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DOE BioEnergy Technologies Office (BETO) 2023 Project Peer Review

DBTL Infrastructure, Demonstration Projects, and Beachheads

April 3, 2023 Conversion Technologies

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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
- Since 2016, DBTL infrastructure and demonstration projects have been core ABF activities
 - In FY16, DBTL infrastructure was referred to as the "Foundry" task
 - Demonstration projects included the "P. putida" and "S. venezuelae" tasks
- In 2020, beachheads were added along with the demonstrations
 - As a strategic activity, to make more of biochemical and bioprocess space accessible
 - "Beachhead": a strategic metabolic intermediate that can be converted into several bioproducts
 - "Exemplar": a representative target molecule downstream of a given beachhead





Project Goal and BETO Relevance

- DBTL Infrastructure goal: Design, implement, operationalize, and maintain Design/Build/Test/Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF tasks and enables the overall ABF goal
- Relevance to BETO's goals: DBTL infrastructure supports the ABF and other BETO projects, and can be leveraged by U.S. industry
- Specific research question: How to achieve 10X improvement in Design/Build/Test/Learn cycle efficiency, new IP and manufacturing technologies demonstrated and ready for translation to U.S. industry?



Project Goal and BETO Relevance

- Demonstration projects and beachheads goal: Develop bacteria
 (e.g. *P. putida*), yeast (e.g. *R. toruloides*), and filamentous fungi (e.g.
 A. niger) hosts to efficiently, cost-effectively, and sustainably produce
 beachhead and exemplar pairs to aid commercialization
- Relevance to BETO's goals: Demonstration projects and beachheads assess BETO investments in ABF capabilities, and are are key drivers towards industry collaboration projects
- Specific research question: How to select compelling demonstration projects that showcase the successful use and value of ABF capabilities, and beachheads that strategically make more of biochemical and bioprocess space accessible?







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 webinars for them
 - 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
 - An industry collaboration project leverages ABF 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





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		A. niger (>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		C. glutamicum	1.5X	3X	2X	
Microfluidics screening		C. glutamicum				2X
Microfluidics screening		Rhodobacter	2X		1X	2X
Genomic integration		C. necator	>10X	>10X	1X	10X
Fungal transporters						





