



U.S. DEPARTMENT OF
ENERGY

Energy Efficiency &
Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

Integration and Scaling

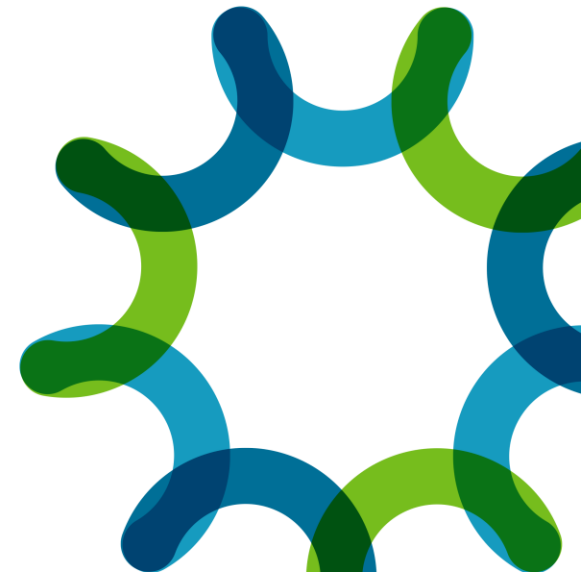
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BETO Peer Review 2019

Technology Session Review Area:

Agile BioFoundry

March 7, 2019



ABF Goal Statement

- **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 that will productionize synthetic biology.
- **Outcomes:** **10X improvement in Design-Build-Test-Learn cycle efficiency**, new host organisms, new IP, and manufacturing technologies effectively translated to U.S. industry ensuring market transformation.
- **Relevance:** Public infrastructure investment that increases **U.S. industrial competitiveness** and enables new opportunities for **private sector growth and jobs**.



Integration and Scaling Goal Statement

- **Goal:**

- Conduct bioreactor cultivations for ABF Target-Host pairs across scales, integrate with Test activities
- Provide a standardized DMR-EH hydrolysate for the ABF and industrial partners as a consistent material for testing
- Expand Test capabilities by on-boarding new bioreactor capacity
- Liaison with other BETO consortia towards integrated activities

- **Outcome:**

- Self-consistent, industrially-relevant testing capabilities and sugar streams across scales
- Establish interactions with other consortia to amplify impact of ABF discoveries made through DBTL activities

- **Relevance:**

- Benchmark DBTL cycle performance and improvement across scales with real-world substrates and process configurations



Quad Chart Overview

Timeline

- Start: October 1, 2016
- End: September 30, 2019
- 83% complete

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19- Project End Date)
DOE Funded	-	\$225k	\$320k	\$1.14MM

Partners:

FY18: LBNL (46.6%); NREL (53.4%)
FY 19: LBNL (33%); NREL (45.2%); SNL (4.4%)
PNNL (17.6%)

Barriers

- Ct-D.* Advanced Bioprocess Development
 - Using scaling together with Test-Learn to inform modeling towards predictable microbe performance across scales
- Ct-K.* Developing Methods for Bioproduct Production
 - Developing improved bioprocessing parameters, informed by DBTL, for ABF target-host pairs
- Ct-L.* Decreasing Development Time for Industrially Relevant Microorganisms

Objective

Conduct bioprocess scale up/down development in support of DBTL activities

End-of-project goal

Demonstrate T-H pair production of at least 3 molecules at 10 g/L, 100 mg/L/hr, at 40% of theoretical yield from DMR-EH at 10 L

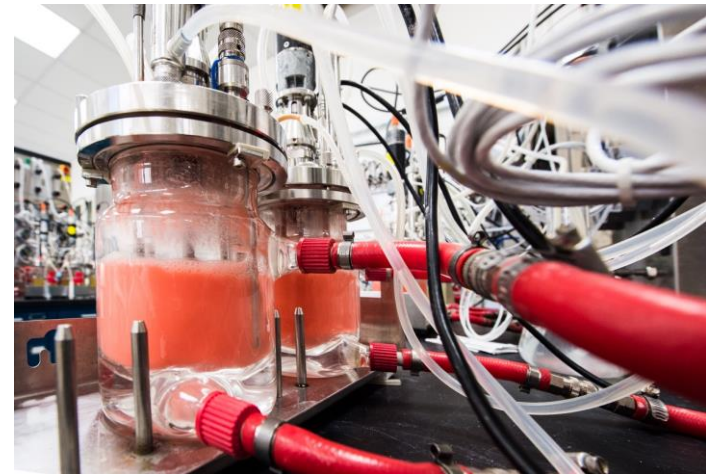
Project overview

History: Task initiated at the inception of the Agile BioFoundry

- First priority to provide consistent hydrolysate for Test
- Intent to conduct pan-scale Test to inform pan-scale Learn

Context: Demonstrated scale-up/down, integration critical for ABF

- Few publicly available pan-scale DBTL activities in synthetic biology
- Each partner conducting Test needs to use consistent relevant substrate



Project goals:

- Produce and deliver hydrolysate to partners and industry
- Conduct pan-scale Test with T-H pairs to inform predictive Learn models
- Act as a liaison between ABF and other BETO consortia
- Maintain bioreactor Test equipment across the ABF
- Provide integrated data to TEA/LCA teams

Management approach

Aim 1: Hydrolysate production



Interface with all partner institutions

- Led by experienced conversion team at NREL
- **Milestones:** hydrolysate production, distribution based on annual requests
- Dedicated tracking system for distributions

Aim 3: Interface with DBTL, Integrated Analysis, industry, and other BETO projects

- Regular meetings with DBTL team to plan and update on bioprocess development
- Calls with ABPDU & IBRF to coordinate Round Robins, in-person updates at All-Hands meeting
- Provide process data to Integrated Analysis team for T-H pairs for modeling milestones

Aim 2: Test-Learn activities across scales and on hydrolysates



Interface with Target-Host Pair teams

- Initiated Round Robin between LBNL and NREL for bioreactor reproducibility
- **Milestones:** providing data to Learn for modeling purposes
- Chose first T-H pair in FY18 for pan-scale Test and Learn based on no. of strains to compare

Technical approach

Critical success factors

- Ability to provide self-consistent hydrolysate to ABF partners for DBTL activities
- Development of predictive models (with Learn team) for scale-up activities
- Ability to provide realistic, relevant data to Integrated Analysis team
- Engagement with industry, other BETO projects to amplify ABF impact through integration

Aim 1: Hydrolysate production



Approach:

- Deacetylation, mechanical refining, enzymatic hydrolysis at the pilot scale.
- Industrially relevant, contains C5/C6 sugars from real biomass, and is a new process relevant to the entire BETO portfolio
- Harnessing advantages in filtration at pilot scale to make clarified, concentrated sugars

Challenges: Solids workup in original batches

Aim 2: Test-Learn activities across scales



Approach:

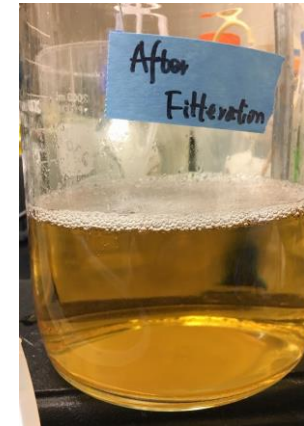
- Conduct Test experiments from μL to kL scale
- Bioprocess development for DBTL activities

