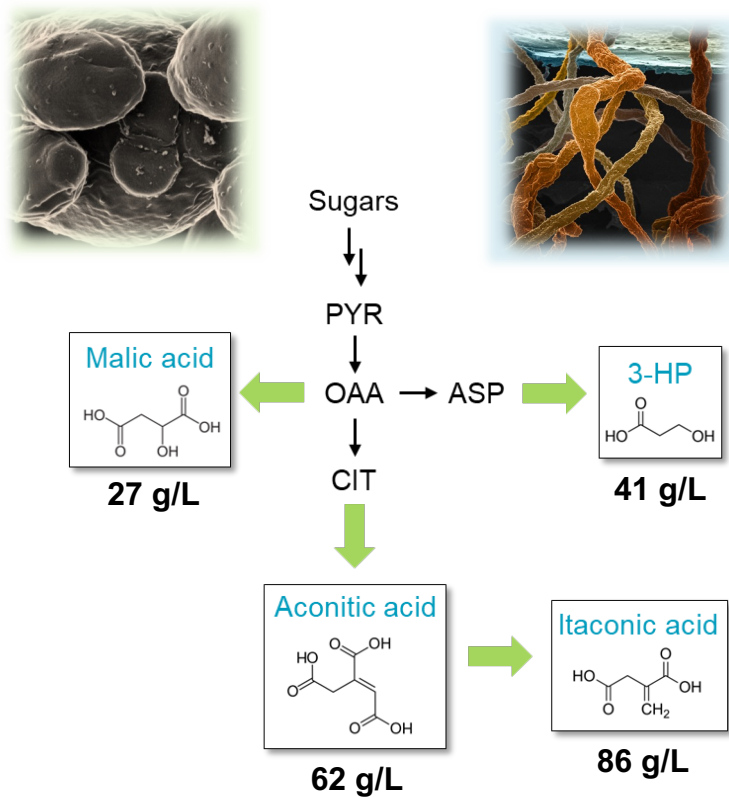
# Highlights – Bacterial Demonstrations

- Engineering xylose utilization enables muconate 33.7 g/L titer at 0.18 g/L/hr
- Ongoing work to utilize arabinose to further increase rate

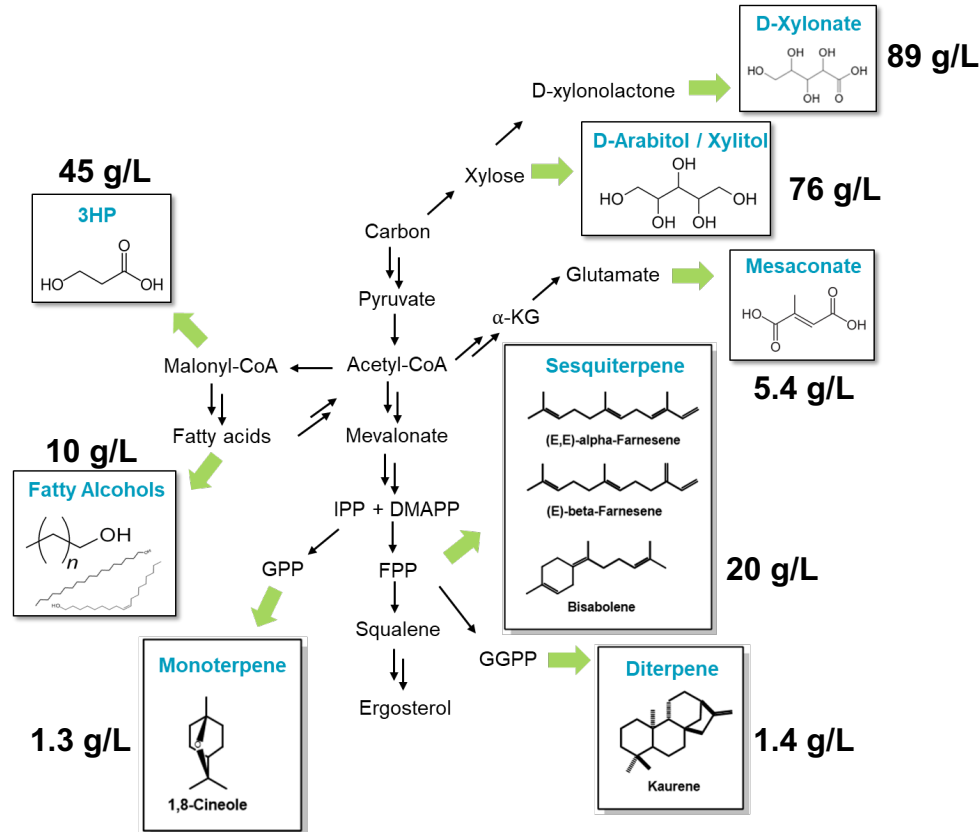
Ling *et al.* Nature Comm. 2022



# Highlights – Fungal Demonstrations



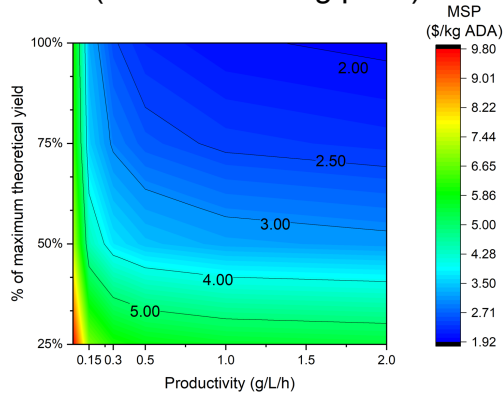
# Highlights – Yeast Demonstrations



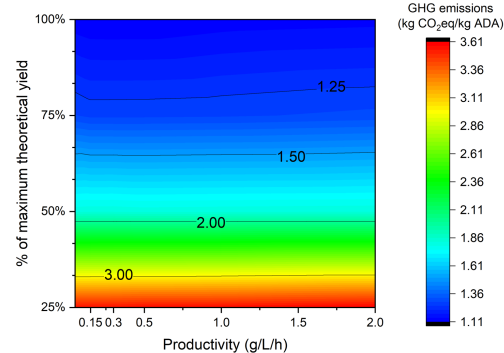
# Highlights – Integrated Analysis

Goal: Develop **techno-economic** and **life cycle analyses (TEA and LCA)** models to quantify the **economic** and **environmental** performance of metabolic pathways of interest to the ABF consortium and provide guidelines for future experimental directions

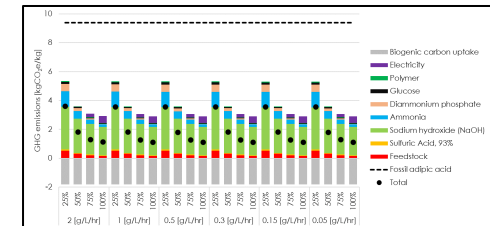
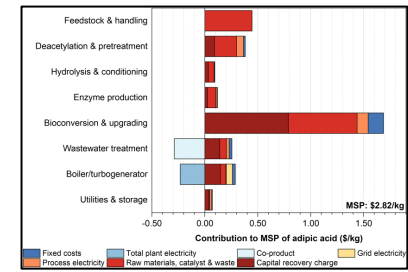
## Economic impact (minimum selling price)



