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**ENERGY** | Energy Efficiency &  
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BIOENERGY TECHNOLOGIES OFFICE

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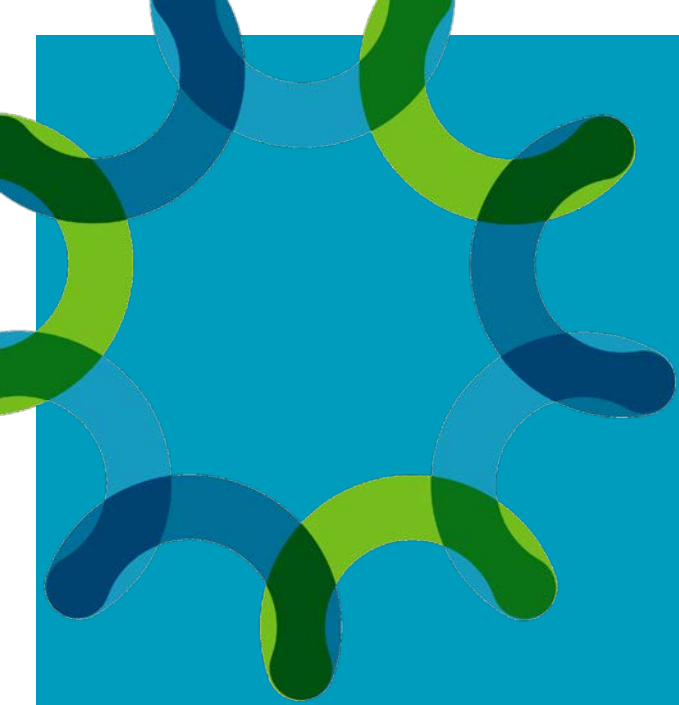
# Agile BioFoundry (ABF) Overview

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Principal Investigator, DOE Agile BioFoundry

BETO Peer Review 2021  
Conversion Technologies  
10:45-11:45AM EST  
March 9, 2021





# Project Overview

# Goal

- **Goal:** Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry to productionize synthetic biology
- **Outcomes:** Development and deployment of technologies enabling commercially relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts
- **Relevance:** \$20M/year public infrastructure investment that increases U.S. industrial competitiveness and enables opportunities for private sector growth and jobs
- **Risks:** Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from



# Public Infrastructure Investment Enables Private Industry

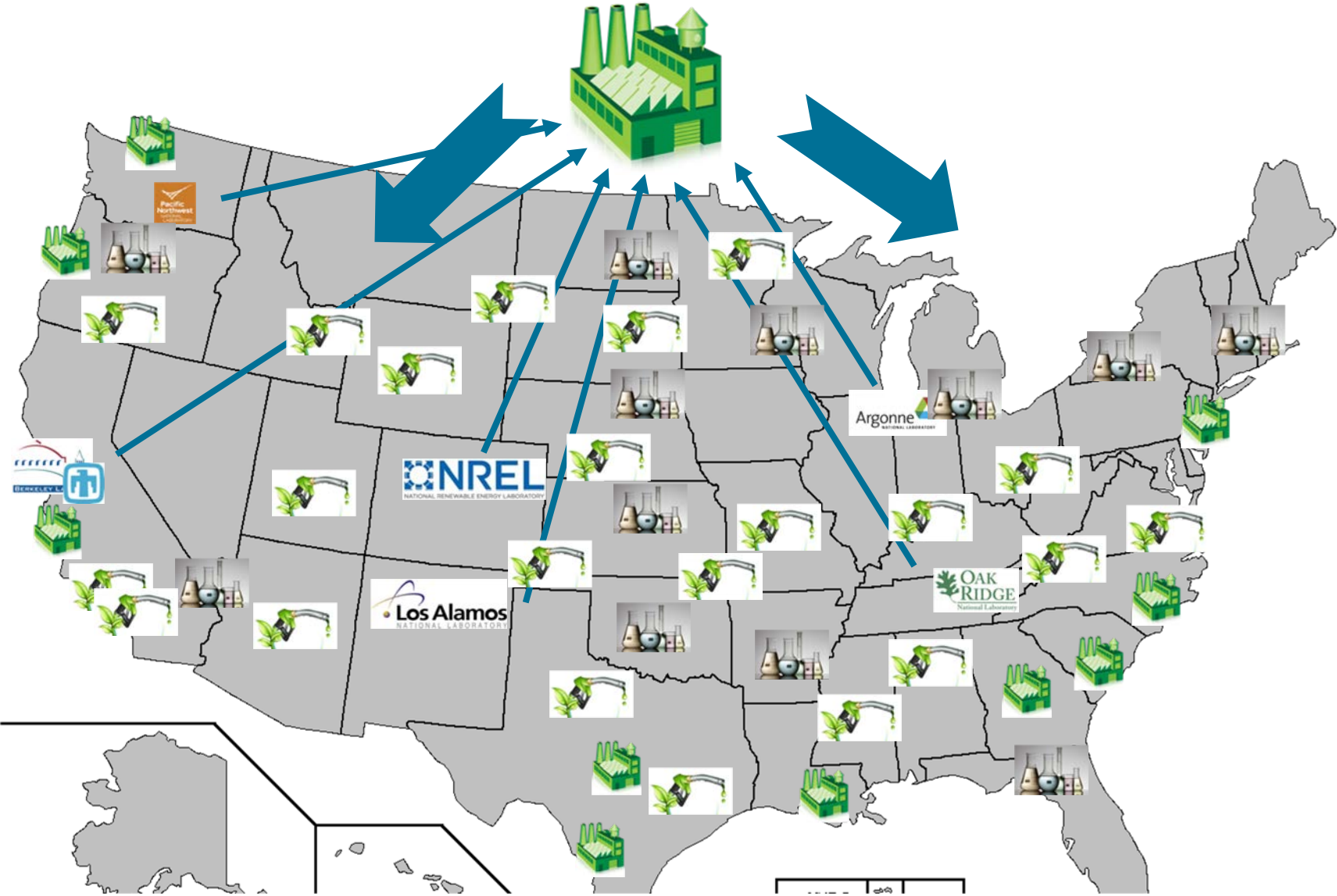
*Public* investment in biomanufacturing infrastructure



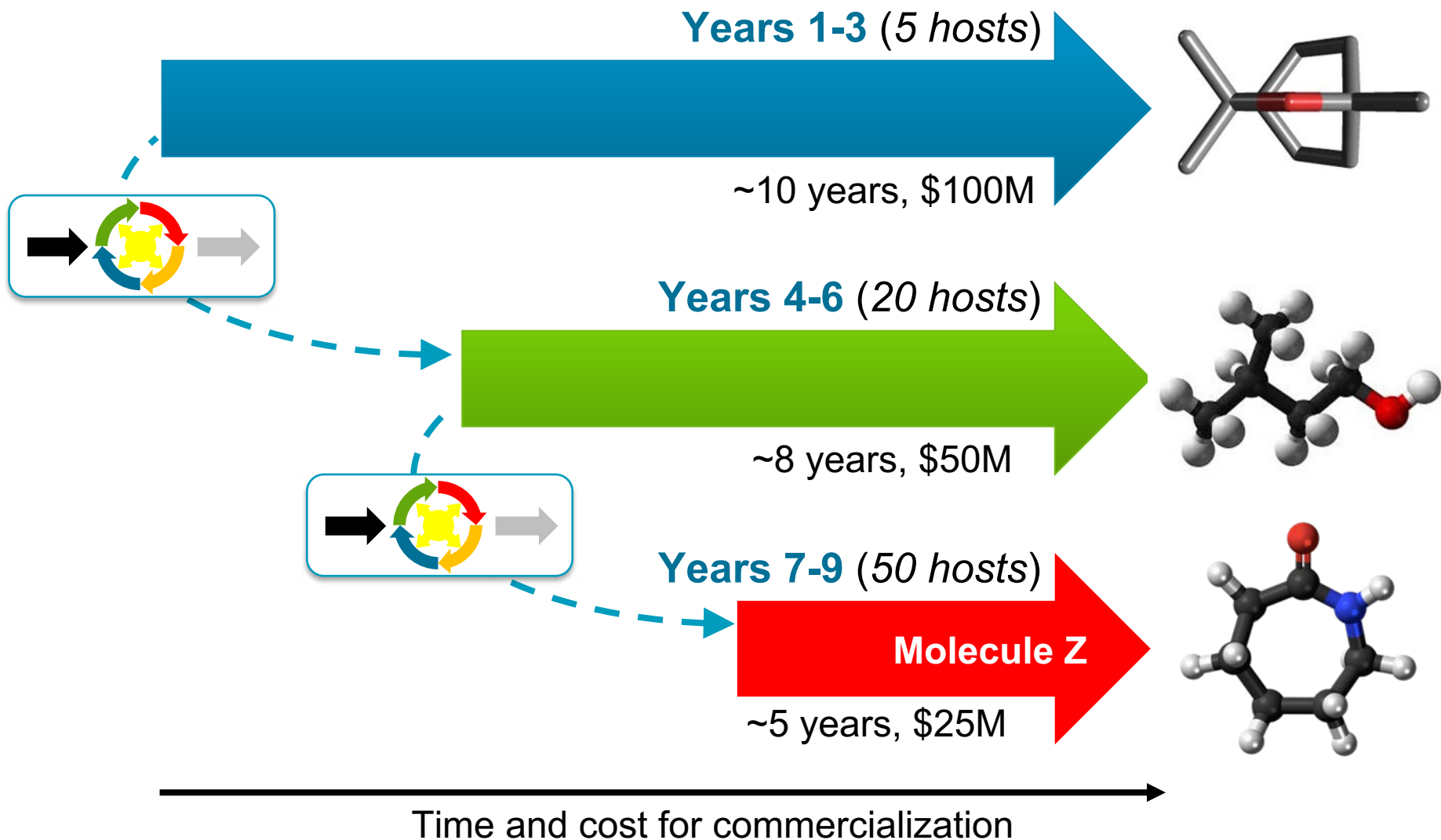
*Private* investment in product development, scaling, and tailoring to unique pathways and products

Adapted from Lyft

# A Distributed Agile BioFoundry



# Agile BioFoundry Will Reduce Time-to-Scale up





# Definitions of ABF parlance / terms

- **Target / Host pair**

- **Target**: the target molecule to be produced (e.g., adipic acid)
- **Host**: the microbial species hosting the metabolic pathway that produces the target (e.g. *P. putida*)
- **Target / Host pair**: a target paired with a specific host to produce it

- **Transfer Target / Tool**

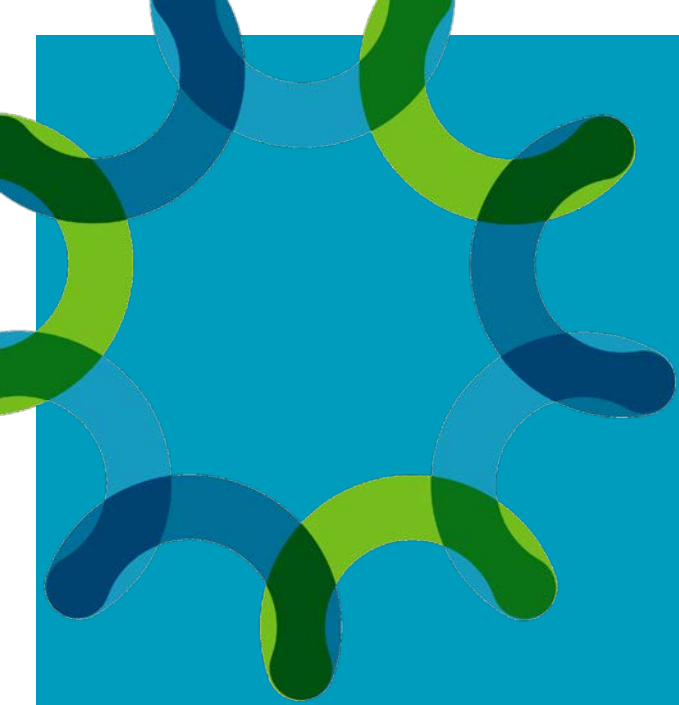
- A target or tool the ABF has already produced in/developed for one host, now paired with a new host

- **Beachhead / Exemplar molecule**

- **“Beachhead”**: a strategic metabolic intermediate that can be converted into several bioproducts
- **“Exemplar”**: a representative target molecule downstream of a given beachhead

- **Platonic DBTL cycle**

- **“Platonic”**: a reference to Plato’s Ideals (e.g. a perfect/abstract vs. an imperfect/physical chair)
- Conceptually similar to [EERE/EPA Fuel Economy Estimates](#) (idealized highway/city driving)
- Consists of a [specified set of unit operations](#) across DBTL, with minimum capacities through each
- Efficiency metrics are individually captured for each unit operation performed, irrespective of other unit operations used in a cycle
  - Wall/clock time, capacity, resource requirements
- Efficiency metric for a Platonic DBTL cycle is estimated from the [underlying unit operation metrics](#)
- [Enables ABF to track efficiency progress better](#) (more data!) even when not every cycle is Platonic
- **“Mini-DBTL”** cycles lack one or more unit operations of the **“Full”/Platonic** DBTL cycle



# 1 - Management



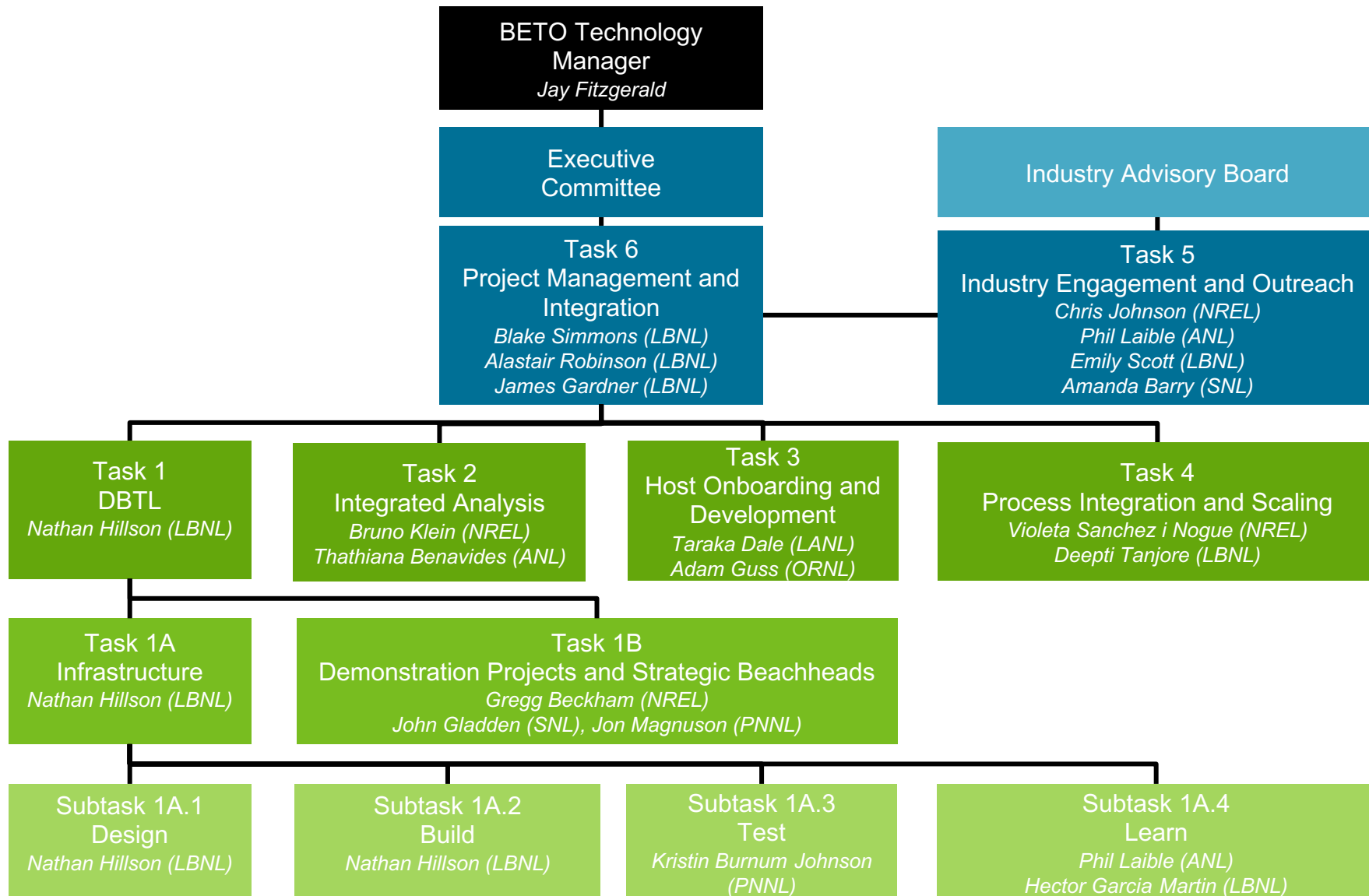
# Project Structure

- **History:** FY17-19: \$41.2M + \$2.7M ABF-Directed Funding Opportunity (DFO)
- **Funding:** FY20-22: planned at \$45.0M + \$15.0M DFO
  - FY20: \$15.0M + \$5.0M DFO
  - FY21: \$15.0M + \$5.0M DFO
  - FY22: \$15.0M + \$5.0M DFO
- **Coordination** with FY18-19 ABF-related FOAs
- **Seven National Lab consortium**
- **Industry Advisory Board** actively engaged
- **Six tasks** for overall project
  - 4 research tasks, 2 management tasks
- **Multiple milestones** per quarter
  - Multiple annual SMART milestones and a 18-month Go/No-Go decision point
- **Monthly and quarterly progress reporting** to BETO