Challenges: Oxygen transfer demands and contamination issues at large scale

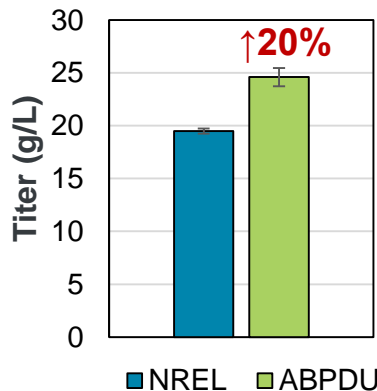
Outline of Technical Accomplishments

Hydrolysate production

Two batches to date implementing process improvements at pilot scale

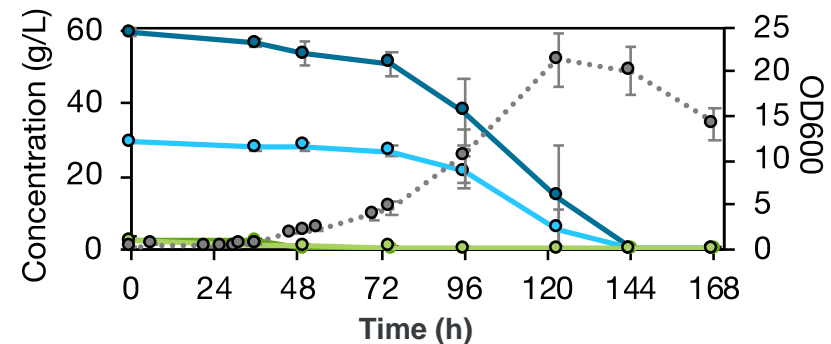


Pan-scale muconate Test/Learn



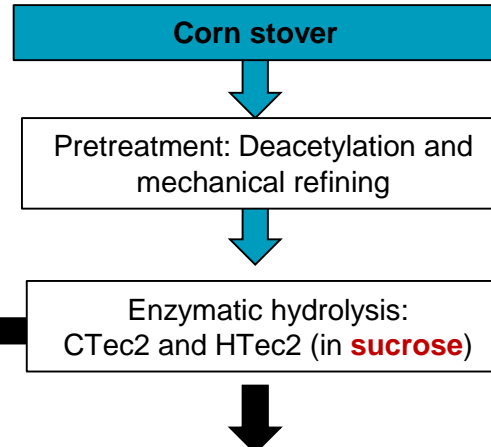
Round Robin study for muconate

Bioprocess development



Hydrolysate production and delivery

ELEMENT NAME	DMR Hydrolysate µg/g
Sodium	241
Calcium	226
Sulfur	96
Magnesium	95
Potassium	93
Phosphorus	73
Manganese	4
Boron	<20
Strontium	1
Aluminum	<1
Scandium	<1
Titanium	<1
Iron	<1
Nickel	<1
Gallium	<1
Germanium	<1
Zirconium	<1
Niobium	<1
Rhodium	<1
Palladium	<1
Silver	<1
Indium	<1
Tin	<1
Antimony	<1
Tellurium	<1
Cesium	<1
Vanadium	<0.5
Barium	0.5
Zinc	0.3
Cobalt	<0.1
Copper	<0.1
Molybdenum	<0.1
Other elements, etc	<0.1
NA, etc	—



	Batch 1	Batch 2
Glucose	87.00	82
Xylose	42.00	39
Arabinose	4.80	4.2
Galactose	<1	<1
Sucrose	11.00	0
Lactate	0.66	2.61
Glycerol	0.17	0.13
Acetate	0.29	0.37



DMR slurry



DMR clarified

Hydrolysate production (FY17, FY18)

- ~ 245 gallons (925 L) of slurry
- Composition similar between batches

Hydrolysate delivery

- Slurry from enzymatic hydrolysis was delivered to partners

Batch #	DMR slurry (gallons)	Delivered	Laboratory
1	35	--	NREL
1	5	2/1/2017	SNL
1	10	3/6/2017	LANL
1	15	3/13/2017	LBNL
1	5	3/13/2017	ORNL
2	45	--	NREL
2	5	1/16/2018	ANL
2	15	3/22/2018	LBNL
2	5	5/3/2017	SNL
2	35	8/9/2017	PNNL
2	35	9/20/2018	LBNL/SNL
2	35	9/20/2018	PNNL
Total Gallons	245		
Total Liters	927		

NA - element not analyzed or quantified by the ICP-AES / ICP-MS scans performed

Pan-scale Test with *P. putida*-muconic acid

Process evaluation across scales:

Provide understanding on strain performance and **cell and process robustness** in different environments

Multi-omics conducted at different scales and three strains (KT2440, GB45, GB62)

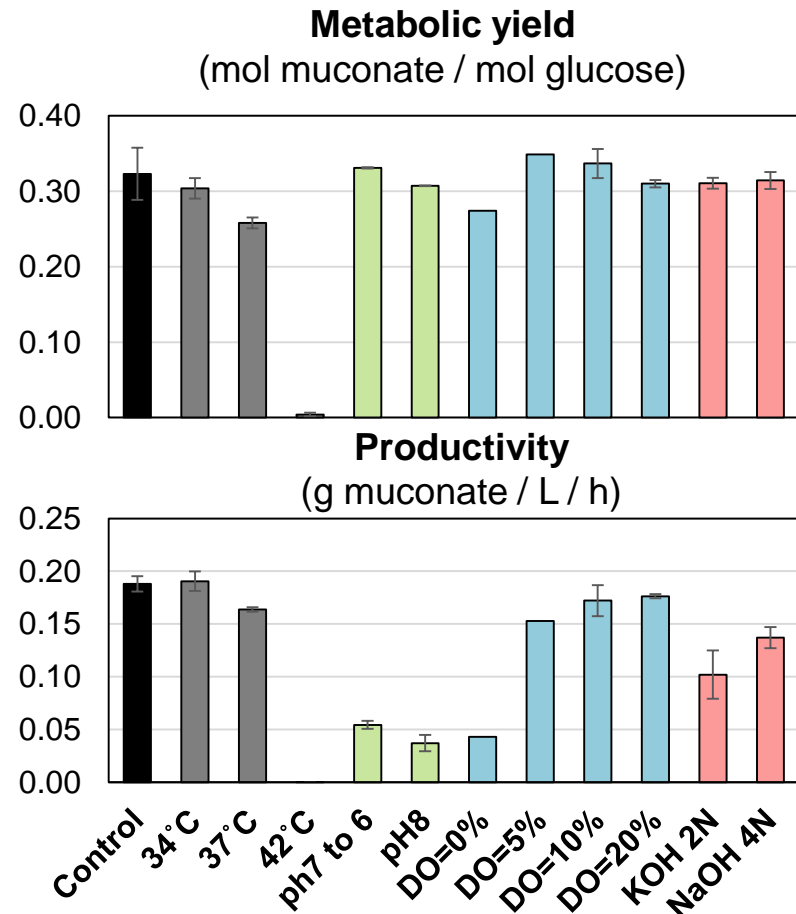
Bioreactor scale:

- 0.001 L (mini-bioreactors)
- 0.5 L
- 2.5 L
- 50 L
- 500 L (only GB45)

Muconate production conditions

Control: Temp=30°C, pH=7, DO=30%, 4N NH₄OH (informed from IA team)

Understanding *P. putida* GB45 robustness in 0.5 L bioreactors before scale-up



Pan-scale Test with *P. putida*-muconic acid

NREL pilot plant



First complete process: from bioreactor to clarified product at scale

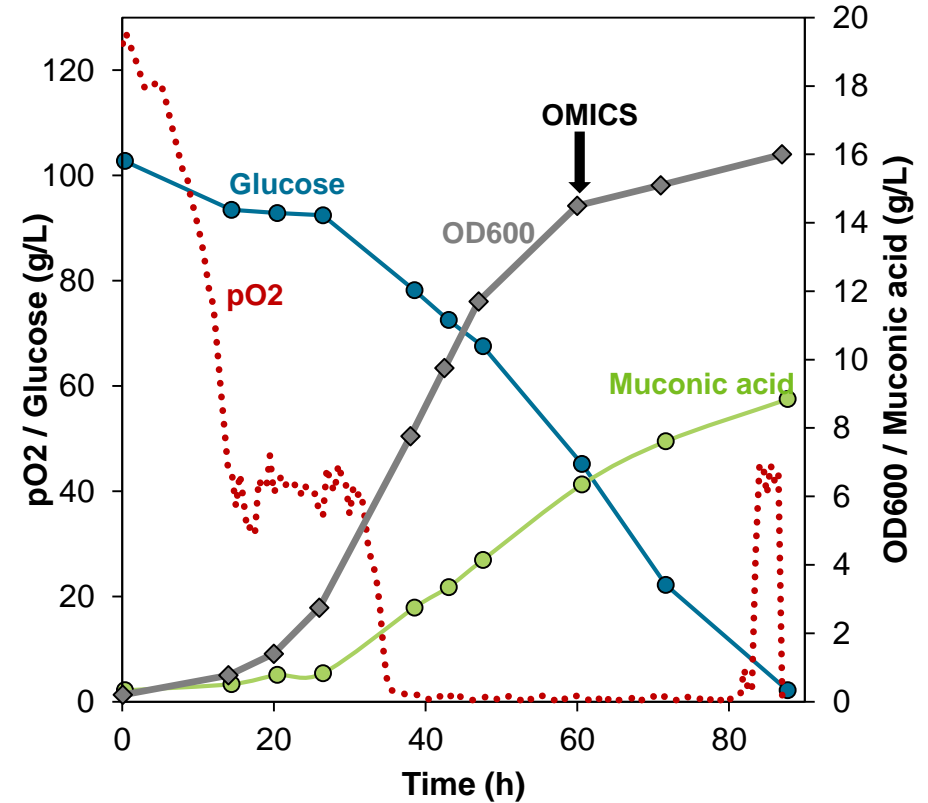
Production in 1,500 L bioreactor (500 L media)