## Environmental impact (greenhouse gas emissions)

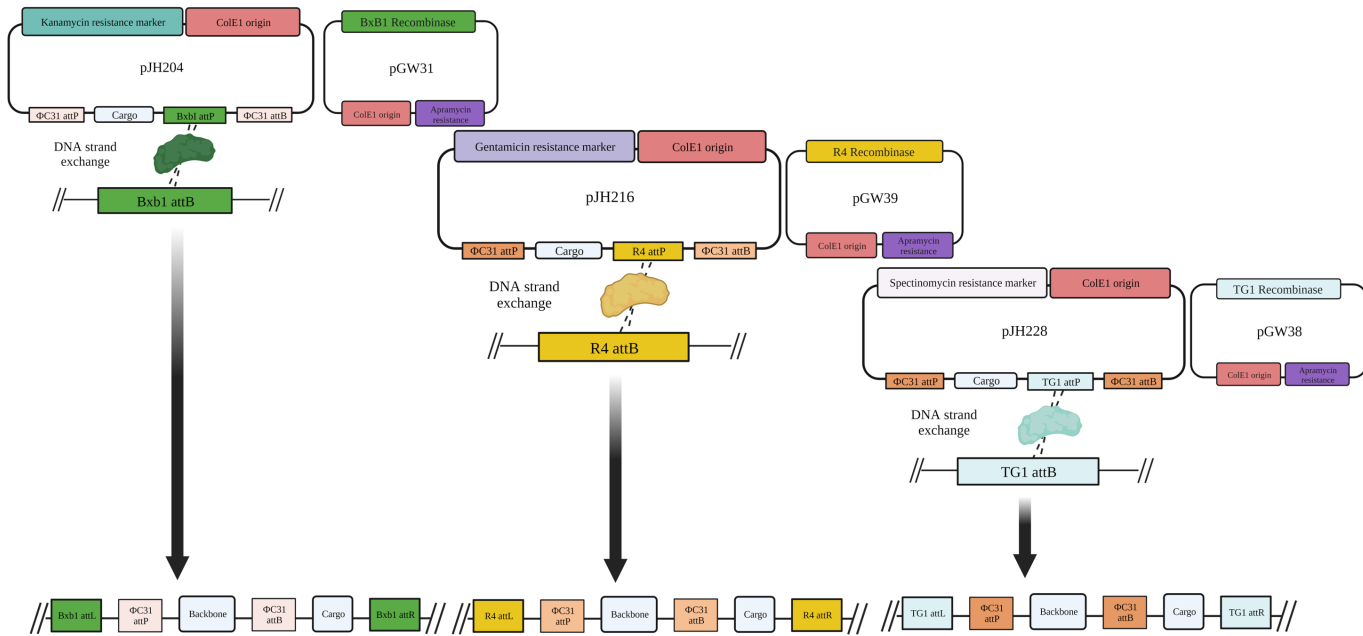


## Breakdown of impacts



- Incorporate key scientific and technical parameters (titer, rate, yield) around product synthesis/recovery steps into integrated process models
- Provide TEA- and LCA-based guidelines to the experimental teams for process development

# Highlights – Host Onboarding and Development



- Expanded “SAGE” DNA integration system to enable high efficiency, simultaneous integration of three plasmids (or libraries of plasmids) into the *P. putida* chromosome
- Simultaneous removal of plasmid backbone allows additional rounds of DNA insertion
- This greatly accelerates strain construction and pathway optimization

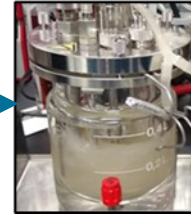
- **Outcome:** Base SAGE technology has been licensed by a company for use in one organism
- **Outcome:** Highly efficient tools that allow combinatorial pathway assembly in the target host for rapid Build

# Highlights – Process Integration and Scale-Up

**Bioprocess development and strain evaluation**



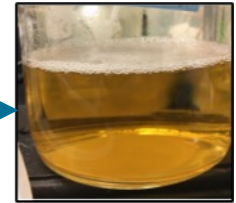
Increase product titers, rates, and yields and testing at different scales



**Lignocellulosic hydrolysate production**

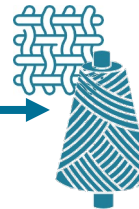


Deacetylation, mechanical refining, enzymatic hydrolysis



**Product delivery to industry and other DOE-BETO funded projects**

Test material properties

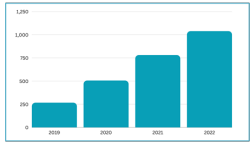


Test downstream product recovery systems

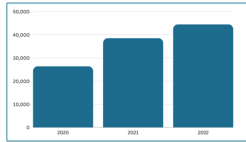


# Highlights – Industry Engagement & Outreach

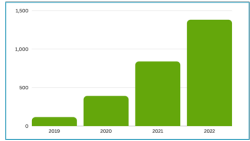
Twitter followers



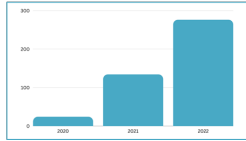
Website pageviews



LinkedIn followers



Mailing list subscribers



## Outreach

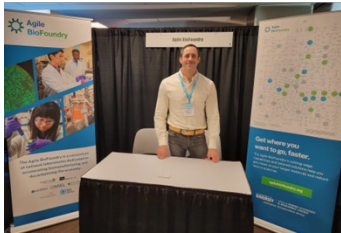
- Website: Main source of visibility to industry
- Newsletter
- Marketing materials
- Social media: LinkedIn, Twitter, YouTube

## Interactions

- IAB Meetings: Quarterly
- Conference attendance
- Annual Industry Day
- Webinar series

## Assessment

- Energy I-Corps Approach
- ~25 Interviews annually
- Surveys



ABF's Christopher Johnson describes our metabolic engineering strategies to enable production targets associated with various beachhead molecules.



Deepti Tanjore, co-lead of ABF's Process Integration and Scaling Task, outlines ABF's fermentation capabilities.

## Companies represented in FY22 roster



# Highlights – Management



Inaugurated partnership with the National Science Foundation, resulting in **6 co-funded projects** in synthetic biology with BETO/ABF-aligned applications.

Selected **3 funds-out projects** as a new initiative to grow relationships with minority-serving institutions (MSIs).

ABF is working to expand access to its capabilities for MSIs and investigators from underrepresented communities.



MSI STEM  
Research & Development  
CONSORTIUM



# Key Milestones

- **FY22Q4 DBTL Infrastructure Annual SMART**
  - Milestone: 2X efficiency improvement in automated DBTL engineering cycle unit operations compared to FY22Q2\_DBTLI\_R1 non-automated baseline efficiencies demonstrated
  - Status: Completed earlier than anticipated in pursuit of related FY21Q4 milestone. Removed through change control, in favor of adding a new milestone for FY23.
- **FY22Q4 Demonstration Project/Beachhead Annual SMART 1**
  - Milestone: At least 10 of the ABF beachheads across onboarded hosts achieve individual titer metrics. Any of the FY21 ABF beachheads that do not enable these production metrics after 1 year of optimization further analyzed to determine whether to continue or sunset based on interest from BETO and industry.
  - Status: Completed, with one beachhead/bioproduct (chorismate/4-aminophenylethanol) sunset.
- **FY22Q4 Demonstration Project/Beachhead Annual SMART 2**
  - Milestone: At least one representative target of a beachhead at a TRY that is able to achieve within 20% of the fossil feedstock incumbent MSP demonstrated, either from DMR-EH hydrolysate or a mock hydrolysate containing hexose and pentose sugars in the appropriate relative concentrations.
  - Status: Achieved for  $\beta$ -ketoadipic acid (BKA).
- **FY22Q4 Host Onboarding and Development Annual SMART**
  - Milestone: Bring a total of at least 15 microbial hosts (15 species) to at least Tier 1, and provide corresponding information, resources, and tools via publicly accessible ABF HObT Website
  - Status: Completed.



# 3 – Impact

# Impact on state of technology/industry if successful

- **Accelerated biomanufacturing commercialization**

- 50% reductions in time to bioprocess scale-up
- Higher probabilities of success, and failing faster, save resources
- Translates to savings of US\$10M(s) and many people years per process
- Quantitative increase in the size (\$\$\$) of the bioeconomy

- **No need to re-establish metabolic routes and hosts**

- Industry can save time and resources, while retaining “last-mile” IP, by building from public beachheads and hosts to bioproducts of interest

- **Greater diversity of publicly available microbial hosts**

- More process conditions and target molecule classes supported through more and increasingly engineerable microbial hosts

- **Likelihood assessments / demonstrations of process transfer**

- De-risked technology transfer across facilities and methods to assess how likely a given process can be successfully transferred

- **Increased access to broadly enabling DBTL infrastructure**

# How disseminating results

- **Impact factor of publications**

- 1900+ citations across 65 publications to date (since FY17)
- 672 citations across 34 publications since FY20
- 14.7 impact factor

- **ROIs, software disclosures, and licenses**

- 17 patent applications
- 10 records of invention
- 9 software disclosures
- 2 licenses

- **Reducing barriers to commercialization**

- ABF collaborators can practice co-developed technologies/processes (CRADA mechanism - exclusive or non-exclusive licensing) or wholly-own developed IP (SPP mechanism)
- ABF's philosophy is to only use technologies that are commercially available or licensable from ABF National Labs, so that collaborators can practice them privately behind their corporate firewalls
- The ABF strives to further reduce barriers to commercialization through technology (transfer across facilities and scales) de-risking