# Six Tasks

- **Task 1: Design-Build-Test-Learn** (*Nathan Hillson* - lead)
  - **Infrastructure:** Integrate design-build-test-learn cycle with process automation
  - **Demonstration Projects and Strategic Beachheads:** Demonstrate uses of DBTL infrastructure and establish and improve routes in microbial hosts to beachhead molecules of high strategic interest
- **Task 2: Integrated Analysis** (*Bruno Klein / Thathiana Benavides* – co-leads)
  - Analyze proposed target and beachhead molecules with TEA and LCA methodologies
- **Task 3: Host Onboarding & Development** (*Taraka Dale / Adam Guss* – co-leads)
  - Onboard additional microbial host organisms and further develop them to higher capability tiers through tool development and data collection
- **Task 4: Process Integration & Scale-up** (*Violeta Sanchez i Nogue / Deepti Tanjore* – co-leads)
  - Provide DMR-EH hydrolysates, and test and scale fermentation to improve titer, rate, and yield
- **Task 5: Industry Engagement & Outreach**  
(*Chris Johnson / Phil Laible / Emily Scott / Amanda Barry* – co-leads)
  - Identify barriers to industry adoption of ABF technologies, expand number and diversity of industry partnerships, and establish a set of metrics for determining impact of ABF technologies on industry
- **Task 6: Management** (*Blake Simmons* - lead)
  - Manage project management, develop internal and external communications, provide deliverables to BETO, and make capital equipment purchases

# Org Chart



# Roles and Responsibilities

- **Executive Committee**

- Composition: PI, Task Leads, Program Managers, BETO Technology Manager, others as needed
- Strategic direction and oversight
- Support collaboration between institutions
- ABF policy guidance
- Conflict resolution and performance management
- Progress tracking
- Demonstration Project / Strategic Beachhead, and Host selection
- Technical challenge identification/resolution

- **Program Managers**

- Alastair Robinson – 50% effort
  - Manage core project
  - Collaboration and progress/metrics tracking tools
  - Ensure adequate communication between labs for integration of whole consortium
  - Primary core project communications and operations point-of-contact
  - BETO reporting for core project
- James Gardner – 50% effort
  - Manage DFO projects and coordination with ABF-related FOAs
  - Manage annual DFO preparations and review/selection process
  - Primary DFO communications and operations point-of-contact
  - BETO reporting for DFO projects

# Communications

- **ABF is an integrated, geographically distributed multi-Lab team**
  - Effective communications are essential
- **Internal**
  - Bi-weekly Executive Committee meetings
  - Bi-weekly ABF Task Lead meetings
  - Weekly to monthly demonstration project/beachhead meetings
  - Weekly software and automation infrastructure user meetings / webinars
  - Monthly activity summary including DBTL cycle reports to BETO
  - Monthly Host Onboarding and Development Task team meetings
  - Monthly Learn team meetings - activities and milestone planning
  - Monthly Industry Outreach and Engagement Task team meetings
  - Quarterly progress / milestone completion reports to BETO
  - Software infrastructure (e.g. ICE, DIVA, EDD, LabKey, AgileBioCyc, Jupyter, github/bitbucket, etc.)
  - Google Platforms – file storage and sharing
  - Annual Learn Summit
  - Annual ABF All-Hands Meeting
- **External**
  - ABF website (agilebiofoundry.org) and social media (@agilebiofoundry)
  - Presentations, posters, booths at domestic and international scientific / technical conferences
  - Publications
  - Quarterly Industry Advisory Board meetings and Industry Listening Days
  - Annual Global BioFoundry Alliance meeting, and monthly webinar series

# National Lab Network



Phil Laible,  
Thathiana Benavides,  
Peter Larsen



Taraka Dale,  
Ramesh Jha, Chris Yeager  
Scott Hennelly



Nathan Hillson,  
Alastair Robinson, James Gardner,  
Deepti Tanjore, Blake Simmons,  
Katy Christiansen, Emily Scott



Gregg Beckham,  
Christopher Johnson,  
Violeta Sanchez i Nogue,  
Bruno Klein



Adam Guss



Jon Magnuson,  
Kristin Burnum-Johnson



John Gladden,  
Amanda Barry,  
Anne Ruffing

Jay Fitzgerald and Kevin Craig



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# Technical Risks and Mitigation Plans

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



# The Agile BioFoundry is complementary to BETO's other projects

- BETO's projects frequently target specific molecules/hosts
- In contrast, the **Agile BioFoundry** is a **broadly enabling platform**
  - **Applicable across biorefinery** fuel or chemical production **processes**
  - **Other BETO projects could leverage** Agile BioFoundry capabilities
    - Methods, workflows, instrumentation, software, expertise
    - Accumulated enzyme/pathway/host/process Learnings and data
- Agile BioFoundry development/assessment through several use cases
  - Sufficient number/diversity of demonstration project molecules/hosts to demonstrate broad utility

# Connections to other BETO projects

- **Other BETO consortia**

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



Co-Optimization of  
Fuels & Engines



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

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

- **Application of Energy I-Corp Learnings:**

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

# ABF and other DOE programs

- These activities actively support the demonstration of the ABF concept
- Complementary ABF domain expertise, infrastructure, and operational TRL range offer opportunities for synergy with other DOE programs
- The ABF is open to working with other DOE funded projects and centers, such as the BRCs and EFRCs
  - Target/host suggestions for ABF
    - Scientists can propose biofuel and bioproduct targets for the ABF to work on and further optimize
  - Technology off-ramping into ABF
    - Early stage DBTL infrastructure (e.g. software, devices, methods) and microbial hosts can be brought into the ABF and further developed and operationalized
  - Shared technical challenges collaboratively addressed
    - Example: DNA construction/experiment data storage – DNAda/EDD co-development
    - Resulting resources made accessible across projects – *P. putida* mutant libraries
  - Provide compelling examples of DOE teams working together
    - Across TRLs and bridging the gap between fundamental and applied science and technology
  - Enhance technology transfer and commercialization efforts

# Global Biofoundries Alliance (GBA)

Global Biofoundries Alliance  
Develop. Inspire. Engage. Evolve.

[Welcome](#) [About the GBA](#) [Information](#) [Members](#) [Working Groups](#) [The Bio-Engineering Cycle](#) [Publications](#) [Contact](#)



The overall objective of GBA is to

1. Develop, promote, and support non-commercial biofoundries established around the world,
2. Intensely collaborate and communicate among biofoundries,
3. Collectively develop responses to technological, operational, and other types of common challenges,
4. Enhance visibility, impact and sustainability of non-commercial biofoundries, and
5. Explore globally relevant and societally impactful grand challenge collaborative projects.

Please use the map to discover more about the alliance members or [click here for the list](#)

- **ABF founding \*member of the GBA in 2019**
  - \*ABF signature to GBA MOU pending DOE/U.S. State Dept. approval
- **ABF leadership and benefits from participating in the GBA**
  - Steering committee co-chair
  - Software, metrology, business model, grand challenges working groups

# ABF Industrial Advisory Board

Companies represented in FY21 roster

**amyris**

 **Trelys**

**CALYSTA**

 **Agilent  
Technologies**

 **GINKGO BIOWORKS™**  
THE ORGANISM COMPANY

 **BASF**  
We create chemistry

 **EBRC**

**teselagen** SYNTHETIC  
BIOTECHNOLOGY EVOLUTION™

  
**zymergen**

**ThermoFisher**  
SCIENTIFIC

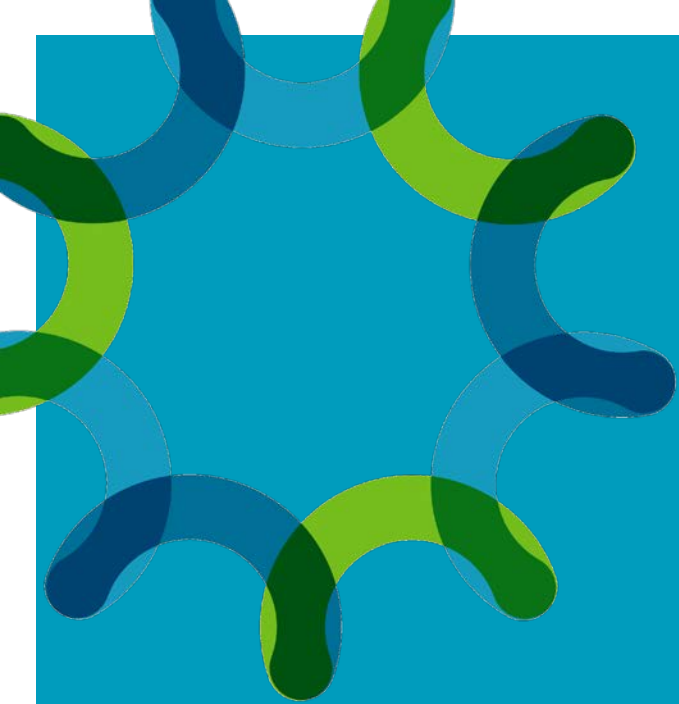
As a means of maintaining industrial relevance and to understand latest pain points and innovations:

Assembled diverse IAB

Established a charter that guides their involvement

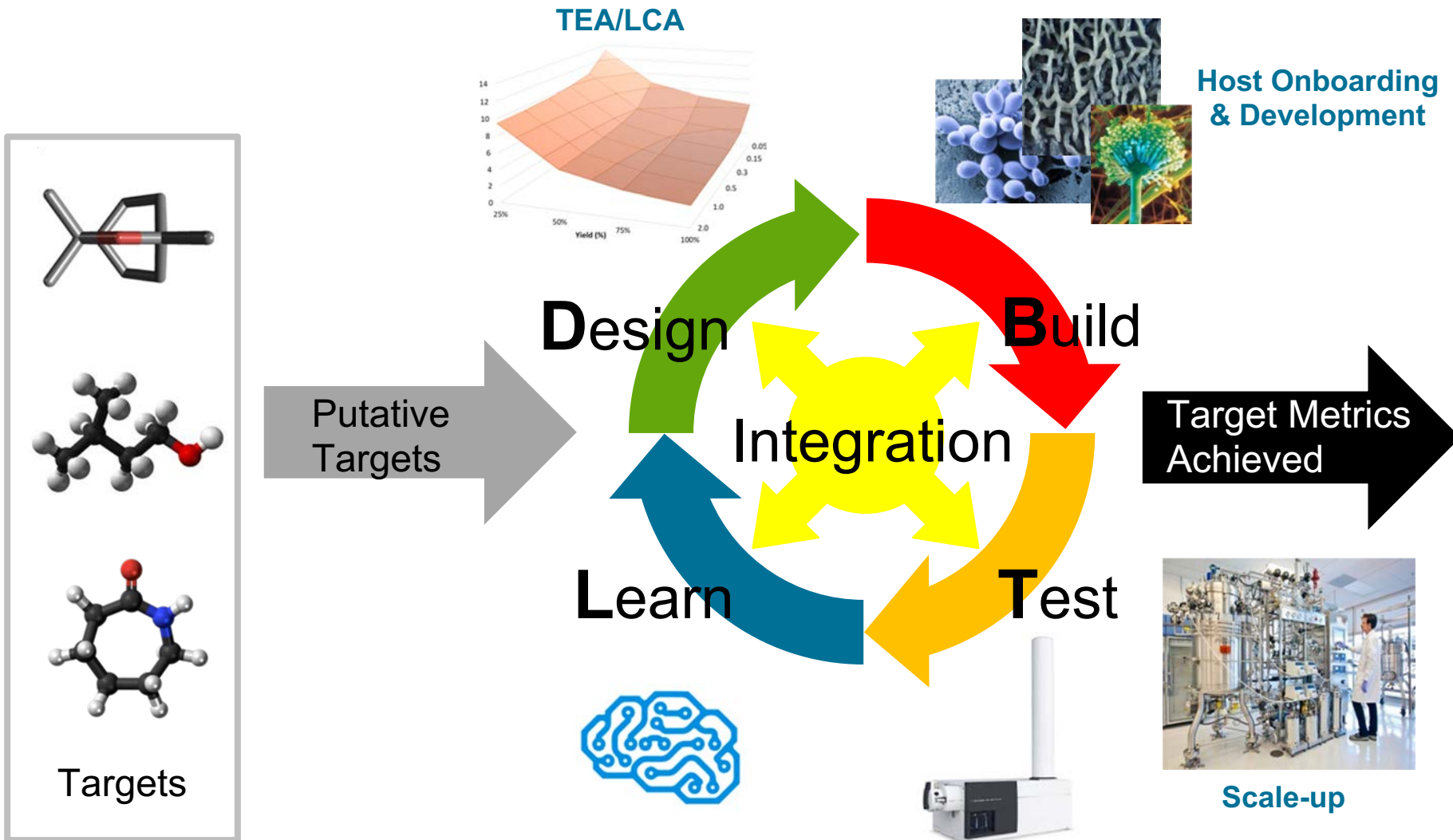
Use as a sounding board for feedback on Foundry:

- Approach
- Operations
- Milestone achievements
- Outreach activities
- Overall impact