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



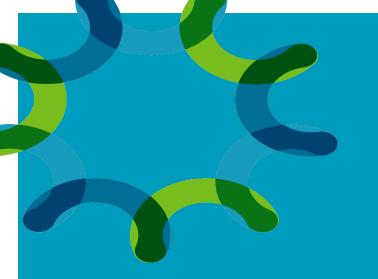


Approach to Diversity, Equity, Inclusion

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

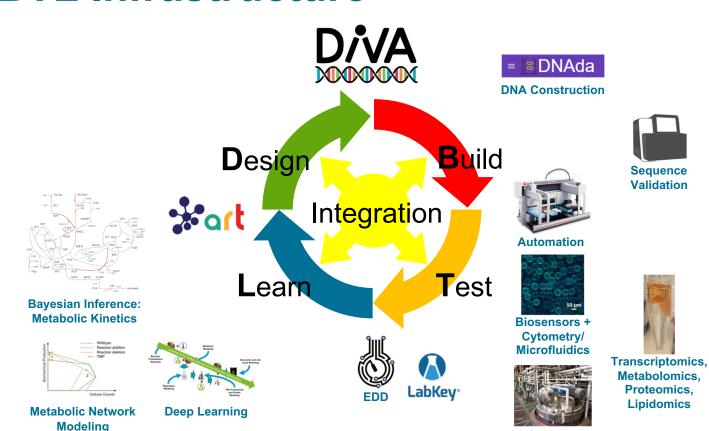






2 - Progress and Outcomes

DBTL Infrastructure

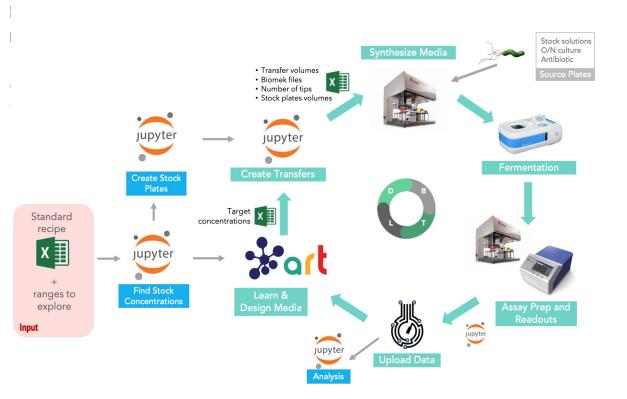




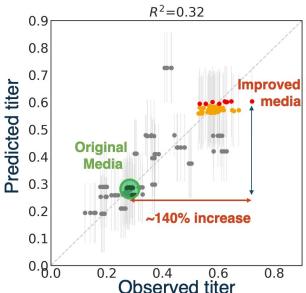


Scale-up

Semi-automated Combinatorial Media Pipeline



Flaviolin (OD340 0.5 ~ 40 mg/L)



In collaboration with JBEI





Better Software Accessibility for the Visually-Impaired

- Improved ABF DIVA and EDD software to better comply with Rehabilitation Act Section 508: WCAG2.1 AA standard criteria
 - Supports BETO Diversity Equity and Inclusion (DEI) emphasis area

ABF contracted TrussWorks

- Expertise in making web applications compliant with Section 508
- Audit baseline DIVA and EDD compliance, generate a list of issues to resolve
- Work with ABF software engineers on identified issues, and instruct process and practices

Assessment, progress, and residual issues

- 165 of the 217 (76%) issues identified for EDD were fixed
- 213 of the 263 (80%) issues identified for DIVA were fixed
- Yet-to-be-fixed issues remain in the issue tracking tables
- Many unfixed EDD issues await migration to a newer version of Bootstrap
- Many unfixed DIVA issues are consequences of third-party software
- Accessibility improvements also improve sighted user experience

Other outreach for feedback and guidance

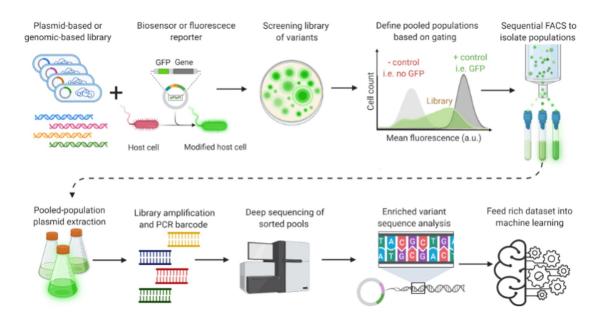
Accessibility experts including Lucy Greco (UC Berkeley) and Annette Greiner (Berkeley Lab)







High-throughput Screening



Bentley et al, Met. Eng. 2020 Pandey & Davison et al, ACS Syn. Biol. 2022 Pardo & Jha et al, Met. Eng. 2020

Outcomes:

 Biosensor promoters optimized, riboregulators screened, and heterogeneous populations from adaptive laboratory evolution experiments sorted

Next: screening genome-scale targeted libraries



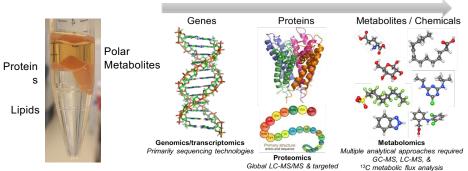


Multi-omics Analysis

- New ABF high-throughput analytical and computational workflow to enable high confidence metabolite profiling using ML.
- Bilbao, Aivett, et al. "PeakDecoder enables machine learning-based metabolite annotation and accurate profiling in multidimensional mass spectrometry measurements" In press, *Nature communications*, 2023.

