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DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

#### **Agile BioFoundry – Host Onboarding and Development**

April 3, 2023 at 3:50pm MST Conversion Technologies

Taraka Dale & Adam Guss Los Alamos National Lab and Oak Ridge National 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 2017, HOD team was created to address industry feedback stating the need for new microbial hosts for bioprocessing
  - Addressing the critical need for robust genetic engineering and strain improvement tools for non-model hosts
  - Introduce phylogenetic and metabolic breadth to industrial microbes

# • In 2022, the Tier System and HObT website were implemented for organization and outreach

- Organizing information and "readiness" of tools for handoff to partners





#### **Project Goal and BETO Relevance**

- Host Onboarding and Development (HOD) goal: Enable industrial bioengineering by identifying and making available new microbial hosts through prioritized strain onboarding and development
- Relevance to BETO's goals: HOD performs pre-competitive research to develop microbial hosts that natively have traits of use in bioengineering, helping to reduce risk and accelerate the adoption of non-model microbes
- Specific research questions: Can we develop generalizable design rules, metabolic engineering tools, and strain improvement approaches for rapidly domesticating microbes with useful phenotypes? Can we efficiently partner with industry to help develop the most useful hosts for specific processes?







# 1 – Approach

#### **Challenges and Approach**

#### • Challenges:

- Non-model microbes can have advantages over well-known model hosts, but genetic tools and approaches are typically not host-agnostic, so tool development is traditionally "artisanal" → high risk, low TRL
- Not every tool/dataset may be needed or feasible for every organism



#### **Risks, Mitigation, and Collaboration**

#### • Risks:

- Developed tools and organisms may not be adopted by stakeholders
- Strain engineering tool development in novel microbes could fail for unknown reasons
- Mitigation:
  - Cast a wide net for organism phenotypes and phylogenetic groups
  - Engage with industry via Funding Opportunities to identify hosts of interest
  - Leverage decades of experience and large collections of genetic "parts" to minimize risk of failure
- Communication and Collaboration: Collaborated with BETO State of Technology (SOT) projects to improve genetic tools in *Clostridium tyrobutyricum* and *Zymomonas mobilis* to accelerate their progress. Interact with ABF Industrial Advisory Board.







#### **Changes made after 2021 Peer Review**

- 2021 Reviewer Comment: [Need] Better dissemination of ABF's capability, including the DBTL tools, developed beachheads, and onboarded strains
- **ABF response:** The HObT website was prioritized not only to organize ABF information, but also to be an externally facing site for information on our strains and tools.
- Scaled back the number of ABF-selected hosts: HOD originally targeted onboarding 20 hosts, but scaled back to focus more deeply on 15.





#### Go/No-Go, Metrics, and DEI

#### Go/No-Go decision

– N/A

#### Economic and Technical Metrics

- Tier 1: minimum set of tools/knowledge that a host needs to be used constructively in the DBTL cycle – all criteria must be met to be considered a Tier 1 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 ≧ 100%

#### • Diversity, Equity, and Inclusion

- HOD efforts follow overall ABF efforts for DEI





#### **Approach: Tier System**

- Used to identify and prioritize tools and knowledge needed for development of a given host
- Facilitates communication and information exchange for host development activities
- Standardizes, guides, and tracks hosts development over time



- Increasing Tier level represents increasing level of tools and baseline knowledge
- Attributes in Tiers 1 and 2 are commonplace, yet essential, tools and knowledge required for a strain to be used constructively in a canonical Design-Build-Test-Learn (DBTL) cycle. *Must meet all Tier 1 attributes to be considered "onboarded*"
- Outcome: Framework to guide onboarding and development for diverse hosts







# 2 – Progress and Outcomes

#### **Progress & Outcomes: Onboarded 15 hosts**

#	Host	Taxon	Tier
			Level
1	Pseudomonas putida	Proteobacteria	2
2	Corynebacterium glutamicum	Actinobacteria	2
3	Bacillus licheniformis	Firmicutes	2
4	Rhodosporidium toruloides	Basidiomycota fungi	2
5	Cupriavidus necator	Proteobacteria	1
6	Zymomonas mobilis	Proteobacteria	1
7	Rhodobacter sphaeroides	Proteobacteria	1
8	Pseudomonas fluorescens	Proteobacteria	1
9	Clostridium ljungdahlii	Firmicutes	1
10	Clostridium tyrobutyricum	Firmicutes	1
11	Aspergillus pseudoterreus	Ascomycota fungi	1
12	Lipomyces starkeyi	Ascomycota fungi	1
13	Pichia kudriavzevii	Ascomycota fungi	1
14	Yarrowia lipolytica	Ascomycota fungi	1
15	Aspergillus niger	Ascomycota fungi	1