## 2 - Approach

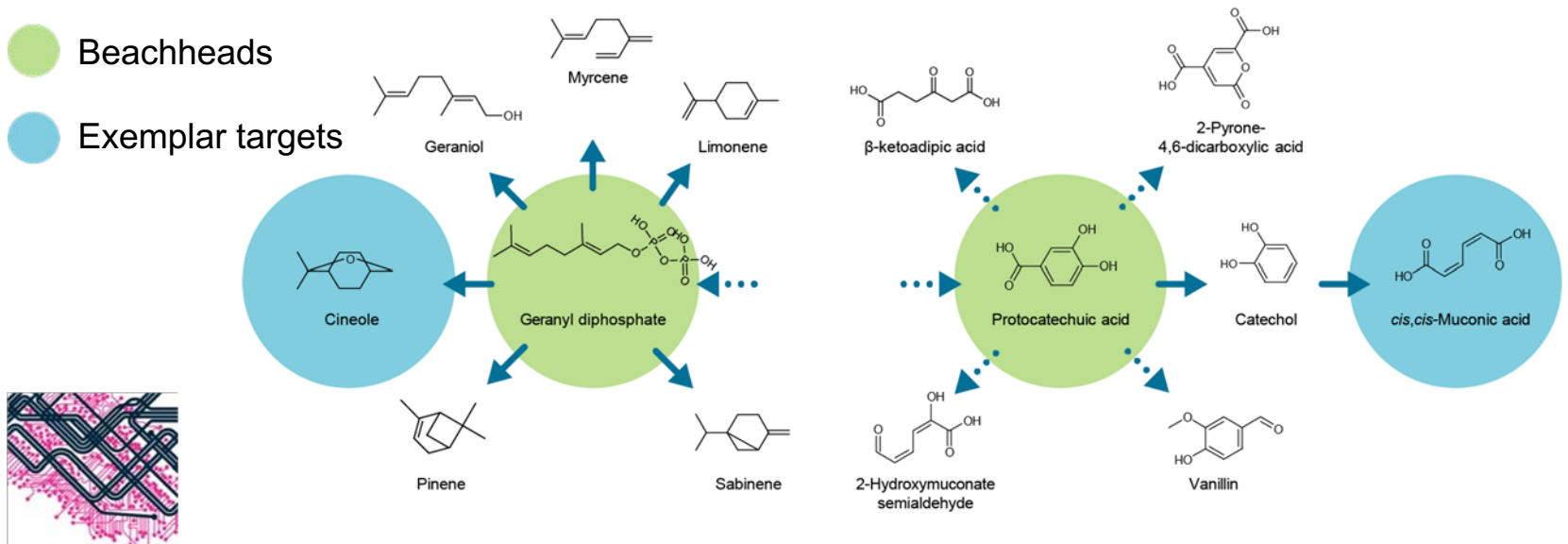
# The Agile BioFoundry Approach





# Beachheads

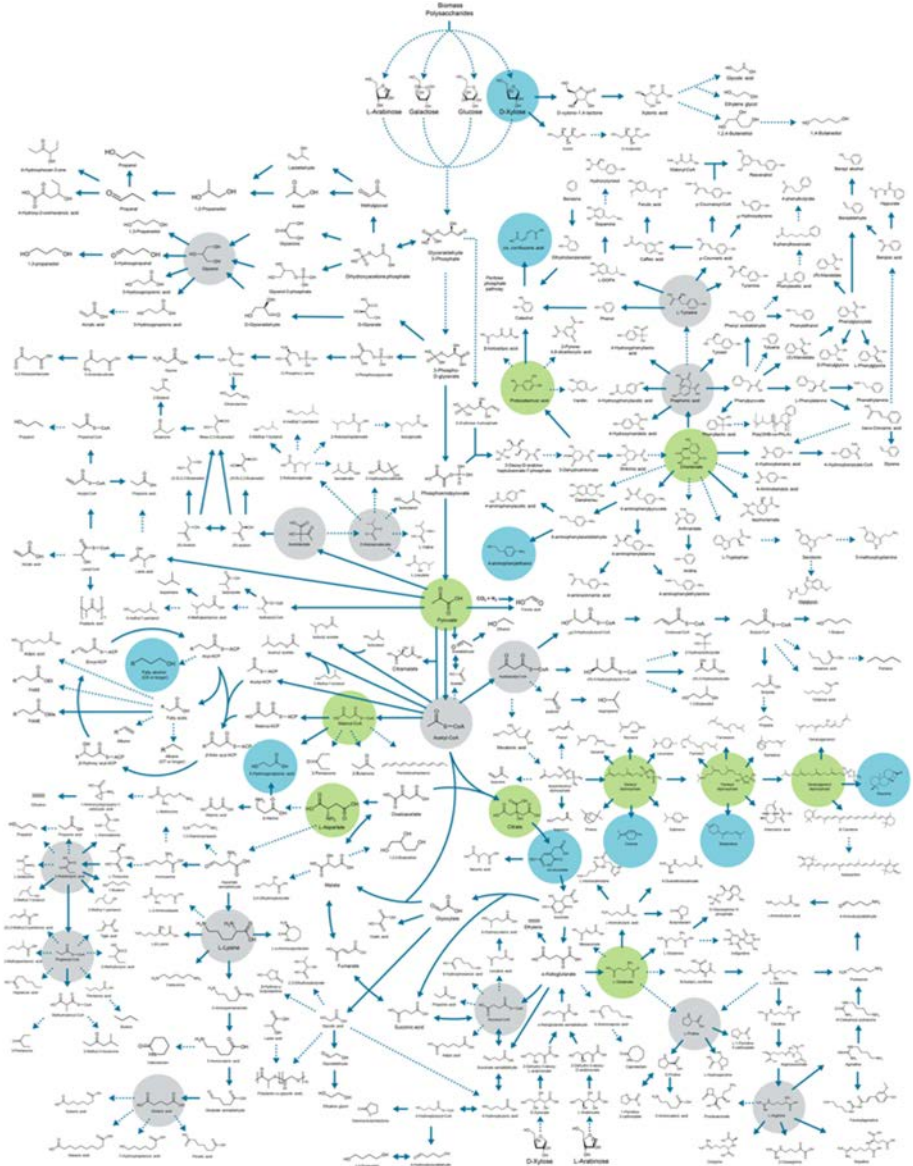
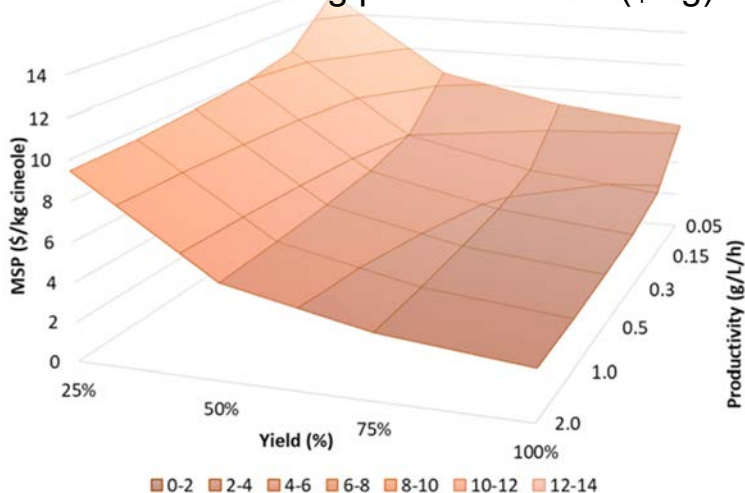
- **Beachheads are metabolic intermediates that can be converted into several bioproducts**
  - Metabolic engineering strategies developed for a given target can be applied to other targets associated with the same beachhead
  - Similar theoretical yields and processing parameters enable TEA and LCA of a single exemplar target product to extend to related products



# Beachheads

- The ABF is developing microbial strains for production of several exemplar products
  - Expanding the number of beachheads we're developing over time
  - Driven by TEA/LCA and industrial relevance

Minimum selling price of cineole (\$/kg)



Adapted by permission from Springer Nature Customer Service Centre GmbH: Nature, Nature Catalysis, A comprehensive metabolic map for production of bio-based chemicals, Lee, S.Y., et al., © 2019

# What makes ABF different than other BETO-funded metabolic engineering projects?

- **The ABF has highly collaborative teams that work together to:**
  - Move target / host pairs and strategic beachheads through the pipeline
  - Build the tools and infrastructure to do so
  - More closely mirror industry in terms of breaking effort into domains (e.g. Test team)
- **Strategic focus on analysis of and routes through beachhead molecules**
  - Requires innovation in TEA/LCA analysis (e.g., via exemplar molecules)
  - Goal is to maximize flux through, as opposed to accumulation of, the beachhead molecule
- **Learn component**
  - Integral to the ABF concept - effective learning from past projects is how we accelerate and increase the efficiency of future work
  - Experiments planned / coordinated to generate Learn-friendly data
- **Infrastructure to support scale / throughput / depth of analysis / Learn**
- **Integrated whole that might be separated in other projects**
  - Including Integrated Analysis (TEA/LCA), Host Onboarding & Development, Scale-up

# How topics are arranged in the talks that follow

- **Some ABF activities straddle Tasks**

- In some cases, we have distributed aspects of a given activity/topic across the ABF Task presentations that follow
- The development and application of biosensors is presented here for illustrative purposes

- **DBTL - Infrastructure**

- Methods / workflows / tools applicable across targets and hosts
- Example: Droplet microfluidic devices / methods development for use with biosensors

- **DBTL - Demonstration Projects / Strategic Beachheads**

- Application of methods / workflows / tools to a specific target / host
- Example: Application of a biosensor to increase TRY for adipic acid/*P. putida*

- **Host Onboarding and Development**

- Development of methods / workflows / tools for a specific host
- Example: Development of *P. putida*-specific aspects of a muconate biosensor

# Changes made in light of 2019 Peer Review

- **DBTL cycle specification and efficiency metrics:**
  - **Specification:** a concrete DBTL cycle specification is now in place
  - **Metrics capture:** we are increasingly automatically capturing efficiency metrics in our workflow-supporting software infrastructure
  - **Software usability:** we are engaging a software firm to improve the user interface and user experience of our software to reduce friction and improve efficiency metrics reporting accuracy and completeness
  - **Quantitative evaluation:** through the Platonic approach, we will be quantitatively estimating efficiency improvements made and identifying the most opportune unit operations(s) to further improve
- **TEA/LCA:**
  - **Beyond preliminary analysis of target/host pairs:** we are innovating our TEA/LCA analyses through the evaluation of strategic beachheads via downstream exemplar molecules. These analyses will identify the most opportune beachheads to pursue

# Top 3 potential challenges

- **Leverage past collaboration learnings with future collaborators**
  - Only portions of past collaborative data or learning methods that do not reveal the underlying primary data may be available
- **Predictive scale-up, and method transferability/reproducibility**
  - Our lack of ability to predict how a process will scale, or how well a method can be transferred across facilities, may limit the impact of our research and development efforts
- **Intellectual framing of strategic beachhead work**
  - It may be difficult to quickly and convincingly convey innovative TEA/LCA approaches for evaluating beachheads and metrics (e.g. flux vs accumulation, metabolic space coverage) to gauge progress in establishing and strengthening beachheads

# Go/no-go decision points

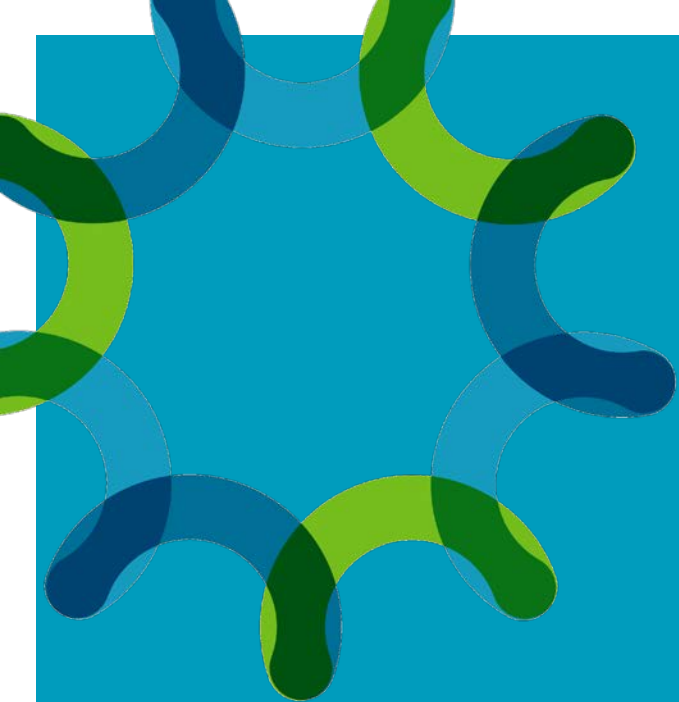
- **Date:** 3/31/2021
- **Description:** 5 target molecules or tools transferred between host organisms that are able to at least achieve 1 g/L or higher in the first host. Successful target molecule transfers will have product titers greater than 1 g/L. For 3 of 5 of these, 2X biological engineering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

Target Molecule/Tool	Original Host	Transfer Host (s)	Efficiency gain
3HP	<i>A. pseudoterreus</i>	<i>A. niger</i> ; <i>R. toruloides</i>	9X; 6X
Muconate	<i>P. putida</i>	<i>C. glutamicum</i>	In progress
Beta ketoadipate	<i>P. putida</i>	<i>C. glutamicum</i>	In progress
Muconate biosensor	<i>P. putida</i>	<i>C. glutamicum</i>	6X
Microfluidic screening	<i>P. putida</i>	<i>C. glutamicum</i> ; <i>Rhodobacter</i>	2X; 2X
Integration tools	<i>P. putida</i>	<i>C. necator</i>	10X
Fungal transporters	<i>A. pseudoterreus</i>	<i>R. toruloides</i>	9X



# Economic/technical metrics

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



# 3 - Impact

# Impact on state of technology/industry if successful

- **Accelerated biomanufacturing commercialization:**
  - 50% reductions in time to bioprocess scale-up
  - Higher probabilities of success, and failing faster, save resources
  - Translates to savings of US\$10M(s) and many people years per process
  - Quantitative increase in the size (\$\$\$) of the bioeconomy
- **No need to re-establish metabolic routes and hosts:**
  - Industry can save time and resources, while retaining “last-mile” IP, by building from public beachheads and hosts to bioproducts of interest
- **Greater diversity of publicly available microbial hosts:**
  - More process conditions and target molecule classes supported through more and increasingly engineerable microbial hosts
- **Likelihood assessments / demonstrations of process transfer:**
  - De-risked technology transfer across facilities and methods to assess how likely a given process can be successfully transferred
- **Increased access to broadly enabling DBTL infrastructure**

# How disseminating results

- **Impact factor of publications:**

- 250+ citations across 50 publications to date (since FY17)
- 67 citations across 17 publications since FY20
- 5.91 impact factor

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

- 16 patent applications
- 6 records of invention
- 7 software disclosures
- 5 licenses

- **Reducing barriers to commercialization:**

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

# Use of IAB + industry surveys to guide project deliverables

- **Industry Advisory Board:**

- Representatives from organizations that are diverse with respect to size, location, specialization
- Provide feedback on approach, operations, metrics, and overall impact

- **One-on-one interviews:**

- Interviews with biotechnology stakeholders including scientists, executives, investors, etc.
- Collect information regarding various topics related to the ABF and synthetic biology industry to guide ABF foci and direction and promote the ABF to potential partners
- 84 interviews conducted since 2017

- **Surveys:**

- Effort to expand information collected to a larger and broader group but logistically challenging