# Summary

- **ABF goal:** Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry to productionize synthetic biology
- **Relevance to BETO's goals:** The ABF empowers BETO to achieve its goals in decreasing development times for industrially-relevant microorganisms, and in advancing bioprocess development
- **Outcomes:** The ABF has developed and demonstrated capabilities that enable commercially-relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts

# Quad Chart Overview

## Timeline

- Project start: *October 1, 2019*
- Project end: *September 30, 2022*

## Project Goal

Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years

## End of Project Milestone

- One representative target at a TRY within 20% of the fossil feedstock incumbent MSP demonstrated, from DMR-EH hydrolysate or mock hydrolysate
- At least 10 of the ABF beachheads across onboarded hosts achieve titer metrics
- Adapt baseline metabolic models for at least 3 ABF hosts to calculate theoretical yield. Calculate the minimum selling price of chemicals to benchmark realistic target chemical markets.
- Bring a total of at least 15 microbial hosts to at least Tier 1 capability, provide corresponding information, resources, and tools via publicly-accessible ABF HOBT website

## Funding Mechanism

AOP

## Project Partners

LBNL (23%), SNL (20%), NREL (18%), PNNL (17%), LANL (8%), ORNL (8%), ANL (6%)

	FY22 Costed	Total Award
DOE Funding	(10/01/2021 – 9/30/2022)	\$15M
Project Cost Share		



# Additional Slides

# Publications, Patents, Presentations, Awards, and Commercialization

## 70 publications, 189 presentations to date

- 54 publications and 115 presentations since FY2019
- The following slides list these publications and presentations

## 17 patents, 10 records of invention, 9 software disclosures, & 2 licenses

- The following slides list these intellectual property assets



# Publications

- Garima Goyal, Zak Costello, Jorge Alonso Guitierrez, Aram Kang, Taek Soon Lee, Hector Garcia Martin, and Nathan J Hillson. (2018) "Parallel Integration and Chromosomal Expansion of Metabolic Pathways" ACS Synthetic Biology DOI: 10.1021/acssynbio.8b00243
- Jha RK, Narayanan N, Pandey N, Bingen JM, Kern TL, Johnson CW, Strauss CEM, Beckham GT, Hennelly SP, Dale T. Sensor-enabled alleviation of product inhibition in chorismate pyruvate-lyase. ACS Synthetic Biology (2019), 10.1021/acssynbio.8b00465.
- Oyetunde, T., Liu, D., Martin, H. G., & Tang, Y. J. "Machine learning framework for assessment of microbial factory performance." PloS one 14.1: e0210558 (2019).
- Ando, D., and Garcia Martin, H. "Genome-Scale 13 C Fluxomics Modeling for Metabolic Engineering of Saccharomyces cerevisiae" Microbial Metabolomics. Humana Press, New York, NY: 317-345 (2019).
- Costello, Zak, and Hector Garcia Martin. "How to Hallucinate Functional Proteins." arXiv preprint arXiv:1903.00458 (2019).
- Chen Y, Vu J, Thompson MG, Sharpless WA, Chan LJG, et al. (2019) A rapid methods development workflow for high-throughput quantitative proteomic applications. PLOS ONE 14(2): e0211582. <https://doi.org/10.1371/journal.pone.0211582>

# Publications

- Paul Opgenorth, Zak Costello, Takuya Okada, Garima Goyal, Yan Chen, Jennifer Gin, Veronica T. Benites, Markus de Raad, Trent R. Northen, Kai Deng, Samuel Deutsch, Edward E.K. Baidoo, Christopher J. Petzold, Nathan J Hillson, Hector Garcia Martin, and Harry R Beller. (2019) "Lessons from two Design-Build-Test-Learn cycles of dodecanol production in *Escherichia coli* aided by machine learning". ACS Synth. Biol., DOI: 10.1021/acssynbio.9b00020 <https://pubs.acs.org/doi/10.1021/acssynbio.9b00020>
- Nathan Hillson, Mark Caddick, Yizhi Cai, Jose A. Carrasco, Matthew Wook Chang, Natalie C. Curach, David J. Bell, Rosalind Le Feuvre, Douglas C. Friedman, Xiongfei Fu, Nicholas D. Gold, Markus J. Herrgård, Maciej B. Holowko, James R. Johnson, Richard A. Johnson, Jay D. Keasling, Richard I. Kitney, Akihiko Kondo, Chenli Liu, Vincent J. J. Martin, Filippo Menolascina, Chiaki Ogino, Nicola J. Patron, Marilene Pavan, Chueh Loo Poh, Isak S. Pretorius, Susan J. Rosser, Nigel S. Scrutton, Marko Storch, Hille Tekotte, Evelyn Travnik, Claudia E. Vickers, Wen Shan Yew, Yingjin Yuan, Huimin Zhao & Paul S. Freemont. (2019) "Building a global alliance of biofoundries". Nature Communications, 10:2040 <https://doi.org/10.1038/s41467-019-10079-2>

# Publications

- Jesus F. Barajas, Ryan P. McAndrew, Mitchell G. Thompson, Tyler W. H. Backman, Bo Pang, Tristan de Rond, Jose H. Pereira, Veronica T. Benites, Héctor García Martín, Edward E. K. Baidoo, Nathan J. Hillson, Paul D. Adams, and Jay D. Keasling. (2019) "Structural insights into dehydratase substrate selection for the borrelidin and fluvirucin polyketide synthases" J Ind Microbiol Biotechnol <https://doi.org/10.1007/s10295-019-02189-z>
- Jesus F. Barajas, Maren Wehrs, Milton To, Lauchlin Cruickshanks, Rochelle Urban, Adrienne McKee, John Gladden, Ed-Been-Goh, Margaret E. Brown, Diane Pierotti, James M. Carothers, Aindrila Mukhopadhyay, Jay D. Keasling, Jeffrey L. Fortman, Steven W. Singer, Constance B. Bailey. (2019) "Isolation and characterization of bacterial cellulase producers for biomass deconstruction: A microbiology laboratory course" J Microbiology and Biology Education. Just accepted. <https://doi.org/10.1128/jmbe.v20i2.1723>
- Mitchel G. Thompson, Jacquelyn Blake-Hedges, Pablo Cruz-Morales, Jesus F. Barajas, Samuel C. Curran, Christopher B. Eiben, Nicholas C. Harris, Veronica T. Benites, Jennifer W. Gin, William A. Sharpless, Jose H. Pereira, Edward E.K. Baidoo, Christopher C. Petzold, Paul D. Adams, Adam P. Arkin, Adam M. Deutschbauer, Jay D. Keasling (2019) "Massively parallel fitness profiling reveals multiple novel enzymes in pseudomonas putida lysine metabolism" MBio. <https://doi.org/10.1128/mBio.02577-18>

# Publications

- Chen, Yan; Guenther, Joel; Gin, Jennifer; Chan, Leanne Jade; Costello, Zak; Ogorzalek, Tadeusz; Tran, Huu; Blake-Hedges, Jacquelyn; Keasling, Jay D; Adams, Paul; Garcia Martin, Hector; Hillson, Nathan; Petzold, Christopher. (2019) "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" Journal of Proteome Research Manuscript ID: pr-2019-00455n Submitted July 8, 2019 Accepted 8/22/2019 <https://doi.org/10.1021/acs.jproteome.9b00455>
- Carbonell, P., Radivojevic, T. and Garcia Martin, H., 2019. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. ACS Synthetic Biology 1474-1477 (2019)
- Roell, G.W., Carr, R.R., Campbell, T., Shang, Z., Henson, W.R., Czajka, J.J., Martín, H.G., Zhang, F., Foston, M., Dantas, G. and Moon, T.S. A concerted systems biology analysis of phenol metabolism in *Rhodococcus opacus* PD630. Metabolic engineering (2019).
- Tijana Radivojevic, Elena Akhmatskaya, "Modified Hamiltonian Monte Carlo for Bayesian inference", Statistics and Computing, <https://doi.org/10.1007/s11222-019-09885-x>
- Pablo Carbonell, Tijana Radivojevic, Héctor García Martín "Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation", ACS Synth. Biol. 2019, 8, 1474-1477