Cells/broth separation in tangential flow filtration system



Product concentration in evaporator (down to ~150 L)



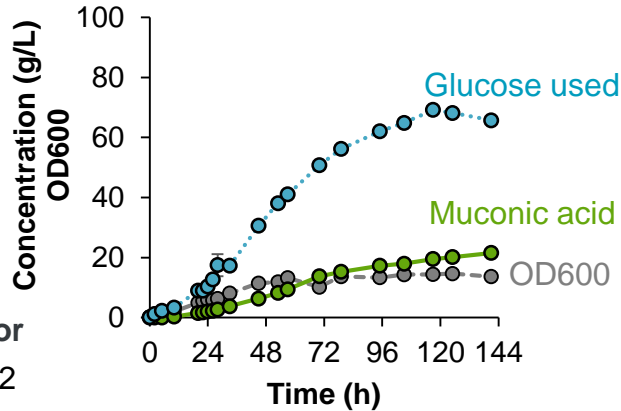
- Yield 3-fold lower than expected
- Contamination by *Enterobacter* sp. (facultative anaerobe)
- Equipment limitations (low K_{LA} , O_2 mass transfer coefficient)

Round Robin for bioreactor cultivation reproducibility

IBRF



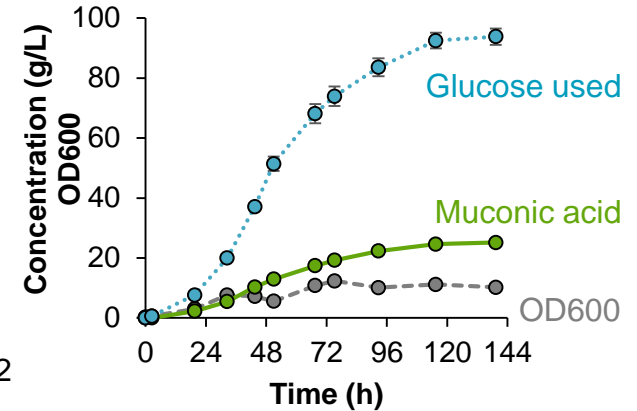
0.5 L bioreactor
P. putida GB62



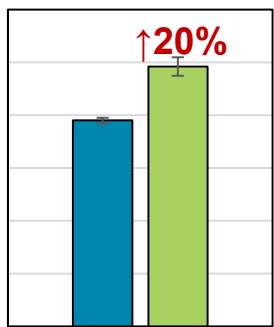
ABPDU



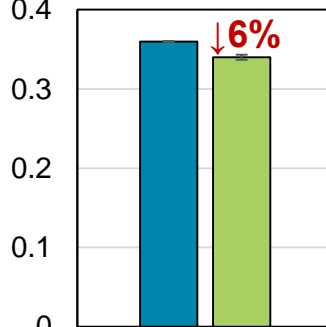
2 L bioreactor
P. putida GB62



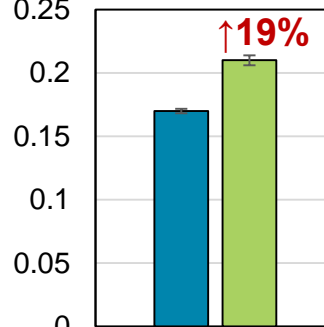
Titers (g/L)



Yields (mol/mol)



Productivity (g/L/h)



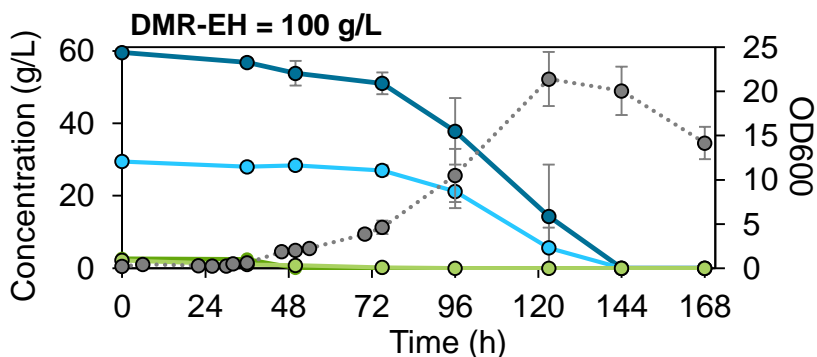
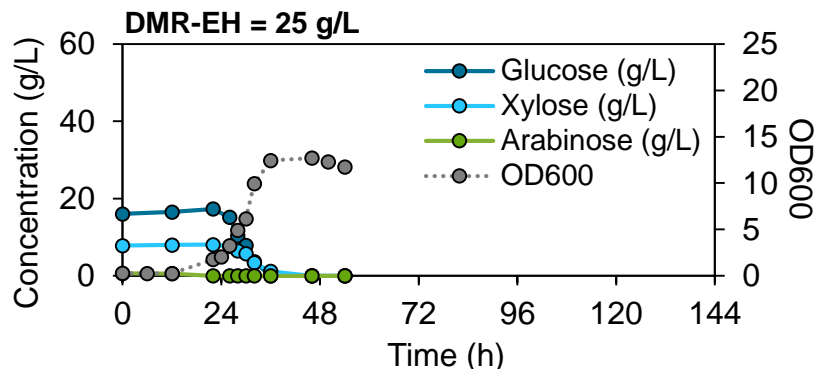
- Cultivation parameters replicated between facilities
- **Outcome:** TRY differences $\leq 20\%$, considered acceptable
- Many factors that can generate differences (e.g. bioreactor configuration, oxygen at different elevations, water, etc.)
- *R. toruloides* will be evaluated in a second Round Robin in FY19

Bioprocess development in support of DBTL activities

Pseudomonas putida

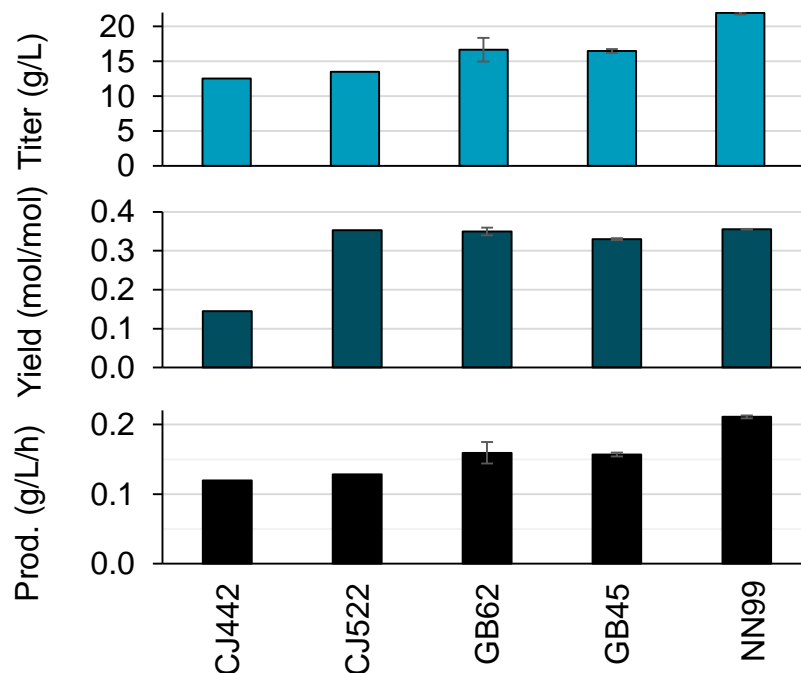
Sugar co-utilization experiments in bioreactor (0.5 L)

- DMR-EH clarified and diluted for strain evaluation (10, 25, 50, 100 g/L)