# Commercial partnerships

**LYGOS**

UC San Diego

**ZYMOCHEM**

NEXT-GENERATION MICROBES  
FOR INDUSTRIAL CHEMICALS



**UNIVERSITY OF  
GEORGIA**



**UNIVERSITY of  
WASHINGTON**



**invaio**

**Berkeley**  
UNIVERSITY OF CALIFORNIA



**KALION**



**UNIVERSITY OF  
DELAWARE**

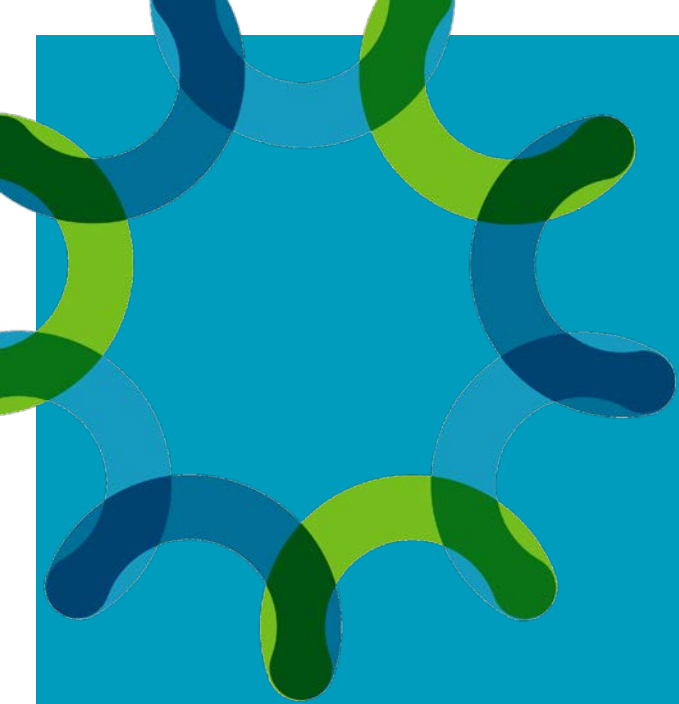
**teselagen**  
BIOTECHNOLOGY

**LanzaTech**

**KIVERDI**

# Memoranda of Understanding (MOU)

- **NSF + DOE (pending)**: There are ongoing discussions between the NSF and DOE towards coordinated funding for collaborations between NSF-supported academic PIs and DFO-supported ABF teams. An MOU is pending
- **Global Biofoundries Alliance (pending)**: The ABF signature to the GBA MOU is pending DOE/U.S. State Dept. approval



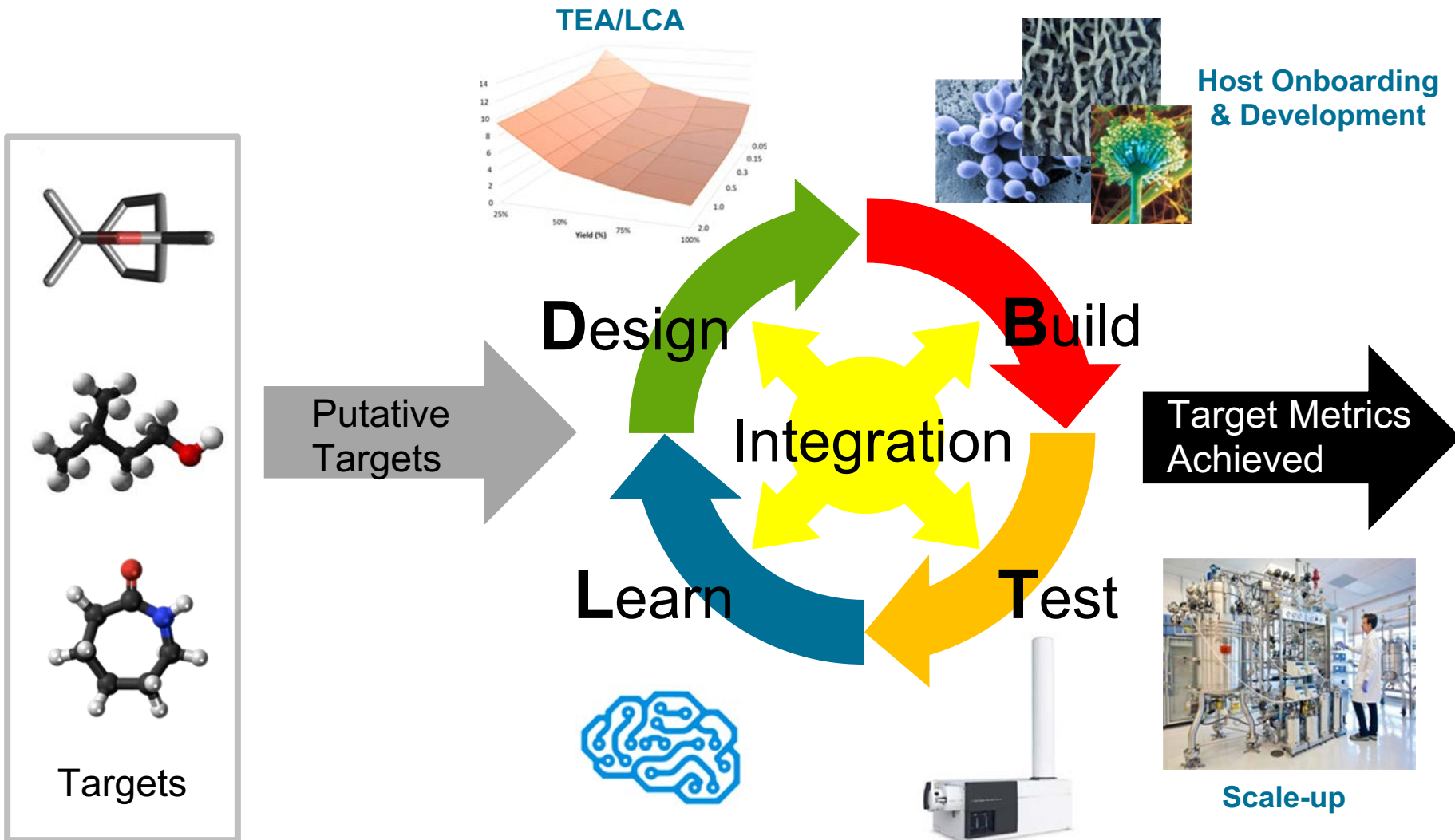
# 4 - Progress and Outcomes



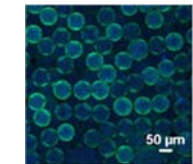
# Progress made towards goals

- **Acceleration of biomanufacturing commercialization:**
  - **Collaboration:** Industrial and academic collaboration projects will be presented the over the next two days. As leading indicators, progress therein is very promising (e.g., Lygos - 20X increase in isobutyrate titers)! Through the outcomes of these collaborations over time, the ABF endeavors to definitively establish end-to-end impacts on time from bioprocess conception to scale-up and commercialization
  - **Internal:** The ABF, as assessed through target/tool transfer and DBTL efficiency metrics, along with established beachheads and hosts, is itself making good progress towards this goal
- **Broadly enabling DBTL infrastructure**
- **Onboarding and development of public beachheads and hosts**
- **Assessments and demonstrations of bioprocess transferability**
  - DBTL infrastructure presentation immediately follows
  - Target and Host Engineering, and the Host Onboarding and Development ABF presentations are later this afternoon
  - These subsequent presentations will detail our progress
  - The following slides will offer concise highlights thereof

# The Agile BioFoundry Approach



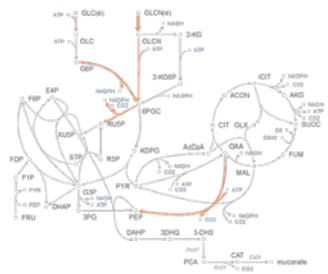
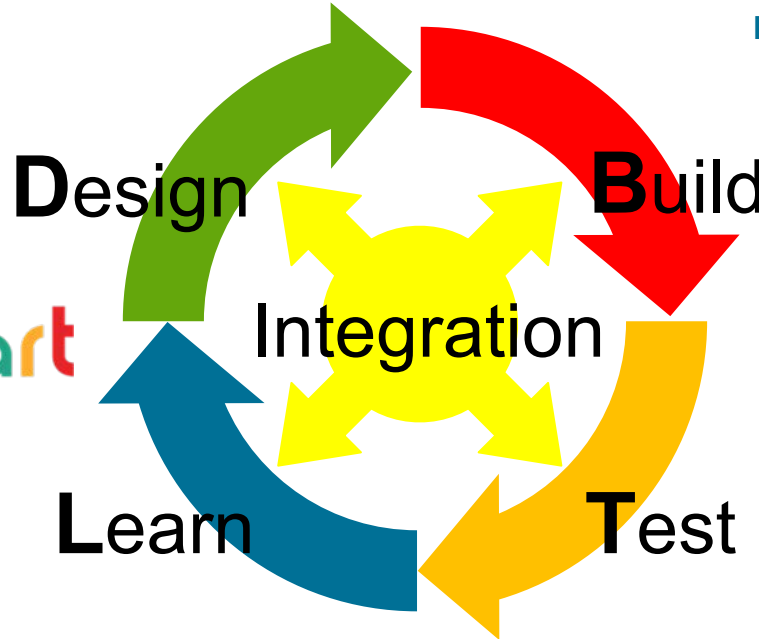
# Highlights - DBTL infrastructure



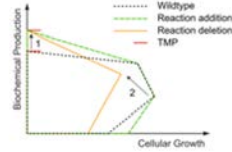
Transcriptomics,  
Metabolomics,  
Proteomics,  
Lipidomics



Scale-up



Bayesian Inference:  
Metabolic Kinetics



Metabolic Network  
Modeling



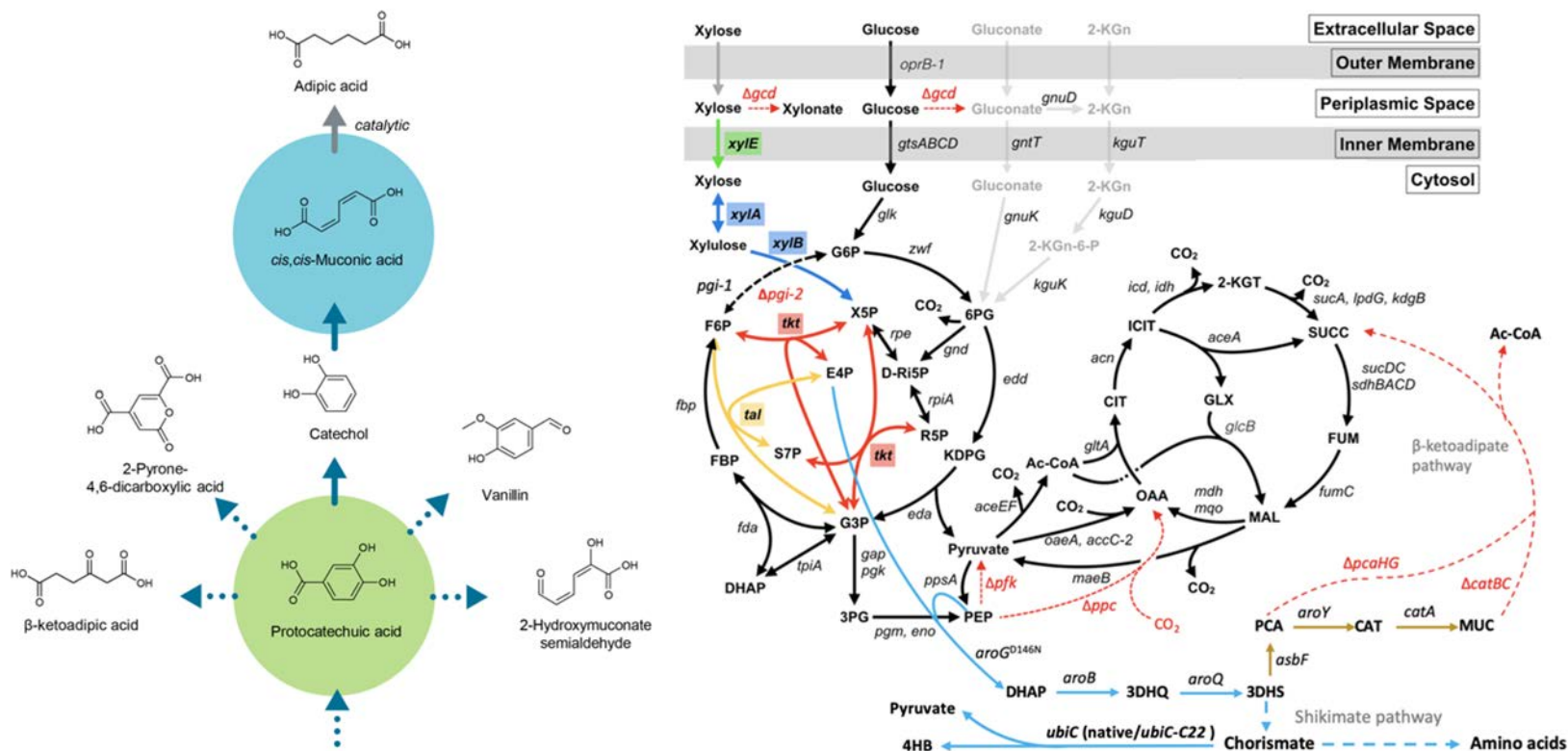
Deep Learning



# Highlights – *P. putida*

## Efforts on protocatechuic acid (beachhead) and muconic acid (exemplar)

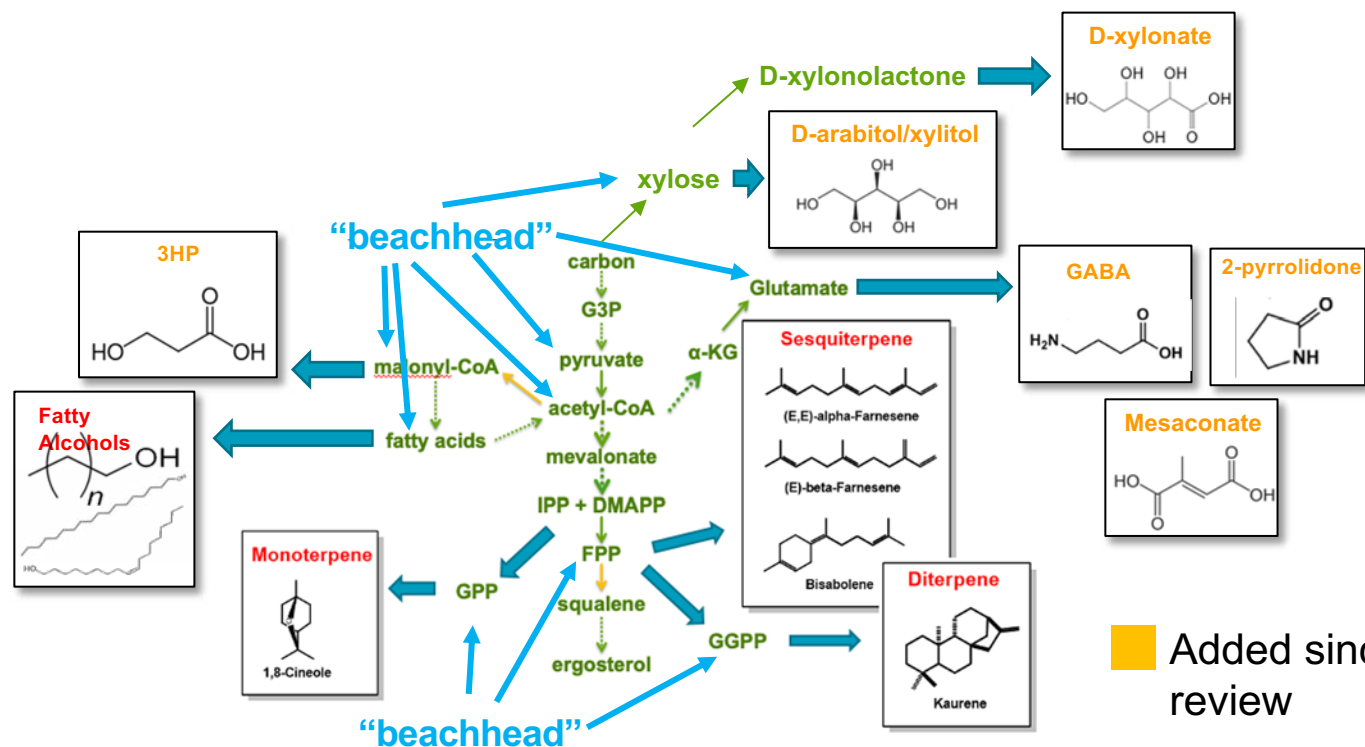
- *P. putida* engineered to consume glucose, xylose, and arabinose simultaneously
- Applied DBTL to substantially improve muconate rate (main TEA/LCA driver)
- Developed advanced biosensors and genetic tools for this important chassis
- Transferring targets and tools to *C. glutamicum* – other BH/EX pairs in progress