# Publications

- Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachúa, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson, Gregg T. Beckham, "Engineering glucose metabolism for enhanced muconic acid production in *Pseudomonas putida* KT2440", in press at Metabolic Eng.
- Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. Engineered *Pseudomonas putida* KT2440 co-utilizes galactose and glucose. *Biotechnol Biofuels* 12, 295 (2019) doi:10.1186/s13068-019-1627-0
- Christopher B. Eiben, Tristan de Rond, Clayton Bloszies, Jennifer Gin, Jennifer Chiniquy, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Oliver Fiehn, Jay D. Keasling. (2019) "Mevalonate Pathway Promiscuity Enables Noncanonical Terpene Production", *ACS Synth. Biol.* <https://doi.org/10.1021/acssynbio.9b00230>
- Yan Chen, Deepwanita Banerjee, Aindrila Mukhopadhyay, Christopher J. Petzold. (2020) "Systems and synthetic biology tools for advanced bioproduction hosts", *Curr. Op. Biotechnol.* <https://doi.org/10.1016/j.copbio.2019.12.007>

# Publications

- Jacquelyn M. Blake-Hedges, Jose Henrique Pereira, Pablo Cruz-Morales, Mitchell G. Thompson, Jesus F. Barajas, Jeffrey Chen, Rohith N. Krishna, Leanne Jade G. Chan, Danika Nimlos, Catalina Alonso-Martinez, Edward E. K. Baidoo, Yan Chen, Jennifer W. Gin, Leonard Katz, Christopher J. Petzold, Paul D. Adams, Jay D. Keasling. (2019) "Structural Mechanism of Regioselectivity in an Unusual Bacterial Acyl-CoA Dehydrogenase", J. Am. Chem. Soc. <https://doi.org/10.1021/jacs.9b09187>
- Thompson, Mitchell G., Allison N. Pearson, Jesus F. Barajas, Pablo Cruz-Morales, Nima Sedaghatian, Zak Costello, Megan E. Garber et al. "Identification, characterization, and application of a highly sensitive lactam biosensor from *Pseudomonas putida*." ACS Synthetic Biology (2019).
- Production of ent-kaurene from lignocellulosic hydrolysate in *Rhodospiridium toruloides*. Geiselman GM, Zhuang X, Kirby J, Tran-Gyamfi MB, Prah JP, Sundstrom ER, Gao Y, Munoz Munoz N, Nicora CD, Clay DM, Papa G, Burnum-Johnson KE, Magnuson JK, Tanjore D, Skerker JM, Gladden JM. Microb Cell Fact. 2020 Feb 5;19(1):24.

# Publications

- Chen, Yan; Guenther, Joel; Gin, Jennifer; Chan, Leanne Jade; Costello, Zak; Ogorzalek, Tadeusz; Tran, Huu; Blake-Hedges, Jacquelyn; Keasling, Jay D; Adams, Paul; Garcia Martin, Hector; Hillson, Nathan; Petzold, Christopher. (2019) "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" Journal of Proteome Research Manuscript ID: pr-2019-00455n Submitted July 8, 2019 Accepted 8/22/2019 <https://doi.org/10.1021/acs.jproteome.9b00455>
- Carbonell, P., Radivojevic, T. and García Martín, H., 2019. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. ACS Synthetic Biology 1474-1477 (2019)
- Roell, G.W., Carr, R.R., Campbell, T., Shang, Z., Henson, W.R., Czajka, J.J., Martín, H.G., Zhang, F., Foston, M., Dantas, G. and Moon, T.S. A concerted systems biology analysis of phenol metabolism in *Rhodococcus opacus* PD630. Metabolic engineering (2019).
- Pablo Carbonell, Tijana Radivojevic, Héctor García Martín "Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation", ACS Synth. Biol. 2019, 8, 1474-1477

# Publications

- Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachua, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson, Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson, Gregg T. Beckham. Engineering glucose metabolism for enhanced muconic acid production in *Pseudomonas putida* KT2440. *Metabolic Engineering* (2020), 59, 64-75
- Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. Engineered *Pseudomonas putida* KT2440 co-utilizes galactose and glucose. *Biotechnol Biofuels* 12, 295 (2019) doi:10.1186/s13068-019-1627-0
- Radivojevic, T., Costello, Z., Workman, K., & Martin, H. G. (2020). A machine learning Automated Recommendation Tool for synthetic biology. *Nature Communications*, 11(1), 1-14.
- Zhang, Jie, Søren D. Petersen, Tijana Radivojevic, Andrés Ramirez, Andrés Pérez-Manríquez, Eduardo Abeliuk, Benjamín J. Sánchez et al. "Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism." *Nature Communications* 11, no. 1 (2020): 1-13.



# Publications

- Ernst Oberortner, Robert Evans, Xianwei Meng, Sangeeta Nath, Hector Plahar, Lisa Simirenko, Angela Tarver, Samuel Deutsch, Nathan J. Hillson, and Jan-Fang Cheng. (2020) "An Integrated Computer-Aided Design and Manufacturing Workflow for Synthetic Biology". In: Chandran S., George K. (eds) DNA Cloning and Assembly. Methods in Molecular Biology, vol 2205. Humana, New York, NY. [https://doi.org/10.1007/978-1-0716-0908-8\\_1](https://doi.org/10.1007/978-1-0716-0908-8_1)
- Gledon Doçi, Lukas Fuchs, Yash Kharbanda, Paul Schickling, Valentin Zulkower, Nathan Hillson, Ernst Oberortner, Neil Swainston, Johannes Kabisch. (2020) "DNA Scanner: a web application for comparing DNA synthesis feasibility, price, and turnaround time across vendors". OUP Synthetic Biology, ysaa011, <https://doi.org/10.1093/synbio/ysaa011>
- Deng, S., Z. Dai, M. Swita, K.R. Pomraning, B.A. Hofstad, E.A. Panisko, S. Baker and J.K. Magnuson 2020. Deletion analysis of the itaconic acid biosynthesis gene cluster components in *Aspergillus pseudoterreus* ATCC32359. Appl Microbiol Biotechnol 104, 3981-3992.

# Publications

- Yuqian Gao, Thomas L. Fillmore, Nathalie Munoz Munoz, Gayle J. Bentley, Christopher W. Johnson, Jamie Meadows, Meagan C. Burnet, Anna K. Lipton, Aivett Bilbao Pena, Daniel J. Orton, Young-Mo Kim, Jeremy D. Zucker, Joonhoon Kim, Ronald J. Moore, Errol W. Robinson, Scott E. Baker, Bobbie-Jo M. Webb-Robertson, John M. Gladden, Gregg T. Beckham, Jon K. Magnuson, Kristin E. Burnum-Johnson\*, High-throughput targeted proteomics assays for quantifying large-scale pathway proteins in *Pseudomonas putida* KT2440, in press at *Frontiers Bioeng. Biotechnol.*
- Isabel Pardo‡, Ramesh Jha‡, Molly Gaddis, Ryan Bermel, Felicia Bratti, Molly Gaddis, Emily McIntyre, William E. Michener, Ellen L. Neidle, Taraka Dale, Gregg T. Beckham\*, Christopher W. Johnson\*, Gene amplification, laboratory evolution, and biosensor screening reveal Muck as a terephthalic acid transporter in *Acinetobacter baylyi* ADP1, *Metabolic Eng.* (2020), 62, 260-274.
- Joshua R. Elmore, Gara N. Dexter, Davinia Salvachúa, Marykate O'Brien, Dawn M. Klingeman, Kent Gorday, Joshua K. Michener, Darren J. Peterson, Gregg T. Beckham, Adam M. Guss\*, Engineering *Pseudomonas putida* simultaneously catabolizes five major components of lignocellulosic biomass: Glucose, xylose, arabinose, p-coumaric acid, and acetic acid, *Metabolic Eng.* (2020), 62, 62-71.