Muconic acid production from glucose in bioreactor (0.5 L)

- Evaluation of engineered/evolved strains
- Evaluate media and feeding strategies

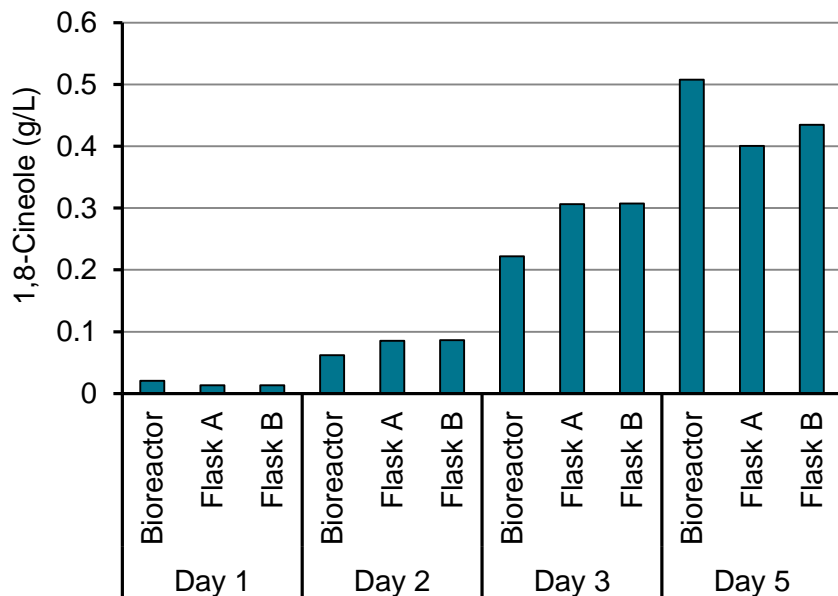


Bioprocess development in support of DBTL activities

Rhodosporidium toruloides

1,8-cineole production in bioreactor (1 L) and 250 mL shake flasks in tandem

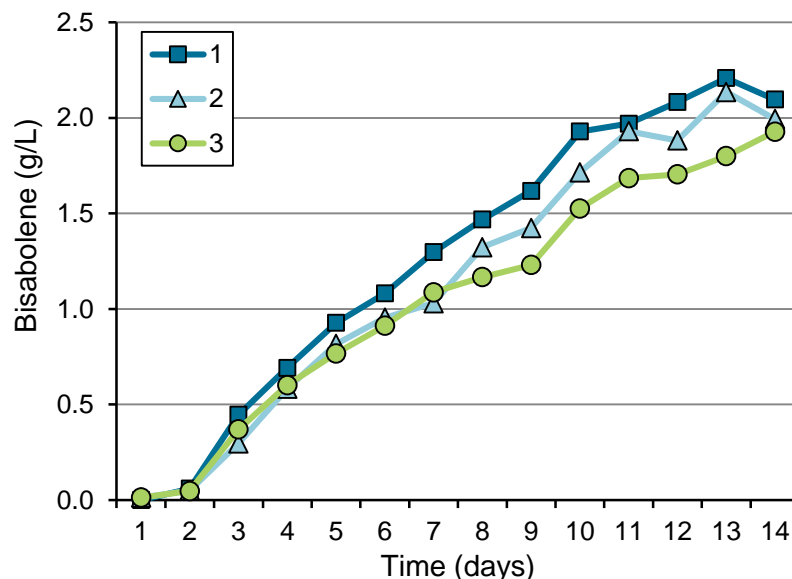
- Best performing strain released 146 mg/L of cineole from 100 g/L glucose during screening
- Tests in lignocellulosic media (75% DMR hydrolysate + 10 g/L yeast extract) revealed improved titer in bioreactor after Day 5



Bisabolene fermentation with defined nitrogen: ammonium sulfate

Media: 75% DMR hydrolysate plus

- **1:** 10 g/L yeast extract
- **2:** 5 g/L $(\text{NH}_4)_2\text{SO}_4$ + 100 mM KPO_4 + Fe (pH 6.0)
- **3:** 5 g/L $(\text{NH}_4)_2\text{SO}_4$ + 100 mM KPO_4 + Fe (pH 6.0) – no pH adjustment



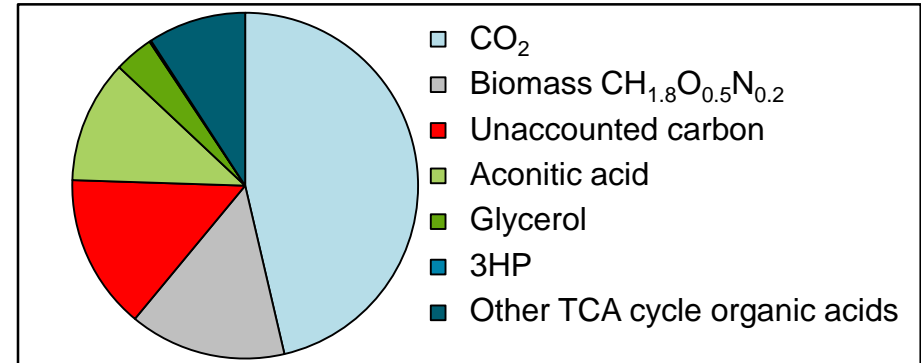
Bioprocess development in support of DBTL activities

Aspergillus pseudoterreus

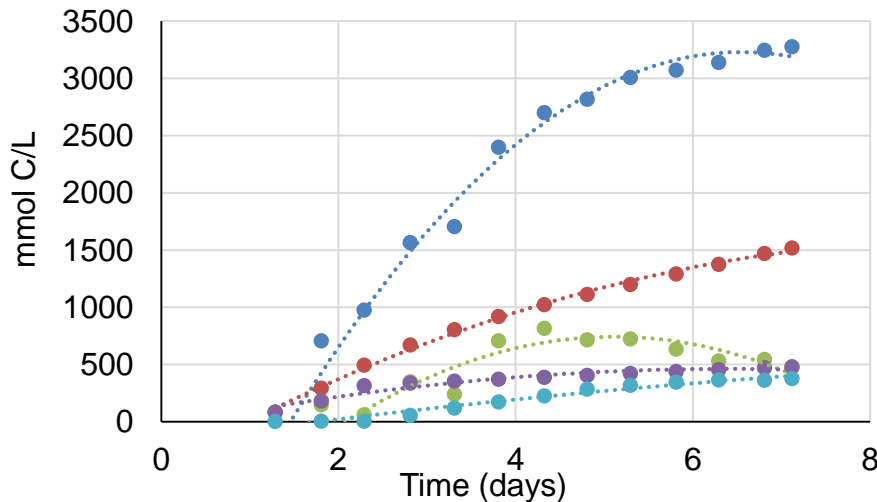
3-hydroxypropionic acid production in bioreactor (30 L)

- Best strain: 1.6 g/L 3HP from 100 g/L
- Riscaldati Production Medium: minimal medium, 100 g/L glucose

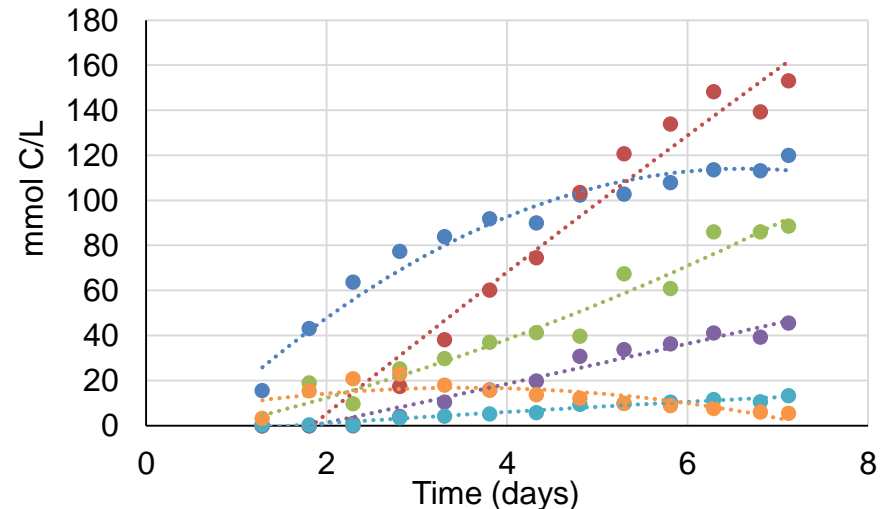
Closing carbon balance for FBA



- Glucose (consumed)
- Unaccounted carbon
- Aconitic acid
- CO₂
- Biomass CH_{1.8}O_{0.5}N_{0.2}



- Glycerol
- Citric acid
- Malic acid
- Isocitric acid
- α-ketoglutaric acid
- 3HP



Expanding state-of-the-art bioreactor capacity in the ABF

ABPDU

Test capacity:
↑ 3-fold



- Automated liquid handling
- Off-gas analysis
- Internal stirring (shear) to mimic larger scale
- 250 mL bioreactors (adequate sampling volume)



Sartorius Ambr250 HTP
system online in Q2 2019

IBRF

Test capacity:
↑ 6-fold



SegFlow autosampler
(for 8 bioreactors)



**YSI (at-line glucose
and xylose analysis)**



BiolectorPro
(32-48 minibioreactors)

Relevance

Goal: Bioprocess development to inform DBTL activities, provide consistent industrial sugar streams, expand Test capabilities, liaise with other BETO consortia for integration

Why is this project important, what is the relevance to BETO/bioenergy goals?