- Nine bacterial strains were
  onboarded to Tier 1 or higher
- Six fungal strains were onboarded to Tier 1 or higher
- Four hosts are at Tier 2 or above
- The Tier status of ABF hosts is available in HObT

#### Milestone Completion:

- FY21Q2\_HOD\_R1. Elevate 2-3 onboarded hosts by at least one Tier.
- FY21Q4\_HOD\_AS1. Complete onboarding of at least 10 new / existing Hosts to at least Tier 1
- FY22Q4\_HOD\_AS1. Bring a total of at least 15 microbial hosts to at least Tier 1, and provide corresponding information via HObT website



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#### **Progress & Outcomes: Host Onboarding Tool (HObT)**

<b>Cymomonas mobilis</b> rroteobacteria	Tier 1	Pseudomonas fluorescens Proteobacteria
ublications		8 publications
associated parts		6 associated parts
associated strains		1 associated strain
experiments		1 experiment
protocols		0 protocols
st update: May 6, 2022	details »	Last update: Sep 15, 2022
hodosporidium toruloides IFO0880	Tier 2	Bacillus licheniformis Firmicutes
a sublications		
		4 publications
associated strains		4 associated parts
protocole		
protocols		i protocol
	detaile »	Last update: Son 7, 2022

- Recently released major version 3.5.0
- Integrated with ICE, EDD and protocols.io for automated data retrieval, along with manual data entry for attributes, Tier progress and publications.

но	bT		Search				€ <u>Login</u> Register
<b>P</b> Pi	Pseudomonas putida KT2440 Proteobacteria						
Attri	butes 0 Publications 17	Parts 30	Strains 99	Protocols 0	Experiments 51	Tier Progress	
• 1	fier 1						✔ Completed
• 1	Fier 2						✔ Completed
• 1	▼ Tier 3 In progress						
	CRITERIA	DETAILS				STATUS	
	Biosensor(s)	one or more FRE molecules (i.e. Al	T or transcription factor-b P, NADH, etc.) or produc	based biosensors for key n sts	netabolic intermediates, cell status	6	100%
	High throughput screening	for droplet-based microflunsors and/or robotic HPL	uidic screens to identify an C analysis	d isolate strains with superior phe	notypes;	100%	
Advanced genome modification tools one or more CRISPR-CAS, CRISPR/Cpf1, TALEN, etc.			, TALEN, etc. genome mo	dification and/or gene expression t	tools	100%	
	Gene expression tuning	fine level gene tuning for at least 3 operons/genes (e.g., RNA-based regulators, engineered panel of 100% promoters, etc.)			100%		
	Saturated deletion/loss of function libraries	build a saturated deletion library and assay cellular fitness under at least 3 different conditions (e.g., bar-coded transposon libraries)					
Genomic over-expression platform build the genome		build the genomic	tic over-expression library and assay cellular fitness under at least 3 different conditions				
Centralized omics databases, multi-omic deposited experi- data integration and analysis learning utilized		nental data and metadata analvze multi-omic data	a from at least 3 omics exp a to make meaningful pred	periments and deep learning/mach	nine 5 Ids of	0%	

#### https://hobt.agilebiofoundry.org

**Outcome**: Currently maintaining Tier progress details on ABF selected host organisms and associated knowledge and information





#### **Progress & Outcomes: Appraisal Framework for Host Development**

#### **Appraisal Framework**



#### **Milestone Completion:**

FY22Q2\_HOD\_R1. An appraisal framework developed to facilitate decision making for prioritization of on-boarding new hosts versus progression of existing hosts up Tier levels. Framework to be developed based on input from ABF researchers, industry and other stakeholders to determine importance of "breadth" (number of hosts on-boarded) versus "depth" (number of hosts developed to Tier 3/4 level) of ABF host

2.4

Industry

Feedback

2.6



#### Five Appraisal Areas:

- How does it enhance the existing ABF host portfolio?
- How does it meet industry requests?
- Does the ABF have the capabilities ٠ (or interest) to execute the activity?
- Cost/effort of the activity?
- How will the activity benefit the ABF and biomanufacturing industry?



### Progress & Outcomes: Multiplexed insertion of DNA into the *P. putida* chromosome



- 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

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- Outcome: Base SAGE technology has been licensed by a company for use in one organism and adapted to other organisms in multiple CRADA projects
- Outcome: Highly efficient tools that allow combinatorial pathway assembly in the target host for rapid Build



### **Progress & Outcomes: Dual Biosensor in C. glutamicum**



- Single plasmid construct; optimized promoters for cis,cis-muconate and chorismate
- Large promoter libraries (10<sup>4</sup>-10<sup>5</sup> diversity) built; FACS to select top performers
- **Outcome:** Dual biosensor established for simultaneous detection of *cis,cis*-muconic acid and chorismate in *C. glutamicum* builds the foundation for simultaneous screening of cell growth (chorismate) and biochemical production (muconate)







#### **Additional Milestones from FY21/FY22: All Complete**

**FY21Q2\_HOD\_R2**. For SOT projects, high efficiency gene integration tools (e.g., site-specific recombinases and/or CRISPR) developed for both *C. tyrobutyricum* and *Z. mobilis* with a minimum efficiency of 1000 cfu/ug DNA.