# Highlights – *R. toruloides*

## Efforts on expanding beachhead and exemplar targets

- Xylose catabolism elucidated in *R. toruloides*, and are using that knowledge to increase the rate of catabolism and to produce several new bioproducts
- Added three new beachheads, malonyl-CoA, glutamate, and pentose sugars
- Transferred 3HP from *A. pseudoterreus* and used DBTL to improve titer

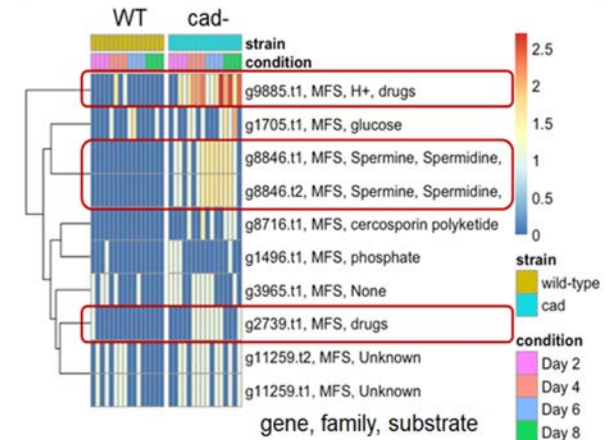


Added since last peer review

# Highlights – *Aspergillus*

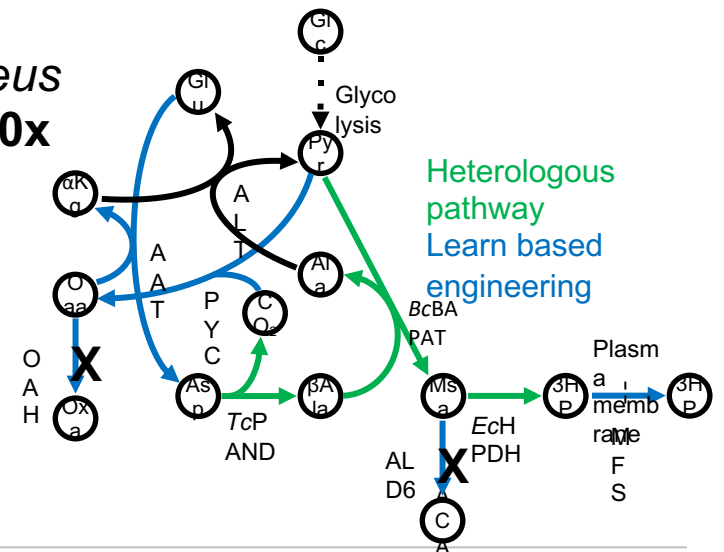
## Efforts on *A. pseudoterreus* citric acid (beachhead) aconitic acid (exemplar)

- Test-Learn identified five aconitic acid transporter gene candidates; Design-Build identified and verified *the transporter gene aex1*
- Titer increased five-fold from 10 g/L to **50 g/L** with the *aex1* transporter gene over-expressed



## Efforts on *A. niger* L-aspartic acid (beachhead) 3-hydroxypropionic acid (exemplar)

- 3HP pathway transferred from *A. pseudoterreus* (undesirable organic acid by-products) with **10x gain** in DB efficiency
- Test-Learn identified candidate 3HP or 3-oxopropanoic acid **degradation genes**
- *A. niger* DBTL strains improved from 3 to **17 g/L 3HP**

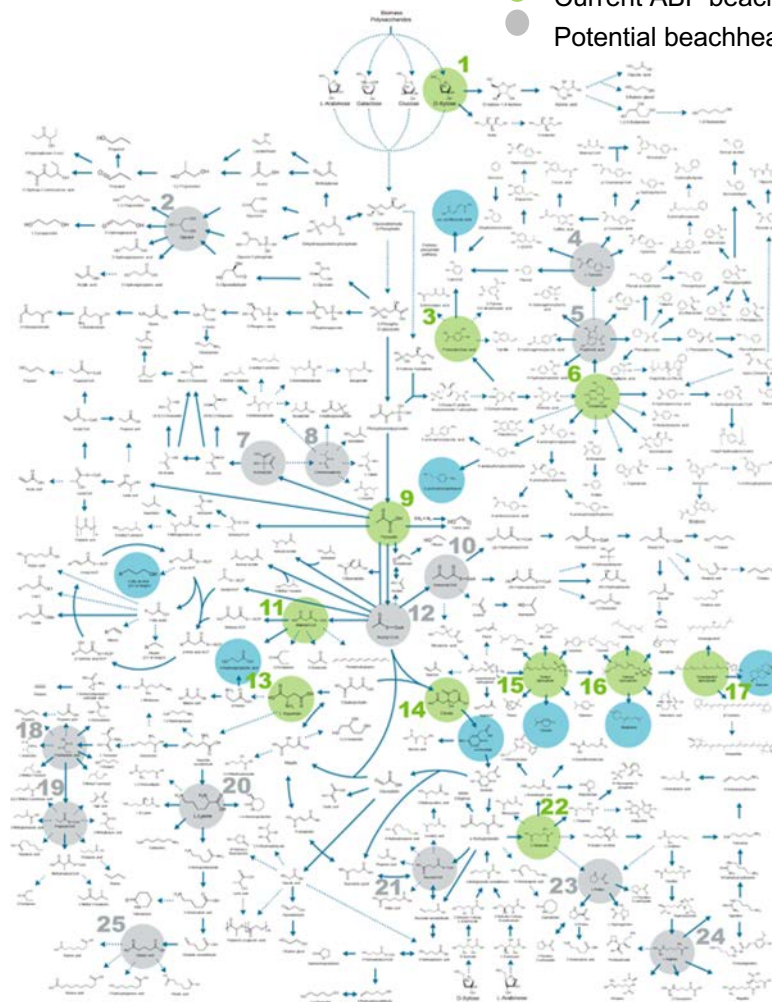




# Highlights - Beachheads

- 01 Xylose
- 02 Glycerol
- 03 Protocatechuic acid
- 04 L-Tyrosine
- 05 Prephenic acid
- 06 Chorismate
- 07 Acetolactate
- 08 2-Ketoisovalerate
- 09 Pyruvate
- 10 Acetoacetyl-CoA
- 11 Malonyl-CoA
- 12 Acetyl-CoA
- 13 L-Aspartate
- 14 Citrate
- 15 Geranyl diphosphate
- 16 Farnesyl diphosphate
- 17 Geranylgeranyl diphosphate
- 18 2-ketobutyric acid
- 19 Propionyl-CoA
- 20 L-Lysine
- 21 Succinyl-CoA
- 22 L-Glutamate
- 23 L-Proline
- 24 L-Arginine
- 25 Glutaric acid

- Current ABF target molecules
- Current ABF beachhead molecules
- Potential beachhead molecules



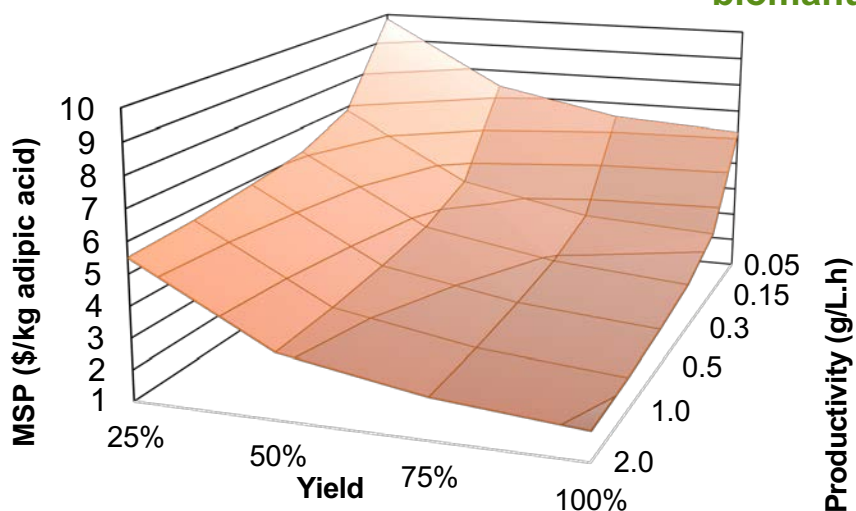
Adapted by permission from Springer Nature Customer Service Centre GmbH: Nature, Nature Catalysis, A comprehensive metabolic map for production of bio-based chemicals, Lee, S.Y., et al., © 2019



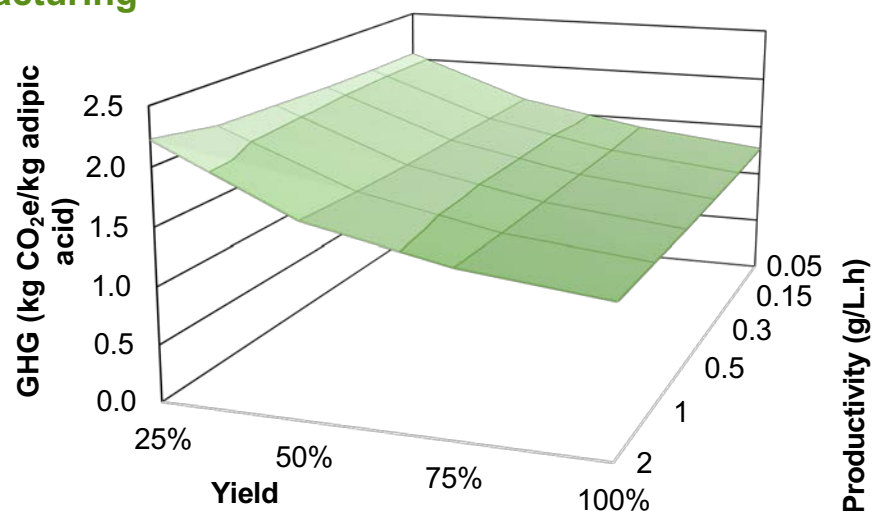
# Highlights - Integrated Analysis

- **Beachhead focus:** TEA/LCA to select an *exemplar* to represent products from each beachhead and to show the value of a whole potential class of related molecules accessible from a beachhead
- **Integrating the ABF metabolic map with IA:** Use metabolic modeling/metabolic map to maintain ABF focus on beachheads and exemplars that could command commodity chemical value
- **Sensitivity scans:** Models will examine multi-dimensional sensitivities, highlight drivers over a range of metrics that the ABF can move towards economic viability/sustainability
- **Integration with other BETO efforts:** Work with other BETO programs (SepCon, PABP, SOTs, Design Cases) to ensure consistency and develop integrated models for biomanufacturing

**Key output: Multidimensional surfaces for TEA/LCA key drivers for prioritizing R&D focus areas – Aim to publish compilation of surfaces for all beachhead-exemplar pairs to inform biomanufacturing**



TEA (adipic acid): strong MSP sensitivity to productivity  
<0.3 g/L-hr, yield <50%

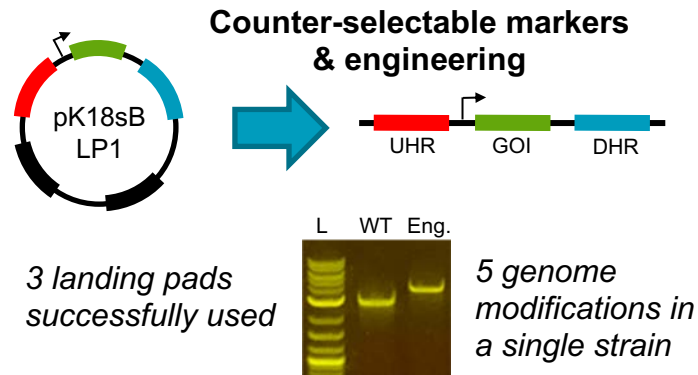


LCA (adipic acid): strong GHG sensitivity to yield,  
relatively independent of productivity

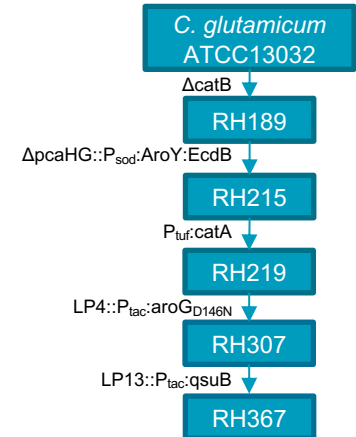
# Highlights – Host Onboarding & Development

## Tier elevation: *Corynebacterium glutamicum*

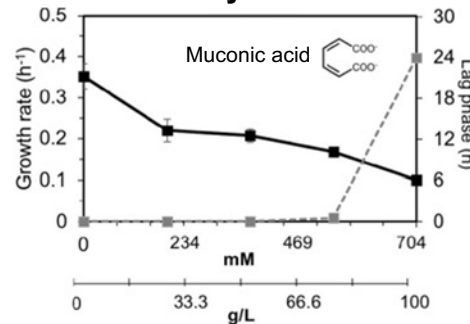
Tier 2		
Criteria	Completion	Details
Substrate utilization panel	✓	10+ carbon sources
Toxicity profiles	✓	1 product, 2 substrates
Bioreactor growth	✓	1 bioreactor run
Genetic stability	✓	5 rounds of modifications
Counter-selectable markers	✓	sacB
Genome integration systems	✓	Homologous and site-specific recombination
Chromosomal safe sites/landing pads	✓	3 used, 15 available in literature
Induction systems	✓	Parts available in ABF
Panel of constitutive promoters, RBSs	✓	Promoters and RBS characterized
Genome scale models	✓	Literature available
Transcriptomic, proteomic, metabolomic datasets	✓	Literature available