# Publications

- Chris Lawson, Jose Manuel Martí, Tijana Radivojevic, Sai Vamshi R. Jonnalagadda, Reinhard Gentz, Nathan J. Hillson, Sean Peisert, Joonhoon Kim, Blake A. Simmons, Christopher J. Petzold, Steven W. Singer, Aindrila Mukhopadhyay, Deepti Tanjore, Josh Dunn, and Hector Garcia Martin. (2020) "Machine learning for metabolic engineering: A review" *Metabolic Engineering* <https://doi.org/10.1016/j.ymben.2020.10.005>
- Riley LA and Guss AM\*. Approaches to genetic tool development for rapid domestication of non-model microorganisms. *Biotechnol* (2021) 14:30. doi: 10.1186/s13068-020-01872-z.
- Somtirtha Roy, Tijana Radivojevic, Mark Forrer, Jose Manuel Marti, Vamshi Jonnalagadda, Tyler Backman, William Morrell, Hector Plahar, Joonhoon Kim, Nathan Hillson, and Hector Garcia Martin. (2021) "Multiomics Data Collection, Visualization, and Utilization for Guiding Metabolic Engineering". *Frontiers in Bioengineering and Biotechnology* 9, 45. DOI=10.3389/fbioe.2020.01009

# Publications

- Pomraning, K., Dai, Z., Munoz, N., Kim, Y., Gao, Y., Deng, S., Kim, J., Hofstad, B., Swita, M., Lemmon, T., Collett, J., Panisko, E., Webb-Robertson, B., Zucker, J., Nicora, C., De Paoli, H., Baker, S., Burnum-Johnson, K., Hillson, N., and Magnuson, J. (2021) Integration of Proteomics and Metabolomics Into the Design, Build, Test, Learn Cycle to Improve 3-Hydroxypropionic Acid Production in *Aspergillus pseudoterreus*. *Frontiers in Bioengineering and Biotechnology*.
- Hector A. Plahar, Thomas N. Rich, Stephen D. Lane, William C. Morrell, Leanne Springthorpe, Oge Nnadi, Elena Aravina, Tiffany Dai, Michael J. Fero, Nathan J. Hillson, and Christopher J. Petzold. (2021) BioParts-A Biological Parts Search Portal and Updates to the ICE Parts Registry Software Platform"" ACS Synthetic Biology DOI: 10.1021/acssynbio.1c00263"
- Chen Ling, George L. Peabody, Davinia Salvachúa, Young-Mo Kim, Colin M. Kneucker, Michela A. Monninger, Nathalie Munoz, Brenton C. Poirier, Kelsey J. Ramirez, Peter C. St. John, Sean P. Woodworth, Jon K. Magnuson, Kristin E. Burnum-Johnson, Gregg T. Beckham\*, Adam M. Guss,\* Christopher W. Johnson,\* Muconic acid production from glucose and xylose in *Pseudomonas putida* KT2440 via evolution and metabolic engineering, in review at *Nature Comm.*

# Publications

- Nicholas A. Rorrer,‡ Sandra F. Notonier,‡ Brandon C. Knott,‡ Brenna A. Black,‡, Avantika Singh,‡ Scott R. Nicholson,‡ Christopher P. Kinchin, Graham P. Schmidt, Alberta C. Carpenter, Kelsey J. Ramirez, Christopher W. Johnson, Davinia Salvachúa, Michael F. Crowley, Gregg T. Beckham\*, Production of B-ketoadipic acid from glucose in *Pseudomonas putida* KT2440 for use in performance-advantaged nylons, in review at Cell Reports Phys. Sci.
- Precise genomic riboregulator control of metabolic flux in microbial systems. Naresh Pandey, Steffi A. Davison, Malathy Krishnamurthy, Daniel Trettel, Chien-Chi Lo, Shawn Starkenburg, Katherine L. Wozniak, Theresa Kern, Sean D. Reardon, Clifford J. Unkefer, Scott P. Hennelly and Taraka Dale. Submitted to ACS Synthetic Biology December 2021.
- Chen Y, Kaplan Lease N, Gin JW, Ogorzalek TL, Adams PD, Hillson NJ, et al. (2022) Modular automated bottom-up proteomic sample preparation for high-throughput applications. PLoS ONE 17(2): e0264467. <https://doi.org/10.1371/journal.pone.0264467>

# Publications

- Making Security Viral: Shifting Engineering Biology Culture and Publishing Rebecca Mackelprang, Katarzyna P. Adamala, Emily R. Aurand, James C. Diggans, Andrew D. Ellington, Samuel Weiss Evans, J. L. Clem Fortman, Nathan J. Hillson, Albert W. Hinman, Farren J. Isaacs, June I. Medford, Shadi Mamaghani, Tae Seok Moon, Megan J. Palmer, Jean Peccoud, Elizabeth A. Vitalis, India Hook-Barnard, and Douglas C. Friedman ACS Synthetic Biology 2022 11 (2), 522-527 DOI: 10.1021/acssynbio.1c00324
- Hyun Gyu Lim, Kevin Rychel, Anand V. Sastry, Gayle J. Bentley, Joshua Mueller, Heidi S. Schindel, Peter E. Larsen, Philip D. Laible, Adam M. Guss, Wei Niu, Christopher W. Johnson, Gregg T. Beckham, Adam M. Feist, Bernhard Palsson, Machine-learning from Pseudomonas putida KT2440 transcriptomes reveals its transcriptional regulatory network, Metabolic Eng. (2022) 72, 297-310.
- Lim HG, Rychel K, Sastry AV, Bentley GJ, Mueller J, Schindel HS, Larsen PE, Laible PD, Guss AM, Niu W, Johnson CW, Beckham GT, Feist AM, Palsson BO. Machine-learning from Pseudomonas putida KT2440 transcriptomes reveals its transcriptional regulatory network. Metab Eng. 2022 Jul;72:297-310. doi: 10.1016/j.ymben.2022.04.004. Epub 2022 Apr 27.

# Publications

- Chen Ling, George L. Peabody, Davinia Salvachúa, Young-Mo Kim, Colin M. Kneucker, Michela A. Monninger, Nathalie Munoz, Brenton C. Poirier, Kelsey J. Ramirez, Peter C. St. John, Sean P. Woodworth, Jon K. Magnuson, Kristin E. Burnum-Johnson, Adam M. Guss,\* Christopher W. Johnson,\* Gregg T. Beckham\*, Muconic acid production from glucose and xylose in *Pseudomonas putida* KT2440 via evolution and metabolic engineering, *Nature Comm.* (2022) 13, 4925
- Hector Garcia Martin\*, Tijana Radivojevic, Jeremy Zucker, Kristofer Bouchard, Jess Sustarich, Sean Peisert, Dan Arnold, Nathan Hillson, Gyorgy Babnigg, Jose Manuel Marti, Christopher J. Mungall, Gregg T. Beckham, Lucas Waldburger, James Carothers, ShivShankar Sundaram, Deb Agarwal, Blake A. Simmons, Tyler Backman, Deepanwita Banerjee, Deepti Tanjore, Lavanya Ramakrishnan, Anup Singh, Perspectives for self-driving labs in synthetic biology, submitted to *Curr. Opin. Biotech*
- N. Pandey, S.A. Davison, M. Krishnamurthy, D.S. Trettel, C. Lo, S. Starckenburg, K.L. Wozniak, T.L. Kern, S.D. Reardon, C.J. Unkefer, S.P. Hennelly and T. Dale. 2022. Precise genomic riboregulator control of metabolic flux in microbial systems. *ACS Syn. Biol*  
<https://doi.org/10.1021/acssynbio.1c00638>

# Publications

- Pomraning K.R., Z. Dai, N. Munoz Munoz, Y. Kim, Y. Gao, S. Deng, and T.L. Lemmon, et al. 2022. "Itaconic acid production is regulated by *laeA* in *Aspergillus pseudoterreus*." *Metabolic Engineering Communications*
- Kyle R. Pomraning; Shuang Deng; Joonhoon Kim; Kristen B. Campbell; Ana L. Robles; Beth Hofstad; Nathalie Munoz; Yuqian Gao; Teresa Lemmon; Marie S. Swita; Jeremy D. Zucker; Young-Mo Kim; Kristin E. Burnum-Johnson; Jon K. Magnuson; Ziyu Dai. 2022 "Metabolic engineering to improve production of 3-hydroxypropionic acid from corn-stover hydrolysate in *Aspergillus* species." *Metabolic Engineering*
- Kevin J. McNaught, Eugene Kuatsjah, Michael Zahn, Érica T. Prates, Huiling Shao, Gayle J. Bentley, Andrew R. Pickford, Josephine N. Gruber, Kelley V. Hestmark, Daniel A. Jacobson, Brenton C. Poirier, Chen Ling, Myrsini San Marchi, William E. Michener, Carrie D. Nicora, Jacob N. Sanders, Caralyn J. Szostkiewicz, Dušan Veličković, Mowei Zhou, Nathalie Munoz, Young-Mo Kim, Jon K. Magnuson, Kristin E. Burnum-Johnson, K.N. Houk, John E. McGeehan, Christopher W. Johnson, Gregg T. Beckham, "Initiation of fatty acid biosynthesis in *Pseudomonas putida* KT2440", *Metabolic Engineering*, Volume 76, 2023, Pages 193-203, <https://doi.org/10.1016/j.ymben.2023.02.006>