Adapted SAGE recombination system to each organism and demonstrated site-specific recombination

**FY22Q3\_HODSOT\_R1.** Efficient selection/counter-selection system demonstrated for genetic marker recycling by stacking three chromosomal modifications in *Clostridium tyrobutyricum* toward developing an SOT biocatalyst with increased efficiency in conversion of biomass to fuel precursors

Developed uracil phosphoribosyl transferase (*upp*) as a counter-selectable marker. Used this tool to delete the master regulator of sporulation (*spo0A*) and two gene clusters related to motility. The presence of each gene/cluster is hypothesized to be detrimental to growth in a bioreactor and is being tested by the collaborative team.







# 3 – Impact

#### Impact: Industry Pull

# Reduced barriers to using non-model organisms:

- Reduced risk for adopting strains with varied phylogeny, feedstock usage and metabolic capacity
- External stakeholder feedback consistently shows that onboarding new hosts and developing host tools would be beneficial to industry
- Based on needs & described by the IEO Task, during Directed Funding Opportunities, <u>companies</u> <u>are interested in leveraging ABF tool development</u> <u>expertise for non-model organisms</u>
- To date, ~50% (18/37) of funded externalpartnership projects (DFO, etc) have included an HOD component

"Expand host range and make new organisms scalable ....Great use of national lab resources"

"Onboard a bunch of organisms with a lot of tools. Avoid companies needing to license and go into different organisms to avoid IP issues"

"Organisms that represent a broad range of metabolic space and manufacturing potential"

*"Make a database. Standardized strain descriptions. Growth conditions. Tools used. Substrate ranges. How transformable."* 

#### Quotes from Industry



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#### **Impact: Information Dissemination**

#### Public access to strains and tools

- Publications, conference presentations, patent applications
- Agile BioFoundry and HObT websites; web portal for each host, with links to protocols and data







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#### **Impact: Relevance to BETO & Biomanufacturing**

- Assist other BETO projects to accelerate progress across the BETO portfolio, in particular the BETO Conversion State of Technology
- Collaborate with industry via CRADA and FOA projects to aide in host onboarding and develop advanced tools in new hosts

#### Examples of benefits for developing non-model organisms







#### **Summary**

#### Approach

- We onboard and develop metabolic and strain engineering tools for non-model microbial hosts
- HOD activity guided by the Tier System and newly developed Appraisal Framework and communicated by HObT

#### Technical progress

- We have developed genetic tools to accelerate strain engineering including transformation methods, gene deletion/overexpression tools, high throughput tools for Build, biosensors, and more (7 publications)
- All milestones met with 15 hosts onboarded and 4 advanced to Tier 2 or above

#### • Impact

- Tools and hosts are meeting needs and accelerating progress both within the ABF and with external stakeholders (half of external partnerships include HOD element)







## **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					
TRL Range: 2-4					

#### **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%)





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# **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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- 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





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- 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.





- Nicholas A. Rorrer, \$\pm\$ Sandra F. Notonier, \$\pm\$ Brandon C. Knott, \$\pm\$ Brenna A. Black, \$\pm\$, Avantika Singh, \$\pm\$ Scott R. Nicholson, \$\pm\$ 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





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- 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.







- 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. 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







- 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







- 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

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 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.

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- Radivojevic, T., "Automated Recommendation Tool (ART): Design of Experiments via ٠ 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, ٠ Fuels and Chemicals (SBF): Speeding up synthetic biology, April 27, 2021
- Radivojevic, T., "Guiding synthetic biology via Automated Recommendation Tool (ART)", ٠ 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 ٠ chemicals" Student-invited seminar at North Carolina State University. September 27, 2021.
- Radivojevic, T. "Guiding synthetic biology via machine learning and multi-omics technologies", ٠ IWBDA 2021, 13th International Workshop on Bio-Design Automation, September 20, 2021 (virtual)
- Hillson, Nathan J. "Session Introduction", Metabolic Engineering 14, Block 8: Session ٠ Biofoundries, (Virtual via Remo), July 15, 2021

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- 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.

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- 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 (2<sup>nd</sup>)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (1<sup>st</sup>)
- Production of organic acids from Aspergillus pseduoterreus cadA deletion strain (2<sup>nd</sup>)
- 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





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