- Demonstrates Foundry strain performance on industrially relevant substrates
- Distributed Foundry needs self-consistent testing mechanisms

How does this project advance the SOT, contribute to commercial viability of biofuels production?

- Scale-up predictability enabled by Test-Learn will inform bioprocess development and commercialization
- Informs broader community on non-model strain performance on real-world substrates



Technology transfer activities

- Patent applications, publications on Test-Learn activities across scales
- Industry collaboration through DFOs and BEEPs projects
- Downstream processing with other BETO consortia

Future Work

Scaling up solid-liquid separation: Lab scale: 2x centrifugation, 2x filtration



Sugar concentration: ~ 130 g/L monomerics



Pilot scale



TFF



Evaporator

Hydrolysate production: Batch 3

- 180 L concentrated clarified liquor
- > 500 g/L monomeric sugars
- Completion: FY19 Q2

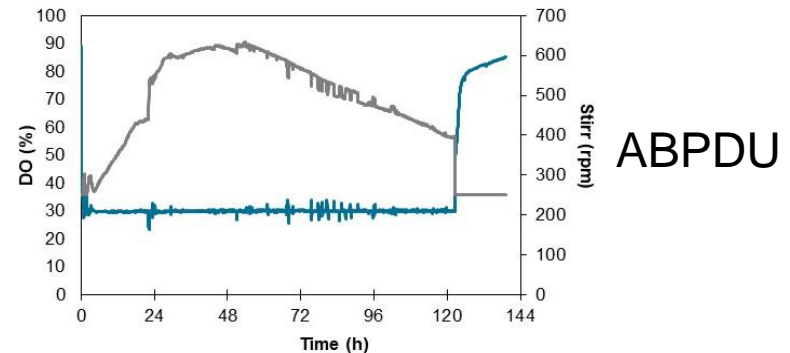
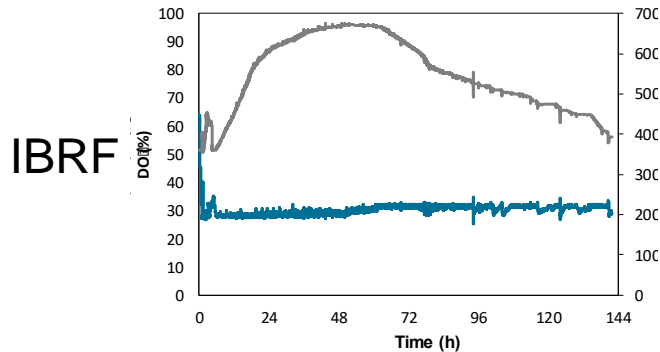
Batch 4: expected completion in Q3 FY19

Future Work

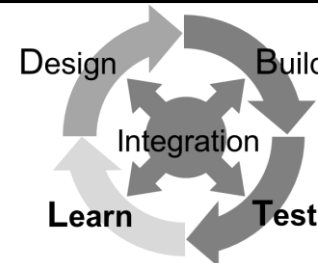
Bioprocess development towards end-of-project milestones:

10 L scale using DMR-EH hydrolysate, with TRY 10 g/L, 100 mg/L/h, 40% of theoretical yield with at least 3 targets

**Additional Round Robin with different T-H pairs/
Correction for environmental and other variables**



Closer collaborations with other BETO consortia (SepCon, FCIC, CCPC, PABP, and ChemCatBio) to further inform the DBTL cycle



- *On-line separations*
- *Feedstock and media considerations for catalysis*
- *Modeling for scale-up*
- *Novel bioproducts*

Future Work

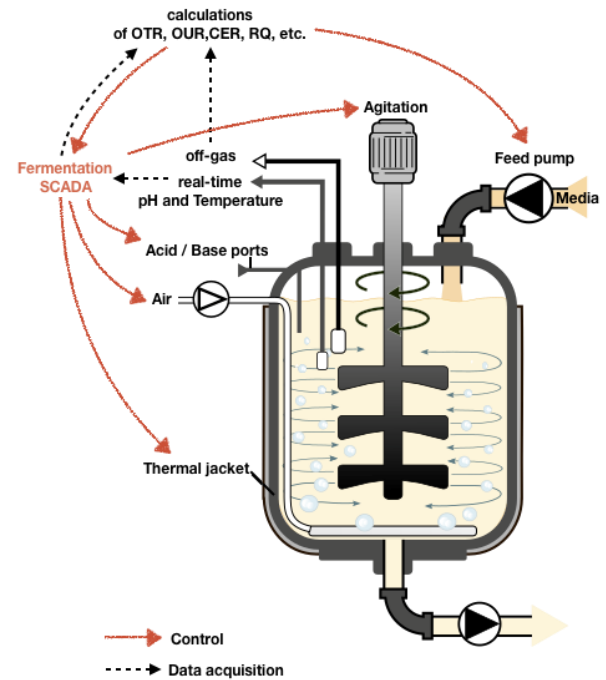
Application of Energy I-Corp Learnings:

1. Better Utilization of Real-time Data for in-line process Control
2. Predictive Scale-Up studies in lab-scale bioreactors

Understand K_{LA} effects on aerobic T-H pairs
BiolectorPro can mimic K_{LA} from ~100 to 450



ABF pan-scale work across the 3 FY17 T-H pairs
Better understanding on equipment needs and bacterial metabolism at different scales



Summary

Overview

- Providing critical raw materials (hydrolysate) for Test and data (bioreactor performance across scales) for Learn

Approach

- Solid interface with other ABF DBTL teams and BETO projects
- Driving progress with state-of-the-art process development

Technical accomplishments

- Produced needed volumes and improved hydrolysate process
- Verified data reproducible between Labs in round robin v1
- Improved strain performance through bioreactor process development
- Initiated pan-scale performance evaluation with key host-target pairs

Relevance

- Enabling industry collaborations in DFO and BEEPS projects
- Advancing feasibility of scaling non-canonical hosts

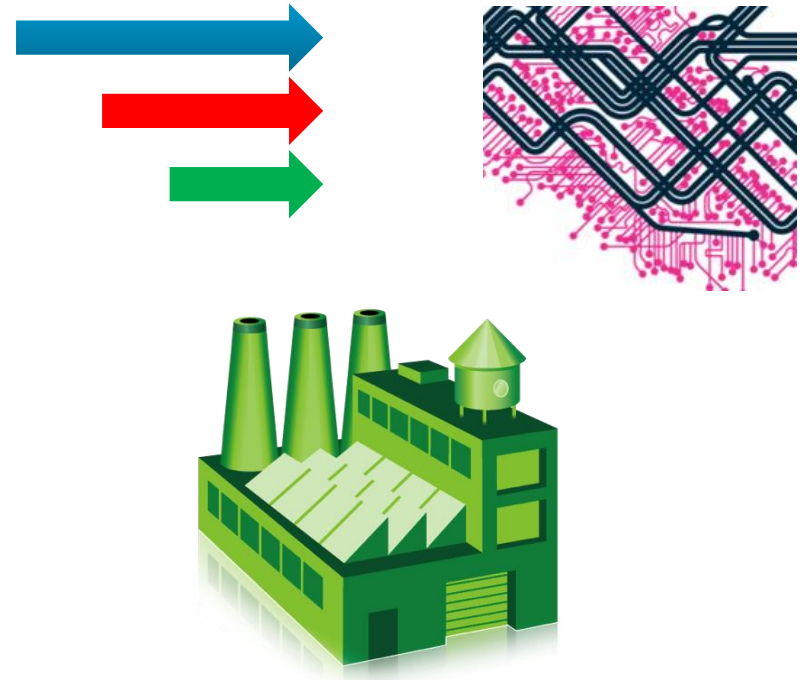
Future work

- Implement added capacity and increase bioreactor Test activities
- Pan-scale process Test + Learn to better inform Design + Build