Single-sample Metabolite, Protein and Lipid Extraction

proteomics, whereby a complex sample is spiked with a stable isotope-labeled





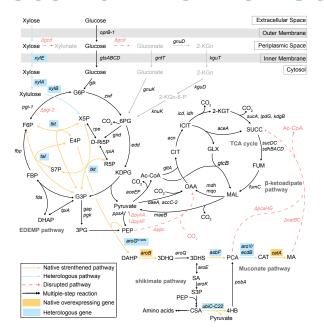
o	peptide that acts as internal standard			
Host	Proteomics (global + targeted)	Metabolomics +Lipidomics		
P. putida	> 920 datasets	> 890 datasets		
A. pseudoterreus	> 590 datasets	> 830 datasets		
A. niger	> 450 datasets	> 200 datasets		
R. toruloides	> 1800 datasets	> 1800 datasets		
Z. mobilis	>70 datasets	>15 datasets		

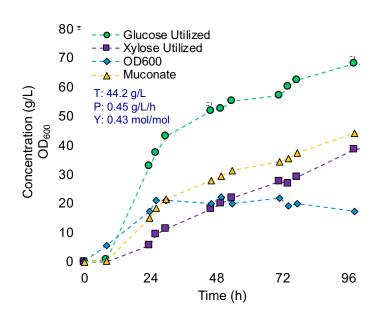
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CRADA	Proteomics (global + targeted)	Metabolomics +Lipidomics
ABF-Superbrewed	> 60 datasets	> 150 datasets
ABF-C16	> 100 datasets	> 500 datasets
ABF-Invaio	>35 datasets	
ABF-UCBerkeley		>150 datasets
ABF-Lygos	> 240 datasets	> 150 datasets
ABF-Agilent	> 30 datasets	> 850 datasets





Bacterial Demonstrations/Beachheads





- Engineering muconic acid production in robust soil bacterium, P. putida
- Work closely with Performance-Advantaged Bioproducts project to show utility of products

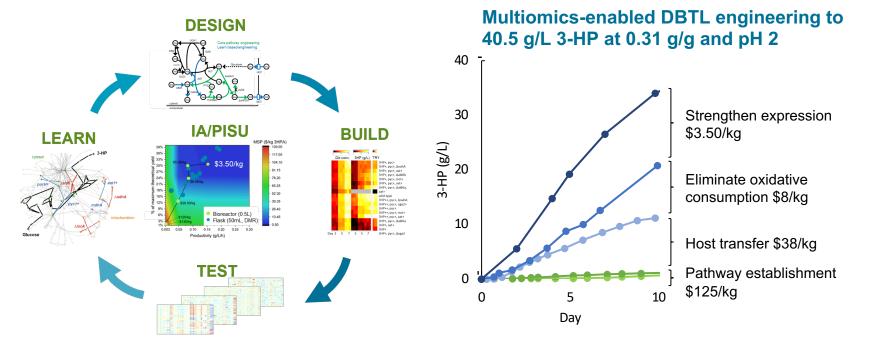




Progress and outcomes on fungal hosts: 3HP

Dai et al. Biotechnol Biofuels 2023

- 3-hydroxypropionic acid is a precursor for large market polyacrylates and other polymers
- Ongoing work to stack Test/Learn ID'd and validated gene targets to increase yield