# Presentations

- Gregg Beckham, Hybrid biological and catalytic processes to manufacture and recycle plastics, Princeton University, November 28th, 2018
- Nathan J. Hillson. “DOE Agile BioFoundry Overview”. Invited Talk, SynBioBeta 2018 visit to ESE, Emeryville, CA, October 1, 2018
- Nathan J. Hillson. “Recent developments at the U.S Department of Energy Agile BioFoundry”. Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 7, 2018
- Nathan J. Hillson. “DIVA (DNA Design, Implementation, Validation Automation) Platform”. Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 8, 2018
- Garcia Martin, H. “Towards a predictive synthetic biology enabled by machine learning and automation”. Ginkgo Bioworks, Boston, MA, November 12, 2018; AIChE annual meeting, Pittsburgh, PA, October 31 2018; Thermo Fisher, San Jose, CA, October 19, 2018; DTRA Tech Watch, Ft. Belvoir, VA, October 10, 2018.
- Garcia Martin, H. “A New Approach to Flux Analysis”. ABF Annual Meeting, Berkeley CA, September 7, 2018.
- Nathan J. Hillson. “BioDesign Department Overview”. Invited Talk, BSE Annual Meeting, Berkeley, CA, January 24, 2019

# Presentations

- Nathan J. Hillson. “Agile BioFoundry Overview”. Invited Talk, BETO Peer Review, Denver, CO, March 7, 2019
- Nathan J. Hillson. “Agile BioFoundry DBTL Infrastructure”. Invited Talk, BETO Peer Review, Denver, CO, March 7, 2019
- Nathan J. Hillson. “DNA Synthesis Science at the U.S. DOE Joint Genome Institute: Biosecurity Sequence Screening and Broader Aspects Review”. Invited Talk, EBRC Spring Retreat 2019, Boston, MA, March 23, 2019
- Nathan J. Hillson. “ABF: plans for the next 3-year cycle”. Invited Talk, BETO Quarterly FY19Q2, Washington DC, March 27, 2019
- Jennifer Chiniquy. “Emery Station East DIVA DNA Sequencing”. Invited Talk, BSE Annual Meeting, Berkeley, CA, January 24, 2019
- Garcia Martin, H. " Towards a predictive synthetic biology enabled by machine learning and automation". EmeryStation Campus, Emeryville, CA, February 7th, 2019.
- Garcia Martin, H. "Metabolic modeling, drug synthesis, and their interaction". LBNL Biosciences Experts Advisory Committee, Berkeley, CA, February 8th, 2019.
- Garcia Martin, H. " Towards a predictive synthetic biology enabled by machine learning and automation". Google X, Mountain View, CA, February 14th, 2019.

# Presentations

- Garima Goyal, Z. Costello, J.A. Gutierrez, A. Kang, T.S. Lee, H.G. Martin, and N.J. Hillson. “PIACE: Parallel Integration and Chromosomal Expansion of metabolic Pathways”. Invited Talk, ACS Conference, Orlando, Florida, April 4, 2019
- Nathan J. Hillson. “DNA synthesis use and biosecurity screening at U.S. DOE projects including the Joint Genome Institute, Joint BioEnergy Institute, and Agile BioFoundry”. Invited Talk, Gene Synthesis Governance Meeting, Johns Hopkins Center for Health Security, St. Regis Hotel, Washington, DC, April 9, 2019
- Nathan J. Hillson. “DOE Agile BioFoundry: Overview and Recent Highlights”. Invited Talk, SBFC 2019 Session ST-2: Global Research Consortia, Seattle, WA, May 1, 2019
- Nathan J. Hillson. “2019 BETO Merit Review Process”. Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. “Machine Learning-assisted MiSeq library loading”. Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. “Global Biofoundries Alliance: 2019 Annual Meeting Report Out”. Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019
- Nathan J. Hillson. “2019 BETO Peer Review Report Out”. Invited Talk, ABF IAB FY19Q3, Zoom Videoconference, May 17, 2019

# Presentations

- Nathan J. Hillson. "ICE/DIVA, EDD, and ART". Invited Talk, Software for Synthetic Biology Workflows Workshop, SEED 2019, New York, NY, June 27, 2019
- Nathan J. Hillson. "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, JBEI Annual Meeting 2019, Monterrey, CA, May 30, 2019
- Henrique C. De Paoli. "Overview of Synthetic Biology principles". Invited Talk, Xu's Research Group, UC Dept. of Materials Science and Engineering, Berkeley, CA, May 13, 2019.
- Nathan J. Hillson "ABF: CRADA updates and FY20-22". Invited Talk, BETO Conversion Call, July 1, 2019
- Wei Xiong and Nathan Hillson, "Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis". Invited Talk, BETO Conversion Call, July 8, 2019
- Ernst Oberortner, Nathan J. Hillson, and Jan-Fang Cheng. "The Operon Refactoring and Construction Assistant (ORCA): Streamlined gene cluster refactoring". Invited Talk, 11th International Workshop on Bio-Design Automation, University of Cambridge, UK July 9, 2019
- Nathan J. Hillson "ABF Overview, FY19 Update, and FY20-22 Plans". Invited Talk, ABF Annual Meeting, Richland, WA, July 30, 2019
- Nathan J. Hillson "FY22Q4\_DBTL\_AS1: 5x efficiency improvements". Invited Talk, ABF Annual Meeting, Richland, WA, July 31, 2019

# Presentations

- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, VIP Visit - Wendy Pulling | Director of ESG Integration University of California Office of the Chief Investment Officer, Emeryville, CA, Aug 6, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: Overview and Recent Highlights". Invited Talk, Tiangong Forum Distinguished Lecture, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 16, 2019
- Nathan J. Hillson "DOE Agile BioFoundry: DBTL Infrastructure". Invited Talk, SynBioYSF 2019, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 17, 2019
- Nathan J. Hillson "U.S. DOE Agile BioFoundry: Overview and Recent Highlights", Invited Talk, Genscript Double Helix Symposium 2019, San Francisco, CA September 30, 2019
- Jennifer Chiniquy "DIVA DNA Sequencing". Invited Talk, 2019 ABF All Hands Meeting, Pacific Northwest National Laboratory, Richland, WA, July 30, 2019.
- Tijana Radivojevic, "Automatic Recommendation Tool", Invited Talk, ABF Annual Meeting 2019, Richland, WA, July 30, 2019
- Nurgul Kaplan."Automated DNA Construction: "from j5 protocol design to Laboratory Robotics" Invited Talk, ABF Annual Meeting 2019, Pasco, WA, July 30, 2019

# Presentations

- Nathan J. Hillson “U.S. DOE Agile BioFoundry: Organization and Capabilities”, Invited Talk, ABF Industry Day 2019, Emeryville, CA October 4, 2019
- Garcia Martin, H. “Machine Learning, Synthetic Biology and Automation: Engineering Life for the Benefit of Society”. NERSC data seminar, Berkeley CA, November 1st, 2019.
- Garcia Martin, H. “ART: a machine learning Automated Recommendation Tool for guiding synthetic biology”. AI4Synbio Symposium, Arlington VA, November 8th, 2019.
- Garcia Martin, H. “Opportunities in the intersection of: Artificial Intelligence & Synthetic Biology & Automation”. Army Science Planning and Strategy Meeting, Burlington MA, November 13th, 2019.
- Nathan J. Hillson “ABF: CRADA updates and FY20-22”. Invited Talk, BETO Conversion Call, July 1, 2019
- Wei Xiong and Nathan Hillson, “Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis”. Invited Talk, BETO Conversion Call, July 8, 2019
- Ernst Oberortner, Nathan J. Hillson, and Jan-Fang Cheng. “The Operon Refactoring and Construction Assistant (ORCA): Streamlined gene cluster refactoring”. Invited Talk, 11th International Workshop on Bio-Design Automation, University of Cambridge, UK July 9, 2019