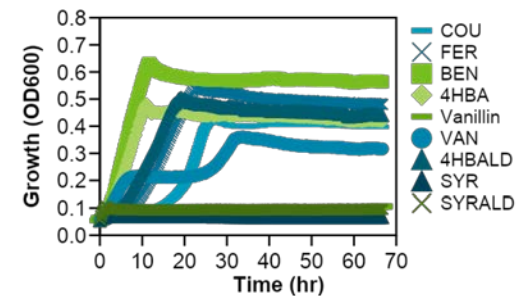
### Genetic stability



### Toxicity Profiles



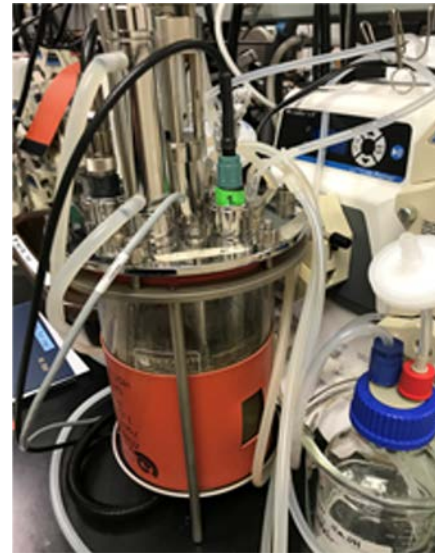
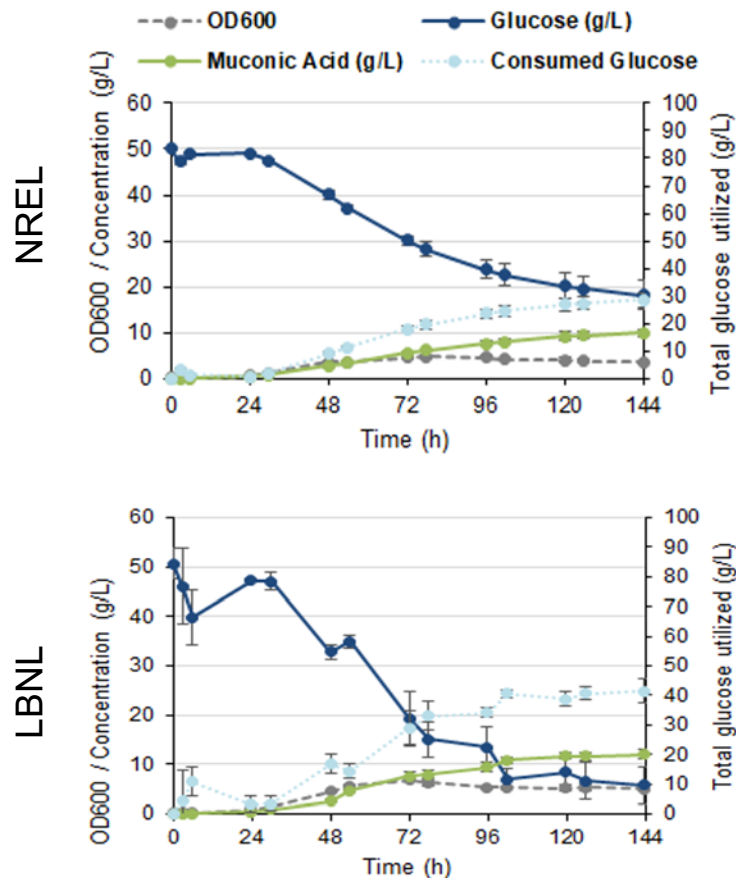
### Substrate utilization



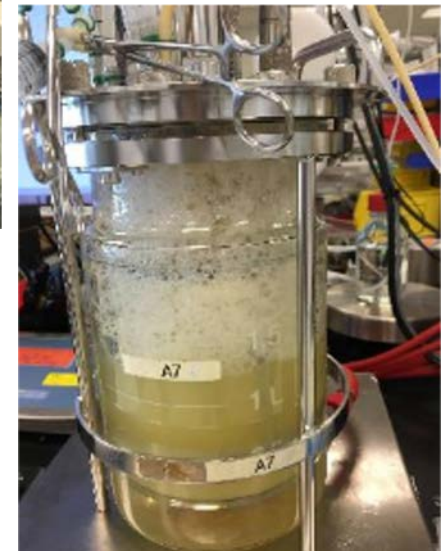
- Culture and transformation procedures onboarded
- Toxicity profiles, substrate utilization, and genetic engineering tools developed
- **Outcome:** *Corynebacterium glutamicum* elevated from Tier 1 to Tier 2 in ABF

# Highlights – Round Robin Scale-up Study

- Increased dissolved oxygen concentrations at lower altitude increased glucose utilization. Product yields did not improve with glucose utilization.



2L Bioreactor



2L Bioreactor

# Highlights – Industry Outreach

- **Revamped ABF website**

- Capabilities section improved in particular
- Webcast series easily accessible to the public to disseminate ABF capability and collaboration information

Themes
Introduction to the ABF
Beachheads / Exemplar Targets with Integrated Analysis Approach for their Selection
Host Onboarding and Development
Test and Learn Capabilities
Scaling Capabilities (Round Robin Experiments)
How to work with the ABF



## Website Metrics

(since Jan 2020)

10K users  
33K pageviews

## Social Media Metrics

(since June 2019)

### Twitter

+419 new followers  
320K tweet impressions  
425 retweets

### LinkedIn

+392 new followers  
697 unique visitors

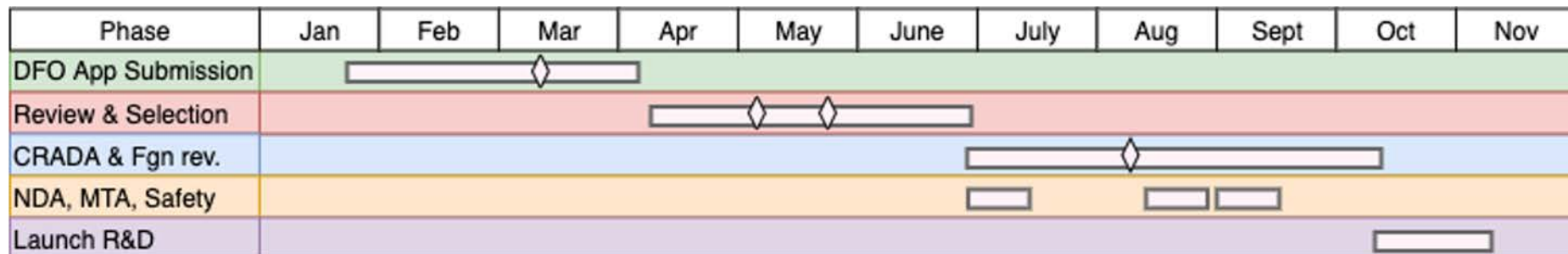
- **Active industry and public engagement**

- 1:1 interviews, online surveys, and interactions at meetings and conferences
- Utilization of online engagement through Twitter and LinkedIn for amplification

# Highlights – Management

- Directed Funding Opportunity Process FY21

<p><b>Goal:</b> Align the ABF with industry’s highest priorities.</p> <p><b>Approach:</b> Leverage the ABF’s full capabilities to help industry solve its toughest challenges.</p> <p><b>Details:</b> \$5M DOE funding (+≥20% partner cost share) available to the National Labs to collaborate with industrial and academic partners.</p>	Days	Description
	0	Announce
	50	Lab lead applicant discussion deadline (i.e., doodle calls)
	25	Application submission deadline; reviewers receive applications
	30	Reviewers meet to normalize scores; Ex Comm receives
	25	Ex Comm selects from top scoring applications within 1.5X DOE \$
	25	DOE reports its review of the selections
	4	Private communications to applicants
	45	Teams complete CRADA SOWs
	100	Teams execute NDA, MTA, CRADA, EH&S (less foreign review)
	20	Teams commence R&D





# Status of key milestones

- **Completed in FY20 (representative):**

- Q1: DBTL cycle defined with specific required unit operations comprised
- Q2: Workplan and methodology established to quantify economic and sustainability implications for “beachhead” molecule pathways through TEA/LCA modeling support
- Q4: Reproducibility of 3 Test unit operations quantified through comparison of results for on-site vs. off-site sample analysis for 3 or more variables
- Q4: Bioreactor ‘round robin’ of 2+ more demonstration project pathways tested

- **On track for completion in FY21 (representative):**

- Q2: (Go/No-Go) 5 metabolic pathways and/or tools transferred between hosts, with 2X improvements in second host, with metrics defined for each case
- Q2: External partnerships facilitated by developing a public ABF biomanufacturing coverage map accessible through the ABF website
- Q4: 1-2 DBTL automation workflows finalized that improve efficiency by => 2X
- Q4: Onboarding of at least 10 Hosts to at least Tier 1 completed

# Risk mitigations - all on-track / ongoing

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

# Summary

- **Goal:** Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry to productionize synthetic biology
- **Outcomes:** Development and deployment of technologies enabling commercially relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts
- **Relevance:** \$20M/year public infrastructure investment that increases U.S. industrial competitiveness and enables opportunities for private sector growth and jobs
- **Risks:** Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from





# How is the ABF contributing to Biden/Harris administration goals?

- **Innovation:** The ABF is a prime example of our National Labs spurring innovations in modern, sustainable infrastructure to deliver a re-invigorated, more resilient, sustainable economy that puts the United States on a path to net-zero emissions. This includes the use of innovative Life-Cycle Assessment capabilities in the ABF's strategic decision making processes
- **Manufacturing:** The ABF's collaboration partners are mobilizing American manufacturing and innovation to commercialize new and better products that can be manufactured using biological feedstocks and materials supplied by small businesses, family farms, and job creators all across our country
- **Global Leadership and Climate Change:** The ABF formally joining the Global BioFoundry Alliance will strengthen and demonstrate our leadership in the global bioeconomy community, much as we are by standing with America's allies and recommitting the United States to the Paris Agreement on climate change

# Quad Chart Overview

## Timeline

- Start: October 1, 2019
- End: September 30, 2022

	FY20	FY21	FY22	Total Active
DOE Funding	\$15M	\$15M	\$15M	\$45M

## Project Partners

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

## Barriers addressed

- *Ct-L*. Decreasing Development Time for Industrially Relevant Microorganisms
- *Ct-D*. Advanced Bioprocess Development

## Project Goal

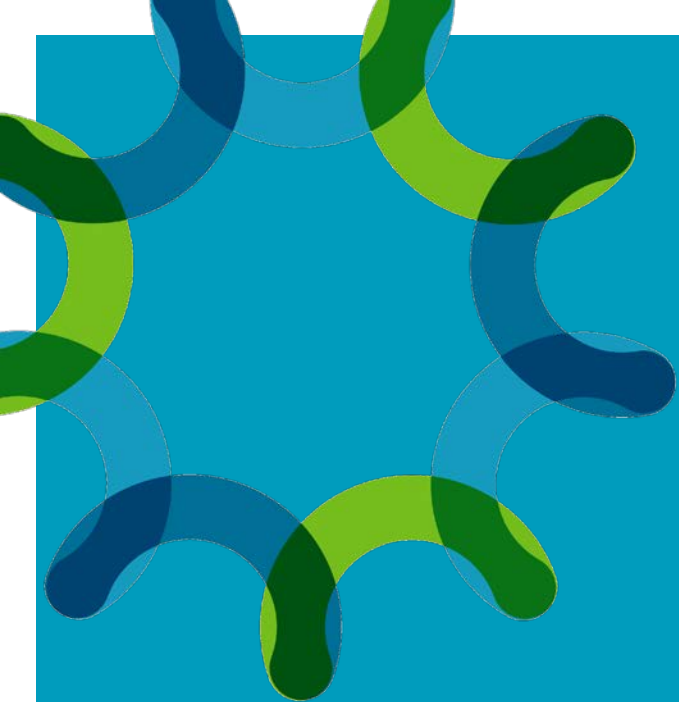
Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry that will productionize synthetic biology

## End of Project Milestone

- 5X efficiency improvement in DBTL engineering cycle
- 20 microbial hosts (20 species) brought to at least Tier 1
- 10-15 beachheads of strategic interest to BETO in at least 3 onboarded hosts
- At least one representative target of a beachhead at a TRY of 80 g/L, 1 g/L/hr, and 70% of theoretical yield

## Funding Mechanism

AOP



# Additional Slides

# Responses to Previous Reviewers' Comments

- **C:** The overall goal of the Agile Biofoundry is to reduce the time and cost of commercialization through the development of a distributed foundry. The foundry is build around the design-build-test-learn paradigm. The team is focusing on the learn component, which is the most challenging step in this process. They are placing particular emphasis on non-intuitive learn predictions, which really amounts to showing that the learn step provides concrete value. Overall, the vision is very compelling. Scale-up and TEA provide the main differentiators from other efforts along with the distributed nature of the foundry. Reasonable milestones are provided though they are somewhat vague. More concrete milestones would strengthen project. Also, it is not clear how the the team will achieve their stated efficiency goals: will it be through improved process knowledge, eliminating bottlenecks, better operational management, or simply increasing capacity?
- **R:** The ABF overview presentation included substantially simplified milestone language. For example, slide 36 listed a FY19Q2 milestone as: “Deep Learning non-intuitive predictions.” However, the actual language for this particular milestone is: “Deep Learning from multi-omics datasets for one or more Crop 1 or Crop 2 targets leads to a set of actionable genetic and / or process modifications (each individually or in combination predicted to increase titer, rate, or yield by 20% or more) to be implemented and evaluated for FY19 Go/No-Go Decision.” As such, the actual milestones are more concrete than the simplified versions presented to the Reviewers. Regarding achieving our stated (10X) efficiency improvement goals, as the Reviewer notes, efficiency is a product of multiple factors, and as such, there are multiple facets through which efficiency could be improved. Whereas in the presentation we did discuss several of these facets (e.g. number of cycles required to obtain a given level of performance, strains/designs needed per cycle, personnel/instrumentation resources used per cycle, cycle time), the Reviewer is correct in that we did not specify precisely through which facet(s) the efficiency goals would be achieved. We have been pursuing an all-the-above strategy, including for example emphasis on Learn to not only reduce the number of cycles and strains required per cycle, but also to enhance operations towards reducing resource requirements and cycle times. For the ABF’s second 3-year AOP cycle, we will quantitatively evaluate efficiency improvements and analyze which facet(s) prove to be the best opportunities for further efficiency gains.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** The ABF was created to develop an infrastructure enabling a rapid DBTL cycle that can achieve a 50% reduction in project development time. The concept is to develop host-target pairs that can be leveraged by industry to go the rest of the way to commercialization. Functional groups are set up to operate like an industry program. There is a very formal project management structure for tracking tasks and milestones, including a 50% time project manager. The team has met most milestones so far, and the accomplishments will be covered in the individual presentations. There has been good industry engagement, and this will be streamlined in the future. Technical aspects of the future work is well defined, though there is some ambiguity around how to measure improvement in DBTL cycle time. TEA work has been good so far for preliminary analysis of the host/target pairs, but will require more emphasis as the projects move forward.
- **R:** The Reviewer is correct in stating that there has been ambiguity in how we measure (improvements to) DBTL cycle time. As of the presentation at Peer Review, we had qualitative but not quantitative definitions of what constitutes a full DBTL (vs. mini-DBTL) cycle. For example, that DBTL requires a minimum set of unit operations, but what that minimum set is had not yet been specified. As such, without a concrete definition, it had not yet been possible to unambiguously measure DBTL cycle time (and as a consequence, improvements thereof). For the ABF's second 3-year AOP cycle, we will have the concrete DBTL specification in place, and we will work towards increasingly automatically capturing cycle time metrics in our workflow-supporting software infrastructure (e.g. DIVA), which will facilitate and standardize how cycle times (and their improvements) are measured. For the ABF's second 3-year AOP cycle, we will place increasing emphasis on TEA and LCA as the project moves forward.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Well organized and clear project despite complexity of the project in regard to having multiple teams across the US involved. Same as previous ABF project, the PIs are encourage to expand on how their approach will help reduce development cycle by 10X as this does not seem to be clear in any of ABF projects.
- **R:** A minor correction to the Reviewer's comment - one outcome of the ABF will be to increase engineering cycle efficiency, not the cycle (time) exclusively. See above regarding the multi-faceted approach to increasing efficiency.
- **C:** Weakness: The DBTL cycle is currently very slow. Some of this could be attributed to the difficulty of genetic manipulations in the chosen organism (Build), but there are still delays in the Test and Learn portions.
- **R:** Agree that DBTL cycle is slow. We stated as much in the presentation and we have plans to define it, measure it, and improve it going forward.
- **C:** Weakness: Could provide a better measure of success. Are 10 g/L, 0.1 g/L/hr, and 40% yield all that are necessary to drive the bioeconomy? Given the potential, more ambitious and relevant targets could be provided. Not clear what the process is for choosing target molecules.
- **R:** At the final end of the project (9-year project objective), our measure of success will be how much we have enabled industry to reduce their bioprocess commercialization timelines. We needed to set more near term SMART TRY milestones against which to gauge our infrastructure/capability development progress. As mentioned in Target/Host presentation responses, some of these are more useful internally rather than a gauge against commercial viability and relevance. Furthermore, relevant TRY metrics depend very much on the particular target. We did have a very detailed process for selecting targets, but didn't have time to fully present it in the overview presentation.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: The objective statement could do with refinement - it is not clear if it means to start scale-up (pass Go/NoGo) or to finish it. A clearer line between this and end of project goals would be helpful. Directionally it makes sense but how those particular quantitative goals were determined is not obvious. Additional advisory members or committees are recommended, in particular, regarding biosafety. Non-technical risks are not very well discussed. One thing that is missing from the articulated plan is personnel development. By integrating National Labs, for example, a traditional view of cultural identity is changing. It would be an upgrade to consider personal development opportunities for those involved in the effort, in other words, consider deliberate training rather than relying on adapt-or-die responses. This is an often overlooked facet to developing entities and can be a disabler if not done correctly.
- **R:** Not likely going to change the objective statement, but a good idea internally to know more specifically and explicitly what we are referring to (i.e. where does the bioprocess commercialization timeline start and end - that we are trying to speed up 50%). Agreed that since the overall objective is a 9-year goal, better connecting end of 3-year AOP cycle project goals to this 9-year objective is a good idea. We will be talking with BETO about additional or supplementary representation on existing advisory panels concerning biosafety/biosecurity. Personnel development isn't really in the scope of a project like the ABF, but rather in the scope of the employing National Labs in the consortium. Point taken and agreed, though, on training rather than adapt-or-die regarding ABF cultural identity; however, there are some staff whose personalities are more predisposed than others to an ABF mindset.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Even if go/no-go goal of non-intuitive predictions is not met, the ABF still has a lot of value for the experimental infrastructure. Thus this may not be the best measure of success. The team should also consider adding an enzyme engineering component to the workflow.
- **R:** Agreed that the ABF will still provide value even if the non-intuitive predictions are not entirely successful. However, un-intuitive Learn is a differentiator for the ABF value proposition, since this is one clear way in which the ABF is unlike many other BETO supported projects. We do do some enzyme engineering / evolution work as part of the ABF, but it is a minor component currently. We will discuss with BETO the possibility of expanding scope of enzyme engineering work.