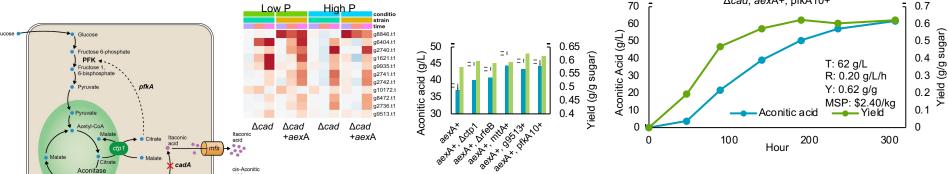




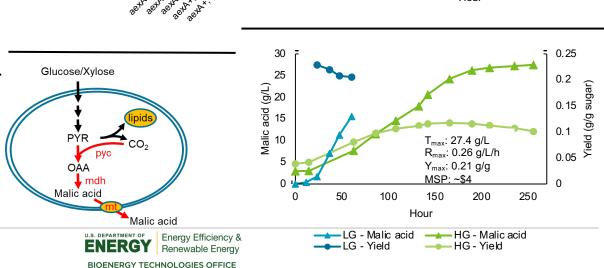
Progress and outcomes on fungal hosts: Aconitic, Malic Deng et al. Metabolic Engineering in review

Aconitic: Engineering of transporters and Test/Learn targets enabled 62 g/L

Ongoing work to stack gene targets and improve robustness of scale-up

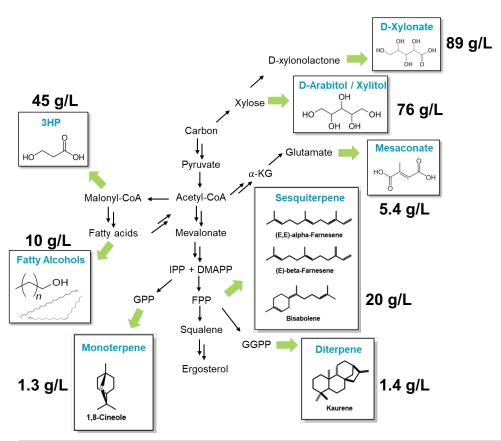


- Malic: Pathway establishment enabled 27 g/L acid from hydrolysate
- Ongoing work to optimize culture conditions (ART) and build genetic targets



Δcad, aexA+, pfkA10+

Yeast Demonstrations/Beachheads



- Engineering many bioproducts in robust yeast, R. toruloides
- Optimize TRY for several bioproducts, such as 3hydroxypropionate, bisabolene, xylonate, and fatty alcohols

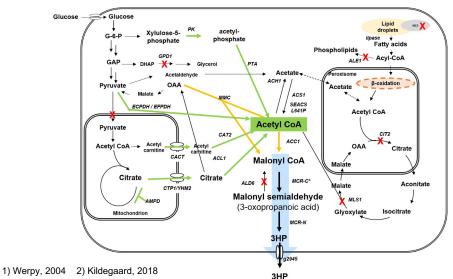


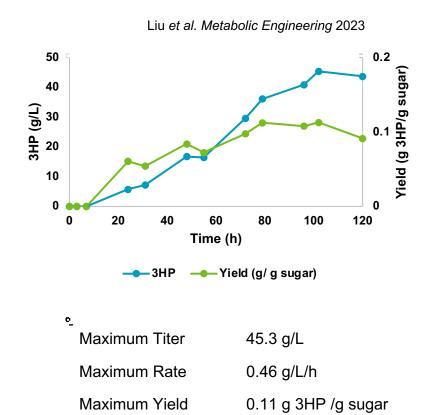




Yeast Demonstrations/Beachheads

- 3HP is the third most promising platform molecule as identified by the DOE¹
- Easily converted into acrylic acid, a market of ~\$19 billion²







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 a adding a new milestone for FY23.

FY22Q4 Demonstration Project/Beachhead Annual SMART 1

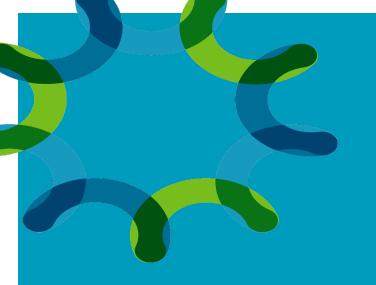
- Milestone: At least 10 of the ABF beachheads across onboarded hosts achieve individual titer metrics. Any of the FY21 ABF beachheads that do not enable these production metrics after 1 year of optimization further analyzed to determine whether to continue or sunset based on interest from BETO and industry.
- 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 β-ketoadipic acid (BKA), with muconic acid at 44 g/L, 0.45 g/L/hr, and 0.43 mol/mol yield