Acknowledgements

- Jay Fitzgerald (BETO)
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- Nathan Hillson
- Alastair Robinson
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- Mark Butcher
- Jeremy Zucker
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- Peter Larsen
- Peter St. John
- Dan Schell
- Jeff Wolfe



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Additional Slides

Responses to Previous Reviewers' Comments

- Weaknesses include geographic separation
 - As a distributed effort, we clearly have faced operational challenges, although these have more than been made up for by the Agile BioFoundry's ability to leverage physical and human resources across distributed national laboratories. The Agile BioFoundry's program manager, together with regular communications across the consortium (via teleconferences, webinars, informatics servers, SharePoint, annual in-person meetings), have helped mitigate communications risks. Sample transfer risks (i.e., sample stability, sample loss) will continue to be assessed through local/proximal compared with remote sample analysis, and to date we have not suffered from any notable sample losses. We are continuing to make progress in addressing disconnects in technology adoption, and it continues to be an operational imperative to standardize workflows and data-exchange formats wherever possible.
- Do not yet have a compelling argument as to why and how their approach will be better than other potential approaches to the problem
 - What sets the Agile BioFoundry apart from other foundries is that we develop and distribute publicly available tools, methods, and strains aimed at broadly benefiting the biofuels and bioproducts industry. Whereas private foundries are incentivized to develop proprietary tools and organisms, the Agile BioFoundry is a publicly funded effort aimed at delivering technology that will enable industry to either leverage our resources through partnership or adopt our methodologies for developing bioproducts. In comparison to the publicly funded Defense Advanced Research Projects Agency Living Foundries program, there are distinct programmatic and technical differences between the aims of the two efforts. Where the Living Foundries program is primarily focused on developing biological pathways to materials that cannot be achieved through transformations of petroleum feedstocks, the Agile BioFoundry is focused developing biological pathways for producing advanced biofuels and renewable, high-volume chemicals.

Responses to Previous Reviewers' Comments (cont.)

- Rationale for their choice of product targets needs to be strengthened
 - The Agile BioFoundry is pursuing multiple target/hosts to demonstrate that the methods, software, and technologies can be productively applied across product classes. The process and rationale for selecting the three target/hosts pairs for FY 2017 (and the 15 pairs for initially prioritized for FY 2017 – FY 2019) was described during the 2017 Peer Review, and the details were provided to BETO. For our FY 2018 and FY 2019 target/host selection processes, in addition to quantitative technical assessments across multiple categories (TEA and Market, LCA, Strategic Value, Scientific Novelty, DOE Relevance, How Designable, How Buildable, How Hostable, How Testable, How Scalable, and Chemical and Biological Safety), we proactively consulted with the Agile BioFoundry Industry Advisory Board to ensure that our prioritized targets and hosts remain aligned with industry's needs.
- Isn't clear that reducing the cycle time to, say, adipic acid, would be generally applicable to other material
 - As will be / has been presented in the Target/Host ABF presentations at the 2019 Peer Review, we have started to diligently measure cycle times across targets and hosts. This is the pre-requisite step to measuring improvements in (i.e., reductions to) cycle time. It should be noted that we are now pursuing multiple targets in the same host (which could suggest how cycle times for the second target have benefitted from improvements for the first target) and the same target in multiple hosts (which could suggest how cycle times in the second host have benefitted from improvements for the first host). While the former is more directly relevant for this previous reviewer's comment, both are important to capture and understand as they both directly affect the Agile BioFoundry's ability to broadly accelerate biomanufacturing process development across targets and hosts.

Responses to Previous Reviewers' Comments (cont.)

- More emphasis should be placed on the performance gap between small-scale culturing and bench-scale fermentation, which is a well-known problem in the field
 - We recognize that there are challenges associated with each increase in process scale, including the transition from high-throughput, small-scale culturing to bench-scale fermentation. Agile BioFoundry workflows leverage design of experiments and small-scale culture to select strains to grow in bench-scale bioreactors. Bench-scale fermentation provides critical data for the “Learn” component of Design-Build-Test-Learn, both to inform future designs and to develop predictive models that may be applied to small-scale experiments. Agile BioFoundry facilities have recently procured Robo/Biolector(Pro) and Ambr250 instrumentation which both serve to bridge the gap between small-scale culturing and bench-scale fermentation.
- PI is encouraged to look deeply into high-throughput fermentation techniques mastered by enzymes and biobased chemicals and fuels companies
 - As mentioned above, towards adopting the techniques practiced and mastered by companies, Agile BioFoundry facilities have recently procured Robo/Biolector(Pro) and Ambr250 high-throughput fermentation instrumentation.
- Encourage the PI to form a strong liaison between fermentation and the high-throughput team
 - There are strong connections between Agile BioFoundry high-throughput and bio-reactor fermentation teams, with staff shared in common between them.

Publications, Patents, Presentations, Awards, and Commercialization

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
- Costello, Zak, and Hector Garcia Martin. "A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data." NPJ systems biology and applications 4.1 (2018): 19. <https://doi.org/10.1038/s41540-018-0054-3>
- Oyetunde, Tolutola, et al. "Leveraging knowledge engineering and machine learning for microbial bio-manufacturing." Biotechnology advances (2018). <https://doi.org/10.1016/j.biotechadv.2018.04.008>
- Amin Zargar, Jesus F. Barajas, Ravi Lal, Jay D. Keasling. "Polyketide Synthases as a Platform for Chemical Product Design" AIChE (2018) <https://doi.org/10.1002/aic.16351>
- Jha RK*, Bingen JM, Johnson CW, Kern TL, Khanna P, Trettel DS, Straus CEM, Beckham GT, Dale T* (2018). A protocatechuate biosensor for Pseudomonas putida KT2440 via promoter and protein evolution. Metabolic Engineering Communications (6) 33-38. <https://doi.org/10.1016/j.meteno.2018.03.001>
- Mitchell G. Thompson, Nima Sedaghatian, Jesus F. Barajas, Maren Wehrs, Constance B. Bailey, Nurgul Kaplan, Nathan J. Hillson, Aindrila Mukhopadhyay & Jay D. Keasling. (2018) "Isolation and characterization of novel mutations in the pSC101 origin that increase copy number". Scientific Reports 8, 1590 doi:10.1038/s41598-018-20016-w
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Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Publications (cont.)

- Morrell, W., Birkel, G., Forrer, M.,; Lopez, T., Backman, T.W.H, Dussault, M., Petzold, C., Baidoo, E., Costello, Z., Ando, D., Alonso Gutierrez, J., George, K., Mukhopadhyay, A., Vaino, I., Keasling, J., Adams, P., Hillson, N., Garcia Martin, H. "The Experiment Data Depot: a web-based software tool for biological experimental data storage, sharing, and visualization" (2017) ACS Synthetic Biology DOI: 10.1021/acssynbio.7b00204
- Eng, C.H.*, Backman, T.W.H.*, Bailey, C.B., Magnan, C., Garcia Martin, H.G., Katz, L., Baldi, P., Keasling, J.D. "ClusterCAD: a computational platform for type I modular polyketide synthase design." (2017) Nucleic Acids Research DOI: 10.1093/nar/gkx893 *Contributed equally
- Barajas, J.F., Blake-Hedges, J., Bailey, C.B., Curran, S., Keasling, J.D. (2017). "Engineered polyketides: Synergy between protein and host level engineering" Synthetic and Systems Biotechnology doi.org/10.1016/j.synbio.2017.08.005
- Shymansky, Christopher M., et al. "Flux-enabled exploration of the role of Sip1 in galactose yeast metabolism." Frontiers in Bioengineering and Biotechnology 5 (2017)

Presentations

- Gregg Beckham, Hybrid biological and catalytic processes to manufacture and recycle plastics, Princeton University, November 28th, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Ginkgo Bioworks, Boston, MA, November 12, 2018
- Nathan J. Hillson. "DIVA (DNA Design, Implementation, Validation Automation) Platform". Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 8, 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
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". AIChE annual meeting, Pittsburgh, PA, October 31 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Thermo Fisher, San Jose, CA, October 19, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". DTRA Tech Watch, Ft. Belvoir, VA, October 10, 2018
- Nathan J. Hillson. "DOE Agile BioFoundry Overview". Invited Talk, SynBioBeta 2018 visit to ESE, Emeryville, CA, October 1, 2018
- Nathan J. Hillson. "ABF Organization, Progress, and FY19 Plans". Invited Talk, ABF All Hands Annual Meeting 2018 (Industry Day), Emeryville, CA, September 12, 2018
- Nathan J. Hillson. "Agile BioFoundry Overview". Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garcia Martin, H. "A new approach to flux analysis". Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Presentations (cont.)