# Publications, Patents, Presentations, Awards, and Commercialization

- **50 publications, 126 presentations to date**
  - 16 publications and 20 presentations since FY20
    - The following slides provide explicit lists thereof
- **2020 R&D 100 Award**
  - Awarded to Smart Microbial Cell Technology for rapid optimization of biocatalysts
  - Special Recognition (Silver Medal) for Market Disruptor in the Services category
- **36 patents, records of invention, software disclosures, & licenses**
  - The following slides list these intellectual property assets

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

- **(Publication)** Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. “Engineered *Pseudomonas putida* KT2440 co-utilizes galactose and glucose.” *Biotechnol Biofuels* 12, 295 (2019).
- **(Publication)** Christopher B. Eiben, Tristan de Rond, Clayton Bloszies, Jennifer Gin, Jennifer Chiniquy, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Oliver Fiehn, Jay D. Keasling. "Mevalonate Pathway Promiscuity Enables Noncanonical Terpene Production", *ACS Synth. Biol.* (2019).
- **(Publication)** Yan Chen, Deepwanita Banerjee, Aindrila Mukhopadhyay, Christopher J. Petzold. “Systems and synthetic biology tools for advanced bioproduction hosts”, *Curr. Op. Biotechnol.* (2020).
- **(Publication)** Jacquelyn M. Blake-Hedges, Jose Henrique Pereira, Pablo Cruz-Morales, Mitchell G. Thompson, Jesus F. Barajas, Jeffrey Chen, Rohith N. Krishna, Leanne Jade G. Chan, Danika Nimlos, Catalina Alonso-Martinez, Edward E. K. Baidoo, Yan Chen, Jennifer W. Gin, Leonard Katz, Christopher J. Petzold, Paul D. Adams, Jay D. Keasling. “Structural Mechanism of Regioselectivity in an Unusual Bacterial Acyl-CoA Dehydrogenase”, *J. Am. Chem. Soc.* (2019).

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

- **(Publication)** Thompson, Mitchell G., Allison N. Pearson, Jesus F. Barajas, Pablo Cruz-Morales, Nima Sedaghatian, Zak Costello, Megan E. Garber et al. "Identification, characterization, and application of a highly sensitive lactam biosensor from *Pseudomonas putida*." ACS Synthetic Biology (2019).
- **(Publication)** Geiselman GM, Zhuang X, Kirby J, Tran-Gyamfi MB, Prael JP, Sundstrom ER, Gao Y, Munoz Munoz N, Nicora CD, Clay DM, Papa G, Burnum-Johnson KE, Magnuson JK, Tanjore D, Skerker JM, Gladden JM. "Production of ent-kaurene from lignocellulosic hydrolysate in *Rhodospiridium toruloides*." Microb Cell Fact. 19(1):24. (2020).
- **(Publication)** Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachúa, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson\*, Gregg T. Beckham\*, "Engineering glucose metabolism for enhanced muconic acid production in *Pseudomonas putida* KT2440," Metabolic Eng. (2020).

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

- **(Publication)** Chen, Y.; Guenther, J.; Gin, Jennifer; Chan, Leanne J.; Costello, Z.; Ogorzalek, T.; Tran, Huu; Blake-Hedges, J.; Keasling, J. D.; Adams, P.; Garcia Martin, H.; Hillson, N.; Petzold, C. "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" Journal of Proteome Research (2019)
- **(Publication)** Isabel Pardo, Ramesh K. Jha, Ryan E. Bermel, Felicia Bratti, Molly Gaddis, Emily McIntyre, William Michener, Ellen L. Neidle, Taraka Dale, Gregg T. Beckham, Christopher W. Johnson. "Gene amplification, laboratory evolution, and biosensor screening reveal MuckK as a terephthalic acid transporter in Acinetobacter baylyi ADP1." Metabolic Engineering, (2020), Vol 62, 260-274
- **(Publication)** Radivojević, T., Costello, Z., Workman, K., & Martin, H. G. "A machine learning Automated Recommendation Tool for synthetic biology." Nature Communications, 11(1), 1-14.(2020).
- **(Publication)** Zhang, J., S. D. Petersen, T. Radivojevic, A. Ramirez, Andrés Pérez-Manríquez, E.Abeliuk, B. J. Sánchez et al. "Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism." Nature Communications 11, no. 1 (2020): 1-13.

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

- **(Publication)** Ernst Oberortner, Robert Evans, Xianwei Meng, Sangeeta Nath, Hector Plahar, Lisa Simirenko, Angela Tarver, Samuel Deutsch, Nathan J. Hillson, and Jan-Fang Cheng. "An Integrated Computer-Aided Design and Manufacturing Workflow for Synthetic Biology". In: Chandran S., George K. (eds) DNA Cloning and Assembly. Methods in Molecular Biology, vol 2205. (2020).
- **(Publication)** Gledon Doçi, Lukas Fuchs, Yash Kharbanda, Paul Schickling, Valentin Zulkower, Nathan Hillson, Ernst Oberortner, Neil Swainston, Johannes Kabisch. "DNA Scanner: a web application for comparing DNA synthesis feasibility, price, and turnaround time across vendors". OUP Synthetic Biology, ysa011 (2020).
- **(Publication)** 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. "Multiomics Data Collection, Visualization, and Utilization for Guiding Metabolic Engineering". Frontiers in Bioengineering and Biotechnology 9, 45 (2021).

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

- **(Publication)** Chris Lawson, Jose Manuel Martí, Tijana Radivojevic, Sai Vamshi R. Jonnalagadda, Reinhard Gentz, Nathan J. Hillson, Sean Peisert, Joonhoon Kim, Blake A. Simmons, Christopher J. Petzold, Steven W. Singer, Aindrila Mukhopadhyay, Deepti Tanjore, Josh Dunn, and Hector Garcia Martin. "Machine learning for metabolic engineering: A review" *Metabolic Engineering* (2020)
- **(Publication)** Riley LA and Guss AM\*. "Approaches to genetic tool development for rapid domestication of non-model microorganisms". *Biotechnol* 14:30 (2021)

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

- **(Presentation)** Nathan J. Hillson “U.S. DOE Agile BioFoundry: Organization and Capabilities”, Invited Talk, ABF Industry Day 2019, Emeryville, CA October 4, 2019
- **(Presentation)** Garcia Martin, H. “Machine Learning, Synthetic Biology and Automation: Engineering Life for the Benefit of Society”. NERSC data seminar, Berkeley CA, November 1st, 2019
- **(Presentation)** Benavides PT, Davis R, Klein, B. “Economic and environmental assessment of biological conversions of Agile BioFoundry (ABF) bio-derived chemicals”. 2nd Bioenergy Sustainability Conference 2020, Virtual meeting, October 15th, 2020
- **(Poster)** Tijana Radivojevic, Zak Costello, Kenneth Workman, Soren Petersen, Jie Zhang, Andres Ramirez, Andres Perez, Eduardo Abeliuk, Benjamin Sanchez, Yu Chen, Mike Fero, Jens Nielsen, Jay Keasling, Michael K. Jensen, Hector Garcia Martin, “ART: A machine learning Automated Recommendation Tool for synthetic biology”, BRC Workshop on AI and ML for Biosystems Design, Washington, DC, February 27, 2020

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

- **(Presentation)** Garcia Martin, H. “ART: a machine learning Automated Recommendation Tool for guiding synthetic biology”. AI4Synbio Symposium, Arlington VA, November 8th, 2019.
- **(Presentation)** Garcia Martin, H. “Opportunities in the intersection of:Artificial Intelligence & Synthetic Biology & Automation”. Army Science Planning and Strategy Meeting, Burlington MA, November 13th, 2019.
- **(Presentation)** “ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- **(Presentation)** Garcia Martin, H. “Opportunities in the intersection of machine learning, synthetic biology, and automation”. ABLC 2020, Virtual meeting, July 10th, 2020.
- **(Presentation)** Garcia Martin, H. “Leveraging machine learning and automation to make synthetic biology predictable”. SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- **(Panel)** Garcia Martin, H. “Sustainable Living Systems”. LA Life Summit, Virtual meeting, October 15th, 2020.



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

- **(Presentation)** T. Radivojevic, “Automatic Recommendation Tool”, Invited Talk, Agile BioFoundry Learn Summit 2020, Argonne/Lemont, IL, March 4, 2020
- **(Presentation)** T. Radivojevic, “Using ART to improve tryptophan production”, Invited Talk, Agile BioFoundry Learn Summit 2020, Argonne/Lemont, IL, March 4, 2020
- **(Presentation)** T. Radivojevic, “Guiding synthetic biology via machine learning”, Invited Talk, Biofuels & Bioproducts Division Meeting, JBEI, Emeryville, CA, March 11, 2020
- **(Presentation)** T. Radivojevic, “ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- **(Presentation)** Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020

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

- **(Presentation)** 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
- **(Presentation)** Garcia Martin, H. "Opportunities in the intersection of machine learning, synthetic biology, and automation". ABLC 2020, Virtual meeting, July 10th, 2020.
- **(Presentation)** Garcia Martin, H. "Leveraging machine learning and automation to make synthetic biology predictable". SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- **(Presentation)** Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020
- **(Presentation)** 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

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

- **License partners**

- University of Georgia
- Kiverdi, Inc.
- LanzaTech, Inc.
- Visolis, Inc.
- Danimer Scientific

- **Patent Applications**

- 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

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

- Patent Applications (cont.)

- A Generative Model for Protein Sequences for the Purpose of Protein Design or Phenotypic Inference
- Predicting Metabolic Pathway Dynamics from Time Series Multiomics Data Using Machine Learning Techniques
- Use of Statistical Learn Approaches to Predict Next Generation Sequencing Subsequence Depth of Coverage
- Mutant transporters for bacterial uptake of terephthalic acid
- Method and strain for sugar conversion
- Engineered Microorganisms for the Production of Intermediates and Final Products (1<sup>st</sup>)
- Engineered Microorganisms for the Production of Intermediates and Final Products (2<sup>nd</sup>)
- Production of organic acids from *Aspergillus pseudo-terreus* cadA deletion strain (1<sup>st</sup>)
- Production of organic acids from *Aspergillus pseudo-terreus* cadA deletion strain (2<sup>nd</sup>)

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

- **Patent Applications (cont.)**

- Genetically engineering an industrial filamentous fungus *Aspergillus niger* for 3-hydroxypropionic acid production
- A specific exporter responsible for aconitic acid high production in *Aspergillus pseudo terreus*

- **Records of Invention**

- Bioproduction of limonene from syngas
- Mutant transporters for bacterial update of terephthalic acid
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids
- A genetic circuit to reduce cell-to-cell production heterogeneity
- High yield conversion of D-xylose to D-arabitol in *R. toruloides*
- Manipulation of tRNA thiolation gene *ncs2* for enhanced production of fatty-acyl-CoA derived chemicals in *R. toruloides*

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

- **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

This presentation does not contain any proprietary, confidential, or otherwise restricted information



U.S. DEPARTMENT OF  
**ENERGY** | Energy Efficiency &  
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BIOENERGY TECHNOLOGIES OFFICE

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# ABF DBTL Infrastructure

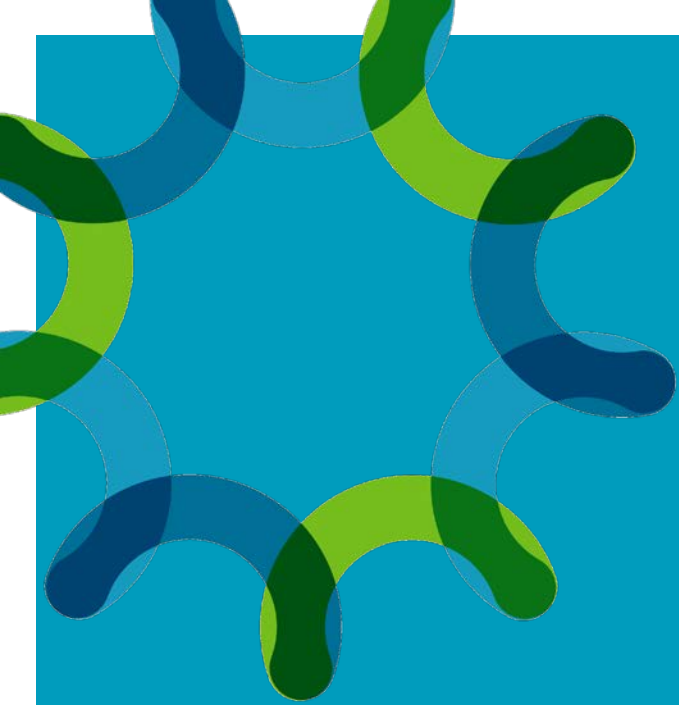
Nathan J. Hillson

Principal Investigator, DOE Agile BioFoundry

BETO Peer Review 2021  
Conversion Technologies  
11:45AM-12:15PM EST  
March 9, 2021