# Presentations

- Nathan J. Hillson “ABF Overview, FY19 Update, and FY20-22 Plans”. Invited Talk, ABF Annual Meeting, Richland, WA, July 30, 2019
- Nathan J. Hillson “FY22Q4\_DBTL\_AS1: 5x efficiency improvements”. Invited Talk, ABF Annual Meeting, Richland, WA, July 31, 2019
- Nathan J. Hillson “DOE Agile BioFoundry: Overview and Recent Highlights”. Invited Talk, VIP Visit - Wendy Pulling | Director of ESG Integration University of California Office of the Chief Investment Officer, Emeryville, CA, Aug 6, 2019
- Nathan J. Hillson “DOE Agile BioFoundry: Overview and Recent Highlights”. Invited Talk, Tiangong Forum Distinguished Lecture, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 16, 2019
- Nathan J. Hillson “DOE Agile BioFoundry: DBTL Infrastructure”. Invited Talk, SynBioYSF 2019, Tianjin Institutes of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS), Tianjin, China, August 17, 2019
- Nathan J. Hillson “U.S. DOE Agile BioFoundry: Overview and Recent Highlights”, Invited Talk, Genscript Double Helix Symposium 2019, San Francisco, CA September 30, 2019
- Jennifer Chiniquy “DIVA DNA Sequencing”. Invited Talk, 2019 ABF All Hands Meeting, Pacific Northwest National Laboratory, Richland, WA, July 30, 2019.

# Presentations

- Tijana Radivojevic, “Automatic Recommendation Tool”, Invited Talk, ABF Annual Meeting 2019, Richland, WA, July 30, 2019
- Nurgul Kaplan.”Automated DNA Construction: “from j5 protocol design to Laboratory Robotics” Invited Talk, ABF Annual Meeting 2019, Pasco, WA, July 30, 2019
- ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- Garcia Martin, H. “Opportunities in the intersection of machine learning, synthetic biology, and automation”. ABLC 2020, Virtual meeting, July 10th, 2020.
- Garcia Martin, H. “Leveraging machine learning and automation to make synthetic biology predictable”. SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020
- Nathan J. Hillson, "Perspectives from the U.S. DOE Agile BioFoundry”, OECD BNCT Virtual Workshop, Session 1: Biofoundries and COVID-19, via Zoom, July 29, 2020
- Nathan J. Hillson, "DIVA, EDD, and ART: Software spanning the Design/Build/Test/Learn cycle", invited talk, COMBINE 2020, via Zoom, October 5, 2020



# Presentations

- Nathan J. Hillson, "DIVA, EDD, and ART: Software spanning the Design/Build/Test/Learn cycle", invited talk, SPARC Workshop (IIT Kharagpur) 2020, via Zoom, October 19, 2020
- Invited talk: Guss AM. "Domestication of non-model microbes for the production of renewable fuels and chemicals" (2021) The Metabolic Engineering Virtual Seminar Series at the University of Texas-Austin. Virtual.
- Garcia Martin, H. "Leveraging machine learning and automation to make bioengineering predictive". The Metabolic Engineering Virtual Seminar, UT Austin, February 12th, 2021.
- Garcia Martin, H. "Machine Learning for Bioengineering". JBEI Board of Directors, March 12th, 2021.
- Nathan J. Hillson, "ABF Overview", invited talk, BETO Peer Review 2021, via Zoom, March 9, 2021
- Nathan J. Hillson, "ABF DBTL Infrastructure", invited talk, BETO Peer Review 2021, via Zoom, March 9, 2021
- Nathan J. Hillson, "ABF Overview", invited talk, BSA ExComm, via Zoom, March 12, 2021
- Guss AM. "Synthetic biology and metabolic engineering of non-model microbes for the production of renewable fuels and chemicals" Seminar at Purdue University Northwest. April 16, 2021.

# Presentations

- Radivojevic, T., “Automated Recommendation Tool (ART): Design of Experiments via Probabilistic Predictive Modeling”, Autonomous Discovery in Science and Engineering Workshop, April 21, 2021 (Invited Tutorial)
- Radivojevic, T., “Guiding synthetic biology via machine learning”, Symposium on Biomaterials, Fuels and Chemicals (SBF): Speeding up synthetic biology, April 27, 2021
- Radivojevic, T., “Guiding synthetic biology via Automated Recommendation Tool (ART)”, Synthetic Biology: Engineering, Evolution & Design (SEED) 2021, Computation, Artificial Intelligence, and Machine Learning for Biological Design Session, June 17, 2021 (Invited Speaker)
- Guss AM. “Domestication of non-model microbes for the production of renewable fuels and chemicals” Student-invited seminar at North Carolina State University. September 27, 2021.
- Radivojevic, T. “Guiding synthetic biology via machine learning and multi-omics technologies”, IWBD 2021, 13th International Workshop on Bio-Design Automation, September 20, 2021 (virtual)
- Hillson, Nathan J. “Session Introduction”, Metabolic Engineering 14, Block 8: Session – Biofoundries, (Virtual via Remo), July 15, 2021

# Presentations

- Hillson, Nathan J. “Updates on DOE assets including Agile BioFoundry, Joint BioEnergy Institute, and Joint Genome Institute”, Digital Biology Engineering Meeting (Air Force) via zoom, September 23, 2021
- Garcia Martin, H. “Machine Learning Tools Can Make Synthetic Biology Predictable”. SIMB, August 9th, 2021.
- Garcia Martin, H. “Leveraging machine learning and automation to make bioengineering predictable”. Indo-US Workshop on Application of Data Science in Biological Systems, September 7th, 2021.
- Garcia Martin, H. “Leveraging machine learning and automation to make bioengineering predictable”. Air Force Research Laboratory MIRAACLE Forum, September 10th, 2021.
- Garcia Martin, H. “Challenges and opportunities in high-throughput data synthesis”. NSF Challenges and Opportunities in Synthesizing Massively Parallel Assays and High-Throughput Datasets Workshop, September 17th, 2021.
- Garcia Martin, H. “Guiding metabolic engineering via kinetic deep learning and multi-omics”. Quantitative Modelling of Cell Metabolism Conference, September 21th, 2021.
- Davison, S. Reprogramming Microbes for Biomanufacturing, Science in 3 at Los Alamos National Laboratory, September 2021

# Presentations

- Wozniak, K. Engineering a Sustainable Future: Fine-tuning Gene Expression for Biomanufacturing, Science in 3 at Los Alamos National Laboratory, September 2021
- Davison, S. Fine-tuning gene expression in bioproduction pathways in diverse bacterial hosts, SIMB Annual Meeting, August.
- Wozniak, K. Targeting global regulatory responses using precise and programmable cis-riboregulators SIMB Annual Meeting, August
- Adam Guss. “Genetic tools and microbial engineering for biological production of sustainable fuels and chemicals” Presented to Weekly Seminar for DOE CCI/SULI Students. October 27, 2021
- Adam Guss. “Domestication of diverse non-model microbes for plastics upcycling and sustainable fuel and chemical production” Biological Sciences Departmental Seminar, Michigan Technical University. Oct 28, 2021.
- Garcia Martin, H. “Guiding metabolic engineering via kinetic deep learning and multi-omics”. Quantitative Modelling of Cell Metabolism Conference, October 20th, 2021.
- Garcia Martin, H. “Machine learning for industrial biotechnology ”. Delft Advanced Course Integrated Multi-Omics approaches for Improvement of Industrial Microbes, November 5th, 2021.