- Hector Plahar. "DIVA Software Platform". Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Tijana Radivojevic. "Automatic Recommendation Tool", Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Jennifer Chiniquy. "DIVA DNA-Seq and DNA Construction", Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garcia Martin, H. "A New Approach to Flux Analysis". ABF Annual Meeting, Berkeley CA, September 7, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, Machine learning for science workshop, Berkeley, CA, September 5, 2018
- Nathan J. Hillson. "Agile BioFoundry Overview". Invited Lightning Talk, LBNL BioSciences Area Retreat 2018, Lafayette, CA, August 30, 2018
- Garcia Martin, H. "Modeling from molecules to ecosystems : opportunities, challenges and vision". Invited talk, BioEpic meeting, Berkeley, CA, August 23, 2018
- Garima Goyal "DIVA DNA Construction". Invited Talk, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- Tijana Radivojevic. "Automatic Recommendation Tool", Invited Talk, JBEI Annual Meeting 2018, Sonoma, CA, August 22, 2018
- Garcia Martin, H. "Opportunities in the intersection of synthetic biology, machine learning and automation". Invited talk, JBEI Annual Meeting, Berkeley, CA, August 20, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, SIMB, Chicago, IL, August 15, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, International Workshop for BioDesign and Automation (IWBDA), Berkeley, CA, August 2nd, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, Biocruces, Bilbao, Spain, July 20, 2018
- Garcia Martin, H. "Machine Learning to Predict Metabolic Pathway Dynamics from Multiomics Data". Invited talk, AI for synthetic biology, Stockholm, Sweden, July 15, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, BCAM, Bilbao, Spain, July 3, 2018
- Nathan J. Hillson, "Berkeley (and other) National Lab(s): Current Biosecurity Frameworks and Strategies in Action", Invited Talk, EBRC meeting - Improving Security Considerations in Engineering Biology Research, Emeryville, CA, June 26, 2018
- Nathan J. Hillson and Hector A. Plahar, "ICE Software Platform", Invited Talk, Software for Synthetic Biology Workflows Workshop, SEED 2018, Scottsdale, Arizona, June 7, 2018
- Gregg Beckham. Developing new processes to valorize lignin and sugars to building-block chemicals and materials, RWTH Aachen University, May 28th, 2018

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Presentations (cont.)

- Gregg Beckham. Adventures in engineering *Pseudomonas putida* for expanded substrate specificity and improved tolerance, RWTH Aachen University, May 28th, 2018
- Hillson, N.J. "Berkeley Lab project activities, biosecurity practices, and their roles within the larger biosecurity landscape". Invited Talk, Working Group on Automation in SynBio, Gryphon Scientific, Takoma Park, MD, May 23, 2018
- Hillson, N.J. "Recent developments at the Agile BioFoundry". Invited Talk, Diligence Ventures/Suzhou Government visit to ABF, Emeryville, CA, May 2, 2018
- Gregg Beckham. Hybrid biological and catalytic processes to manufacture and recycle plastics, MIT, April 27th, 2018
- Hillson, N.J. "Recent developments at the Agile BioFoundry". Invited Talk, 2018 Life Science Symposium - Synthetic Biology and Metabolic Engineering, MilliporeSigma Innovation Center, St. Louis, MO, April 27, 2018
- Garcia Martin, H. "A Machine Learning Approach to Predict Metabolic Pathway Dynamics from Time Series Multiomics Data". Invited talk at Madison Microbiome Meeting at University of Wisconsin, Madison, WI, April 25, 2018.
- Jennifer Chiniquy, Cindi Hoover, Joel Guenther, Nurgul Kaplan, Garima Goyal, Mark Kulawik, Hector Plahar, Zachary Costello, Brian Bushnell, Samuel Deutsch, and Nathan J. Hillson. "Overcoming Challenges in MiSeq DNA Construct Sequence Validation". Invited Poster, DOE JGI User Meeting 2018, San Francisco, CA, March 14, 2018
- "Test" and "Learn" in process research informs design strategy Sundstrom, E. R., M. Mirsiaghi, F. Tachea, N. Sun, T.R. Pray, D. Tanjore. ECO-BIO, Dublin, Ireland, March 5, 2018.
- Garcia Martin, H. "EDD as a data warehouse and Learn facilitator". Invited talk at Argonne National Lab, St. Louis, Lemont, IL, March 5, 2018
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Hector A. Plahar, Annabel Large, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA Services: PCR, Full DNA Construction, and MiSeq Validation". Invited Poster, DOE BER GSP Contractor's Meeting 2018, Tysons Corner, VA, February 27, 2018
- Hillson, N.J. "Three synthetic biology design challenges we face, and how we are approaching them". Invited Talk, Dagstuhl Seminar 18082, Wadern, Germany, February 19, 2018
- Jennifer Chiniquy, Nurgul Kaplan, Garima Goyal. "DIVA DNA-Seq Service", JBEI User Meeting presentation, February 12, 2018.
- Garcia Martin, H. "Metabolic Modeling of -omics Data for Biofuel Production". Invited talk at Bayer, Sacramento, CA, February 2, 2018.
- Garcia Martin, H. "Machine Learning and Mechanistic Models to Predict Biological Outcomes using 'omics Data". Invited talk at Environmental Genomics and Systems Biology retreat, Berkeley, CA, January 19, 2018
- Jesus F. Barajas. "Current progress towards engineered PKS lactam pathways". JBEI/BBD group meeting presentation, December 13, 2017
- Hillson, N.J. "Agile BioFoundry Overview". Invited Talk, iSynBio/SIAT visit to JGI, Walnut Creek, CA, December 9, 2017
- Jennifer Chiniquy, Nurgul Kaplan. "DIVA DNA-Seq Service". ESE User Meeting presentation, November 20, 2017

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Presentations (cont.)

- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Cargill visit to ESE, Emeryville, CA, November 17, 2017
- Hillson, N.J. “Flanking Homology DNA Assembly, Protocol Design Software, and Synthetic DNA”. Invited Talk, Bitesize Bio Webinar, November 15, 2017
- Simmons, B.A. and Hillson, N.J. “The BioDefense Foundry”. Invited Talk, DTRA Tech Watch Briefing, Springfield, VA, November 8, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, University of Wyoming, Laramie, WY, November 3, 2017
- Hillson, N.J. “Parallel Integration and Chromosomal Expansion of Metabolic Pathways”. Invited Talk, University of Wyoming, Laramie, WY, November 3, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Braskem Zoom Teleconference, November 1, 2017
- Hector Garcia Martin. “Modeling of -omics data for Biofuel Production through Synthetic Biology”. EECE Department seminar, Washington University, St. Louis MO, October 20th, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, ABLC Next Tour of ESE (ABF/ABPDU/JBEI), Emerville, CA, October 16, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Berkeley Lab Workshop: Industrialization of engineering biology: from discovery to scale-up, SynBioBeta SF 2017, UCSF Mission Bay, San Francisco, CA, October 3, 2017
- Hillson, N.J. “How the Agile BioFoundry Thinks About Paths to Commercialization”. Invited Talk, SynBio for Defense, Arlington, VA, September 27, 2017
- Hillson, N.J. “BioDefense – the Agile BioFoundry and Predictive Biology”. Invited Talk, Presentation for Dimitri Kusnezov (Chief Scientist, DOE NNSA), Berkeley, CA, September 21, 2017
- Hillson, N.J. “Sustainable development through a synthetic biology foundry”. Invited Talk, CellPress LabLinks - Basic to Applied Science for Sustainable Development, Berkeley, CA, September 18, 2017
- Plahar, H.A. “Software Session: Recent DeviceEditorjs/DIVA/ICE improvements”. Invited Talk, JBEI Annual Meeting, Monterey, CA, September 15, 2017
- Costello, Z. “Software Session: The Automatic Recommendation Tool”. Invited Talk, JBEI Annual Meeting, Monterey, CA, September 15, 2017
- Backman, T.W.H. “ClusterCAD: a computational platform for type I modular polyketide synthase design.” Invited Talk, JBEI Annual Meeting, Monterey, CA, September 14, 2017
- Hillson, N.J. “Agile BioFoundry Update”. Invited Talk, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Plahar, H.A. “ICE/DIVA Software Tutorial”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 29, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- De Paoli, H.C. “A. pseudoterreus 3HP Design and Build”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017.
- Chiniquy J., “DIVA DNA-Seq Service”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Presentations (cont.)