3 – Impact

Impact for demonstration projects

Scientific

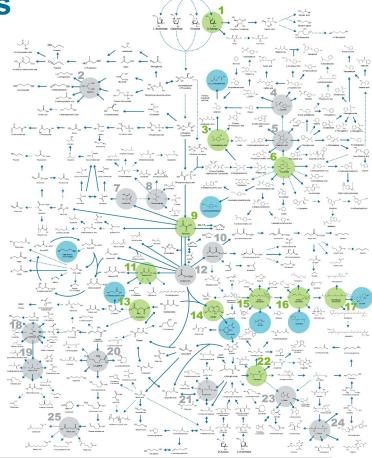
 Peer-reviewed manuscripts and patent applications in bacteria, filamentous fungi, and yeast

Industry

- Helping elevate *P. putida, A. niger, R. toruloides* as chassis for industrial biotech. from carbohydrates
- Working with industry on these hosts via Funding Opportunities and DOE FOAs

Overall

Our demonstration projects efforts focus on developing BH-exemplar pairs for the community and demonstrating cutting-edge ABF tools







Summary – achievements highlighted

DBTL Infrastructure

- Semi-automated combinatorial media pipeline
- Better software accessibility for the visually-impaired
- High-throughput fluorescence screening of promoters, riboregulators, ALE populations
- Multi-omics methodologies and datasets

Bacterial Demonstrations/Beachheads

- Muconate: Titer 44.2 g/L, Productivity 0.45 g/L/h, Yield 0.43 mol/mol

Fungal Demonstrations/Beachheads

- 3HP: Titer 40.5 g/L, Yield 0.31 g/g
- Aconitic acid: Titer 62 g/L, Productivity 0.20 g/L/h, Yield 0.62 g/g
- Malic acid: Titer 27.4 g/L, Productivity 0.26 g/L/h, Yield 0.21 g/g

Yeast Demonstrations/Beachheads

3HP: (Maximum) Titer 45.3 g/L, Productivity 0.46 g/L/h, Yield 0.11 g/g





Quad Chart Overview

Timeline

• Project start: October 1, 2019

• Project end: September 30, 2022

 FY22 Costed
 Total Award

 DOE Funding
 (10/01/2021 – 9/30/2022)
 \$15M

 Project Cost Share
 Cost
 \$15M

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 publiclyaccessible ABF HObT website

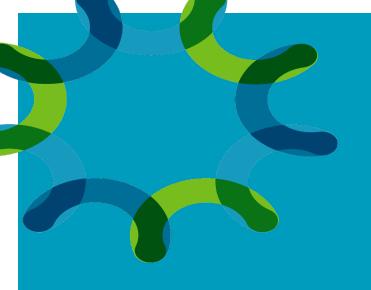
Funding Mechanism

Project Partners

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







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





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- 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. Starkenburg, 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





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- 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





- 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





- 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.





- 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





- 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





- 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





- 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". Al4Synbio 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





- 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.





- 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





- 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.





- 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", IWBDA 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





- 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 MIrACLE 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





- 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 cisriboregulators 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.





- 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





- 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.





- 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





- 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





- 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, 9/22/20222
- 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 update of terepthalic acid
- · Method and strain for sugar conversion
- Engineered Microorganisms for the Production of Intermediates and Final Products (1st)
- Engineered Microorganisms for the Production of Intermediates and Final Products (2nd)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (1st)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (2nd)
- Genetically engineering an industrial filamentous fungus Aspergillus niger for 3hydroxypropionic acid production
- A specific exporter responsible for aconitic acid high production in Aspergillus pseduoterreus





Records of invention

Records of Invention

- Bioproduction of limonene from syngas
- Mutant transporters for bacterial update of terepthalic acid
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3hydroxyacids
- 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