# Presentations

- Hillson, Nathan J. “Opportunities for CABBI collaboration with JBEI (and other DOE assets) in laboratory operations and data management infrastructure”, CABBI seminar, via zoom, October 19, 2021
- Hillson, Nathan J. “Automation in Biotechnology: Challenges and Opportunities”, Panelist at SENAI CETIQT (Brazil), via zoom, October 29, 2021
- Hillson, Nathan J. “ABF Overview and Capabilities”, ABF Industry Day (via zoom), November 19, 2021
- Hector A Plahar, Stephen D. Lane, William C Morrell, Nathan J. Hillson and Christopher J. Petzold. “A Biological Parts Search Portal and Updates to the ICE Parts Registry Platform”, JBEI Annual DOE Review, Dec 6-8, 2021
- Tijana Radivojevic, “Creating a Machine Learning Chassis to Maximize the Efficiency of the DBTL Cycle in Synthetic Biology”, Synthetic Biology-Based Therapeutics Summit, December 9, 2021 (virtual)
- Hillson, Nathan J. “Overview and capabilities of the ABF and opportunities for collaboration with CSU”, Colorado State Seminar Series (via zoom), February 3, 2022
- Hillson, Nathan J. “ABF prospective collaboration with other BETO consortia and projects”, BETO Conversion Call (via MS Teams), March 7, 2022

# Presentations

- Bilbao, A. "Investigating deep learning approaches to advance data processing in Liquid Chromatography, Ion Mobility and Data-Independent Acquisition Mass Spectrometry Omics". 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 9th, 2022.
- Dai Z., K.R. Pomraning, S. Deng, J. Kim, K.B. Campbell, A.L. Robles, and B.A. Hofstad, et al. 05/02/2022. "Assessment and Optimization of 3-hydroxypropionic acid production in industrial filamentous fungus-Aspergillus species." Presented by Z. Dai at 44th Symposium on Biotechnology for Fuels and Chemicals (SBFC 2022), New Orleans, Louisiana
- Adam Guss. "High efficiency DNA integration in diverse non-model microbes for rapid tool and pathway prototyping" SIMB SBFC. New Orleans, LA, May 3, 2022
- Magnuson, J.K.; Beckham, G.T.; Gladden, J.M.; Dale, T.; Guss, A.M.; Laible, P.; Hillson, N.J. 05/03/2022. "The Agile BioFoundry" Presented by Jon Magnuson at the 44th Symposium on Biotechnology for Fuels and Chemicals (SBFC 2022), New Orleans, Louisiana.
- Poirier, B.C. et al. "Mechanical cell disruption enhances the extraction of metabolites from bacterial and fungal species commonly used in metabolic engineering". 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 5-9, 2022.

# Presentations

- Poirier B.C. et al. “Behavior of lactam molecules during aqueous sample preparation and mass spectrometry analysis”. 70th ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis, Minnesota, June 5-9, 2022.
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", ABF Webinar (via zoom) April 29, 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", Lessafre visit to ESE (via zoom) May 6, 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry" (Keynote Presentation) Bioindustry 4.0 – Synthetic Biology & Biofoundry, CHEY Institute – Scientific Innovation Series (via zoom) 12 May 17 (PDT) 18 (KST), 2022
- Hillson, Nathan J. "Overview and Capabilities of the DOE Agile BioFoundry", Wageningen University visit to ESE (via zoom) May 31, 2022
- Hillson, Nathan J. “ABF overview and DBTL Infrastructure”, BETO Conversion Call (via MS Teams), June 13, 2022
- Nathan Hillson. “ABF Decarbonization Efforts”, Invited Talk, LBL Carbon Negative Initiative – Biological Applications – Lightning Talk Session (via zoom) 6/27/2022

# Presentations

- Radivojevic, Tijana. “Guiding synthetic biology via machine learning”, Boston University, April 14, 2022, Guest lecture
- Deng S., J. Kim, K.R. Pomraning, Z. Dai, Y. Gao, N. Munoz Munoz, and Y. Kim, et al. 08/07/2022. "Identification of a specific exporter that enables high production of aconitic acid in *A. pseudoterreus* ATCC32359." Presented by S. Deng at SIMB annual meeting, San Francisco, California.
- Pomraning K.R., Z. Dai, S. Deng, N. Munoz Munoz, Y. Kim, B.A. Hofstad, and Y. Gao, et al. 08/10/2022. "Bioconversion of lignocellulosic feedstocks to 3-hydroxypropionic acid using acidophilic fungi." Presented by K.R. Pomraning at Society for Industrial Microbiology and Biotechnology, San Francisco, California.
- Jha RK. High throughput test tools for industrially relevant microbial chassis, SIMB 2022, San Francisco, August 2022 (invited talk).
- Adam Guss. “Using synthetic biology to solve challenges in plastic waste and renewable chemical production”. Biological Sciences Departmental Seminar, Missouri S&T, Rolla, MO. September 27, 2022.
- Nathan Hillson. “Agile BioFoundry Connections with NSF, MSRDC, and Beyond”, Invited Talk, LBL Biosciences Expert Advisory Committee: connections beyond Biosciences July 8, 2022



# Presentations

- Nathan Hillson. “Biosecurity concern anecdote”, Screening Tools for Genome Engineering and Genome Editing (Inscripta-funded; Sarah Carter organized), via MS Teams, 9/9/2022
- Nathan Hillson. “Webinar Agenda and ABF Overview”, ABF Webinar: Cutting-Edge Technologies for Accelerating Bioproduct Development at the Agile BioFoundry, 9/22/2022
- Garcia Martin, H. “Machine Learning and Automation for Predictive Synthetic Biology”. Basque Center for Applied Mathematics, Bilbao, Spain, July 15th, 2022.

# License partners and patent applications

## Technologies licensed

- Bioproduction of limonene from syngas
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids

## Provisional Patents

- ROI-18-92 U.S. provisional patent applications 63/163,518 63/321,207 63/479,918, not published
- ROI-21-104 U.S. provisional patent application 63/321,332
- ROI-21-63 U.S. provisional patent applications 63/163,518 63/321,207 63/479,918, not published

## Patent Applications

- Post-transcriptional genome regulation in bacteria with next generation CRISPR-Cas tools
- Terephthalate biosensor and applications thereof
- Mutant transporters for bacterial uptake of terephthalic acid
- Alleviating the bottleneck in enzyme evolution and pathway optimization using novel biosensors (Disclosure Title) Modified Biosensors and Biocatalysts and Methods of Use (Application Title)
- Mutant transporters for bacterial uptake of terephthalic acid
- ART: A machine learning Automated Recommendation Tool for guiding synthetic biology

# Patent applications

## Patent Applications (cont.)

- A Generative Model for Protein Sequences for the Purpose of Protein Design or Phenotypic Inference
- Predicting Metabolic Pathway Dynamics from Time Series Multiomics Data Using Machine Learning Techniques
- Use of Statistical Learn Approaches to Predict Next Generation Sequencing Subsequence Depth of Coverage
- Mutant transporters for bacterial uptake of terephthalic acid
- Method and strain for sugar conversion
- Engineered Microorganisms for the Production of Intermediates and Final Products (1<sup>st</sup>)
- Engineered Microorganisms for the Production of Intermediates and Final Products (2<sup>nd</sup>)
- Production of organic acids from *Aspergillus pseudothraus* cadA deletion strain (1<sup>st</sup>)
- Production of organic acids from *Aspergillus pseudothraus* cadA deletion strain (2<sup>nd</sup>)
- Genetically engineering an industrial filamentous fungus *Aspergillus niger* for 3-hydroxypropionic acid production
- A specific exporter responsible for aconitic acid high production in *Aspergillus pseudothraus*

# Records of invention

## Records of Invention

- Bioproduction of limonene from syngas
- Mutant transporters for bacterial uptake of terephthalic acid
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids
- A genetic circuit to reduce cell-to-cell production heterogeneity
- High yield conversion of D-xylose to D-arabitol in *R. toruloides*
- Manipulation of tRNA thiolation gene *ncs2* for enhanced production of fatty-acyl-CoA derived chemicals in *R. toruloides*
- Efficient production of cis, cis-muconic acid from mixed substrates of glucose, D-xylose and L-arabinose
- Whole cell biosensors for industrially relevant polymers precursors
- Engineered Microorganisms for the Production of Intermediates and Final Products
- Method and strain for sugar conversion

# Software disclosures

## Software Disclosures

- Automated Recommendation Tool (ART) v2.0
- Kinetic Learning v0.1
- Automated Recommendation Tool (ART): v1.0
- PIACE: Parallel Integration and Chromosomal Expansion of Metabolic Pathways
- OMG, Omics Mock Generator Library: v0.1.1
- Fermentation Data Processing
- Fermentation Data Manipulation and Analysis Once imported
- DIVA/Device Editor 3.1
- DIVA/Device Editor (DIVA) v6.0.0