- Garcia Martin, H. "Predicting Metabolic Pathway Dynamics by Combining Multiomics Data with Machine Learning and Kinetic Modeling". Invited talk at "Multi-omics for Microbiomes" conference, Pasco, WA, July 31, 2017.
- Johnson, C.W. "Metabolic engineering of *Pseudomonas putida* KT2440 for production of muconic acid from sugar", SIMB Annual Meeting, July 31, 2017
- Hillson, N.J. "j5 Software Through the Years: Insights from Aggregate Public Usage Metrics". Invited lightning talk, World Metrology Day Symposium, Stanford, CA, May 22, 2017.
- Beckham, G.T. "The Agile BioFoundry: Investing in Biomanufacturing Infrastructure", TechConnect World, May 16, 2017
- Derek Vardon. Potential commercialization opportunities for valorization of biomass to polymer precursors. Invited Seminar. Alliance Commercialization and Deployment Committee Meeting, NREL. May 2017.
- Gregg Beckham. The Agile BioFoundry: Investing in Biomanufacturing Infrastructure, TechConnect World, May 16, 2017
- Hillson, N.J. "Overview of the Agile BioFoundry". Invited talk, IMP (Mexican Petroleum Institute) Visit to JBEI, Emeryville, CA, April 21, 2017.

Posters

- J. Meadows, C. Johnson, S. Notonier, Y.M. Kim, S. Tripathy, K. Burnam-Johnson, M. Burnet, J. Magnuson, G. Beckham, N. Hillson, J. Gladden. "Engineering *Pseudomonas putida* KT2440 to produce adipic acid from lignocellulosic components". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Jesus F. Barajas, Jingwei Zhang, Amin Zargar, Bo Pang, Huaxiang Deng, Veronica T. Benites, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Jay D. Keasling. "Development of Valerolactam and Caprolactam Biosynthetic Routes". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Jonathan Diab, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA (Design Implementation Validation Automation) DNA Construction". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Jonathan Diab, Jennifer Chiniquy, Cindi Hoover, Joel Guenther, Nurgul Kaplan, Garima Goyal, Mark Kulawik, Hector Plahar, Zachary Costello, Brian Bushnell, Samuel Deutsch, and Nathan J. Hillson. "MiSeq DNA Construct Sequence Validation". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Edward E.E.K. Baidoo and Veronica Teixeira Benites. "High throughput analysis of isoprenoid pathway intermediates by HILIC-QTOF-MS". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018.
- Isaac Wolf, Carolina Barcelos, Shawn Chang, Nilufer Oguz, Matt Dorsey, Davinia Salvachua, Robert Nelson, Todd Pray, Eric Sundstrom and Deepti Tanjore. "Harmonization of Fermentation for Production of *P. putida*-derived Muconic Acid". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Posters (cont.)

- J. Prah, S. Coradetti, D. Liu, G. Geiselman, T. Pray, J. Gladden, E. Sundstrom, and D. Tanjore. "Insights from Bioreactors make Scale-Down Modeling more Effective". Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Jonathan Diab, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA (Design Implementation Validation Automation) DNA Construction". Invited Poster, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- William Morrell, Mark Forrer, Garrett Birkel, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. "Collaboration with the Experiment Data Depot". Invited Poster, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- Jonathan Diab, Jennifer Chiniquy, Cindi Hoover, Joel Guenther, Nurgul Kaplan, Garima Goyal, Mark Kulawik, Hector Plahar, Zachary Costello, Brian Bushnell, Samuel Deutsch, and Nathan J. Hillson. "MiSeq DNA Construct Sequence Validation". Invited Poster, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- Sarah A LaFrance, Jacob Coble, Thomas Rich, Hector Plahar, Joshua Nixon, Nathan J. Hillson. "VectorEditor: Freely Open-Source Javascript Webapp for DNA Visualization, Annotation, and Editing". Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Annabel Large, Nurgul Kaplan, Jennifer Chiniquy, Garima Goyal, and Nathan Hillson. "Expansion and Optimization of DIVA DNA Sequence Validation Services". Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA (Design Implementation Validation and Automation) DNA Construction". Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "Using DIVA, DeviceEditor, and j5 for DNA Construction". Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- William Morrell, Garrett Birkel, Mark Forrer, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. "The Experiment Data Depot platform". Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Backman, T.W.H., Eng, C.H., Bailey, C.B., Keasling, J.D., Garcia Martin, H. "Software for polyketide synthase (PKS) design". Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA (Design Implementation Validation and Automation) DNA Construction". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "Using DIVA, DeviceEditor, and j5 for DNA Construction". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Posters (cont.)

- Jennifer L. Chiniqy, Cindi A. Hoover, Joel M. Guenther, Nurgul Kaplan, Christopher W. Beitel, Samuel Deutsch, and Nathan J. Hillson. "Towards a High-Throughput Low-Cost Automated DNA Sequence Validation Workflow". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- William Morrell, Garrett Birkel, Mark Forrer, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. "The Experiment Data Depot platform". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Hector A. Plahar, Elena Aravina, Oge Nnadi, Joanna Chen, Paul D. Adams, Jay D. Keasling, and Nathan J. Hillson. "ICE: A Distributed and Interconnected Biological Part Registry". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Jha, R., Narayanan, N., Johnson, C., Beckham, G., Dale, T. "Whole cell biosensing in Pseudomonas putida KT2440". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Pandey N., Krishnamurthy, M., Jha, Ramesh., Hennelly, S., Dale, T. "Riboregulator Development To Increase Metabolic Flux Towards Muconate Production". Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- John Meng, Angela Tarver, Matthew Hamilton, Robert Evans, Lisa Simirenko, Nathan J. Hillson, Jan-Fang Cheng, and Samuel Deutsch. "SynTrack 2: A Scalable DNA Assembly Production Workflow Management". Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Sarah A LaFrance, Jacob Coble, Thomas Rich, Hector Plahar, Joshua Nixon, Nathan J. Hillson. "VectorEditor: Freely Open-Source Javascript Webapp for DNA Visualization, Annotation, and Editing". Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- William Morrell, Garrett Birkel, Mark Forrer, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. "The Experiment Data Depot platform". Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniqy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Nina Stawski, Manjiri Tapaswi, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA (Design, Implementation, Validation Automation) DNA Construction: Wet-Lab Workflow and Software Platform". Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Philip C. Gach, Manasi Rajee, Nurgul Kaplan, Sangeeta Nath, Samuel Deutsch, Jay D. Keasling, Paul D. Adams, Nathan J. Hillson and Anup K. Singh. "A Microfluidic Platform for Combinatorial Gene Assembly, Transformation, Culture and Assay". Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Hillson, N.J. "j5 Software Through the Years: Insights from Aggregate Public Usage Metrics". Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.
- Jennifer L. Chiniqy, Cindi A. Hoover, Joel M. Guenther, Nurgul Kaplan, Christopher W. Beitel, Samuel Deutsch, and Nathan J. Hillson. "Towards a High-Throughput Low-Cost Automated DNA Sequence Validation Workflow". Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

Posters (cont.)

- G. 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 Biofuel Pathways in E. coli". Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Nina Stawski, Manjiri Tapaswi, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. "DIVA (Design, Implementation, Validation Automation) DNA Construction: Wet-Lab Workflow and Software Platform". Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.