# Project Overview

# Goal - Overall ABF

- **Goal:** Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by **establishing a distributed Agile BioFoundry** to productionize synthetic biology
- **Outcomes:** **Development and deployment of technologies** enabling commercially relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts
- **Relevance:** \$20M/year **public infrastructure** investment that increases U.S. industrial competitiveness and enables opportunities for private sector growth and jobs
- **Risks:** **Past learnings** do not transfer well across target molecules and microbial hosts. **Experiment data sets** are of insufficient quality/quantity/consistency to learn from



# Goal - DBTL Infrastructure

- **Goal:** Design, implement, operationalize, and maintain Design-Build-Test-Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF Tasks and enables the overall ABF goal
- **Outcomes:** 10X improvement in Design-Build-Test-Learn cycle efficiency, new IP and manufacturing technologies demonstrated and ready for translation to U.S. industry
- **Relevance:** Public infrastructure investment that supports the ABF and other BETO projects, and that can be leveraged by U.S. industry
- **Risks:** Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from



# Public Infrastructure Investment Enables Private Industry

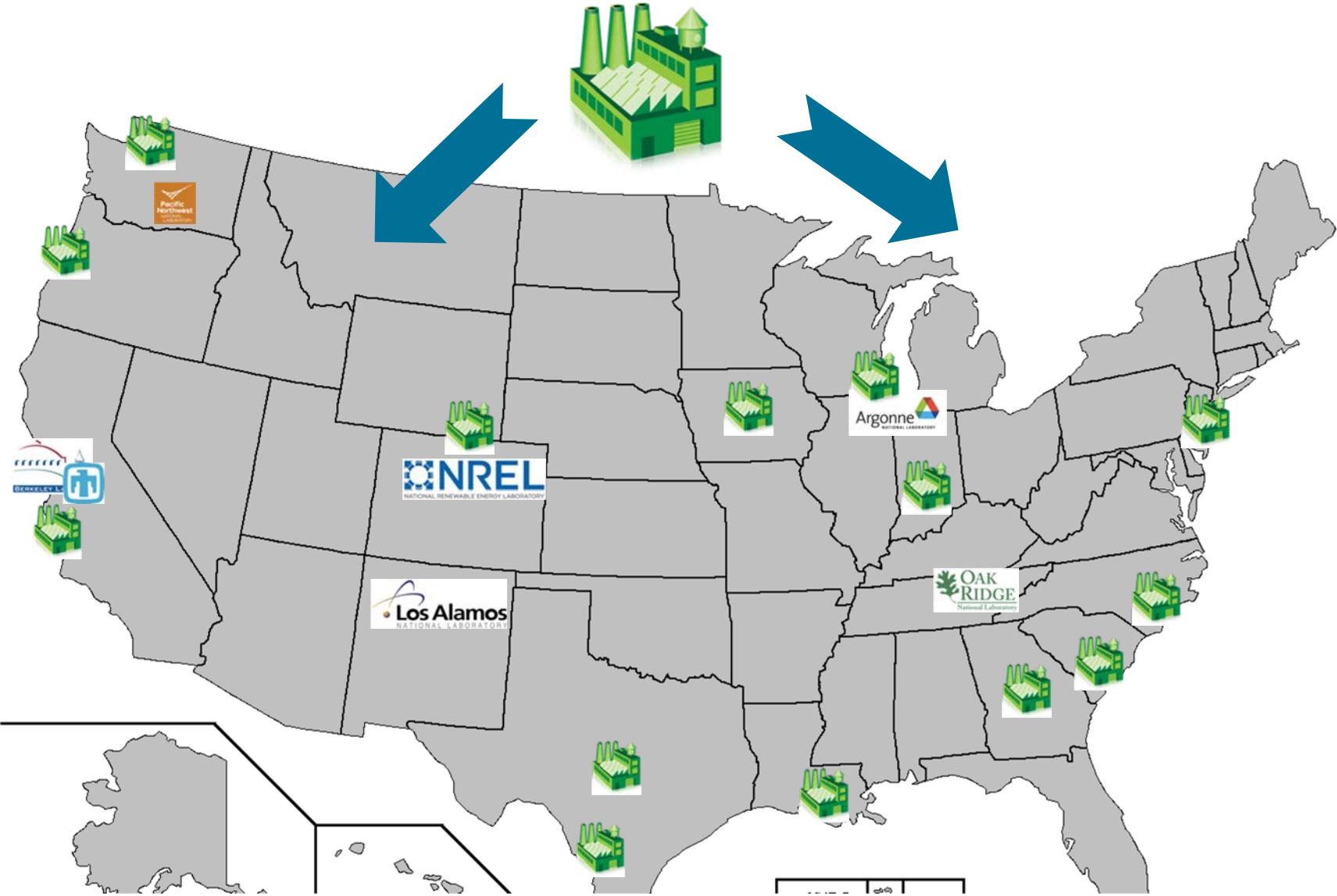
*Public* investment in biomanufacturing infrastructure



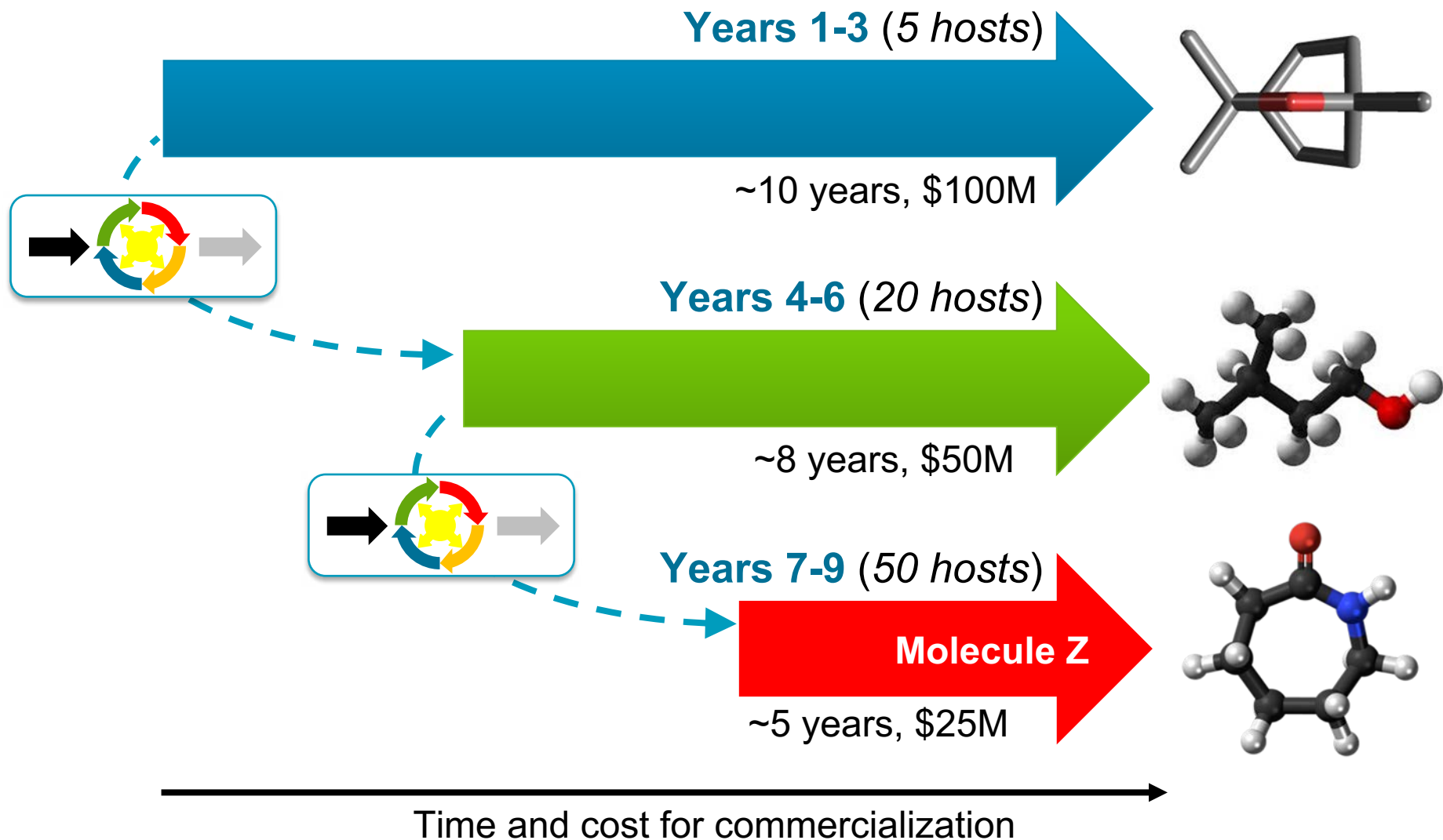
*Private* investment in product development, scaling, and tailoring to unique pathways and products

Adapted from Lyft

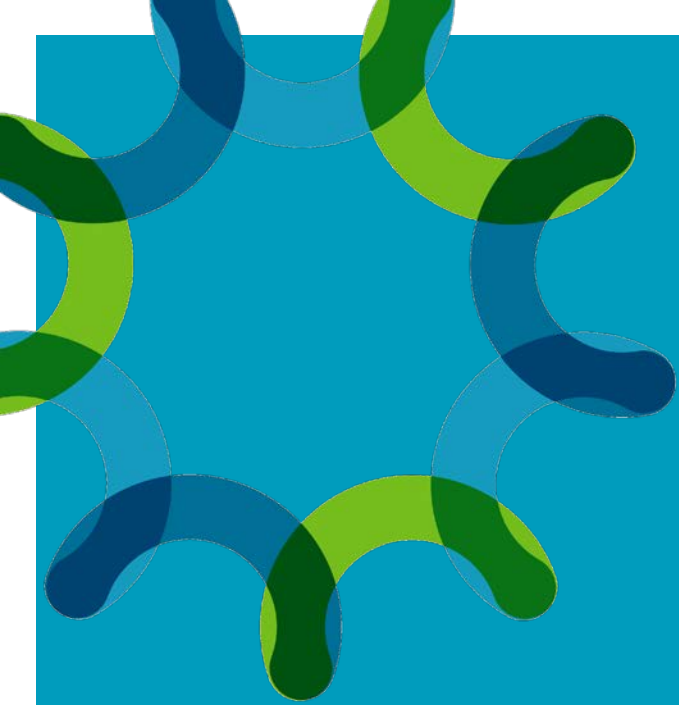
# A Distributed Agile BioFoundry



# Agile BioFoundry Will Reduce Time-to-Scale up







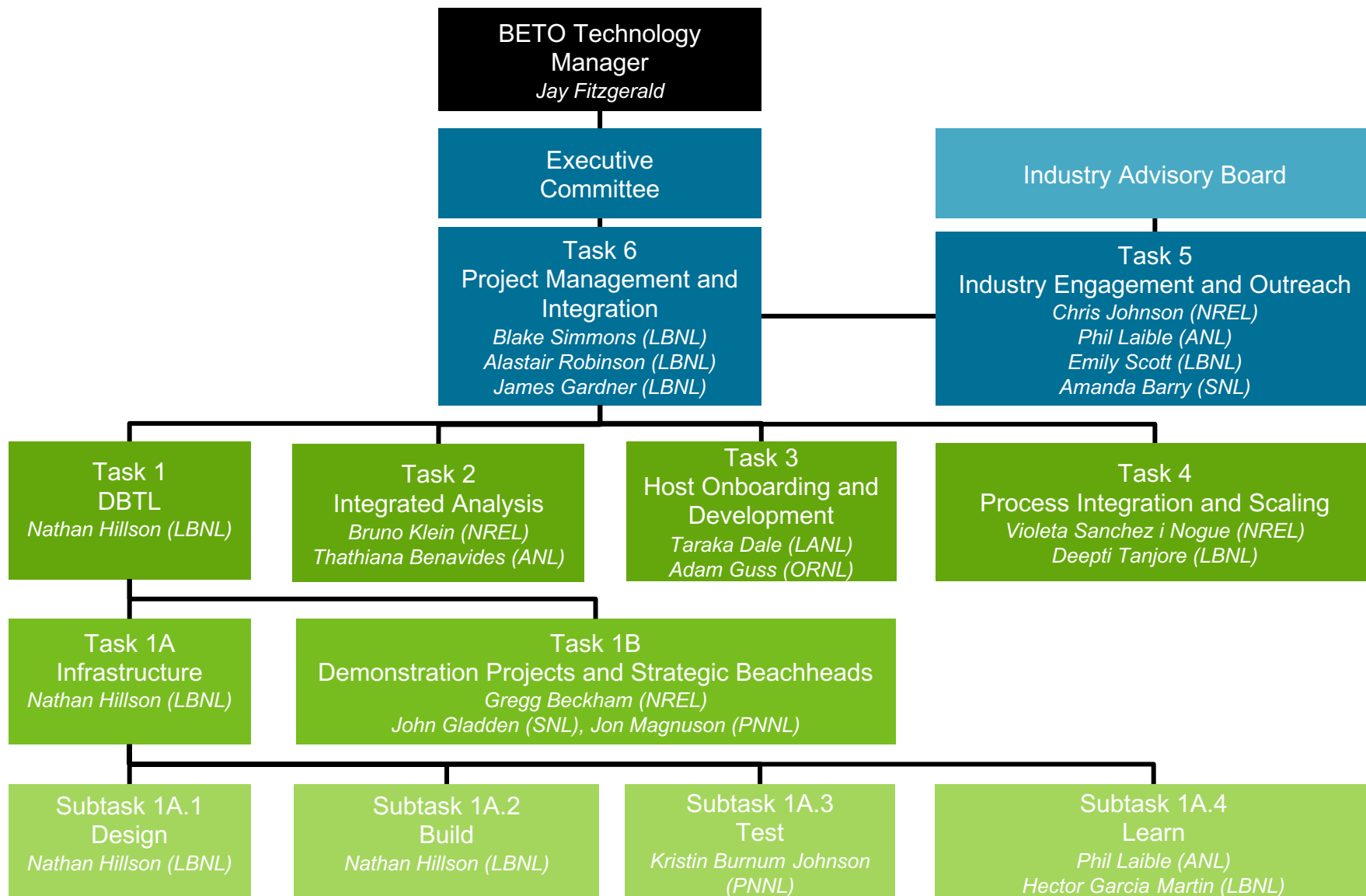
# 1 - Management



# Six Tasks

- **Task 1: Design-Build-Test-Learn** (*Nathan Hillson* - lead)
  - **Infrastructure:** Integrate design-build-test-learn cycle with process automation
  - Demonstration Projects and Strategic Beachheads: Demonstrate uses of DBTL infrastructure and establish and improve routes in microbial hosts to beachhead molecules of high strategic interest
- **Task 2: Integrated Analysis** (*Bruno Klein / Thathiana Benavides* – co-leads)
  - Analyze proposed target and beachhead molecules with TEA and LCA methodologies
- **Task 3: Host Onboarding & Development** (*Taraka Dale / Adam Guss* – co-leads)
  - Onboard additional microbial host organisms and further develop them to higher capability tiers through tool development and data collection
- **Task 4: Process Integration & Scale-up** (*Violeta Sanchez i Nogue / Deepti Tanjore* – co-leads)
  - Provide DMR-EH hydrolysates, and test and scale fermentation to improve titer, rate, and yield
- **Task 5: Industry Engagement & Outreach**  
(*Chris Johnson / Phil Laible / Emily Scott / Amanda Barry* – co-leads)
  - Identify barriers to industry adoption of ABF technologies, expand number and diversity of industry partnerships, and establish a set of metrics for determining impact of ABF technologies on industry
- **Task 6: Management** (*Blake Simmons* - lead)
  - Manage project management, develop internal and external communications, provide deliverables to BETO, and make capital equipment purchases

# Org Chart



# Communications

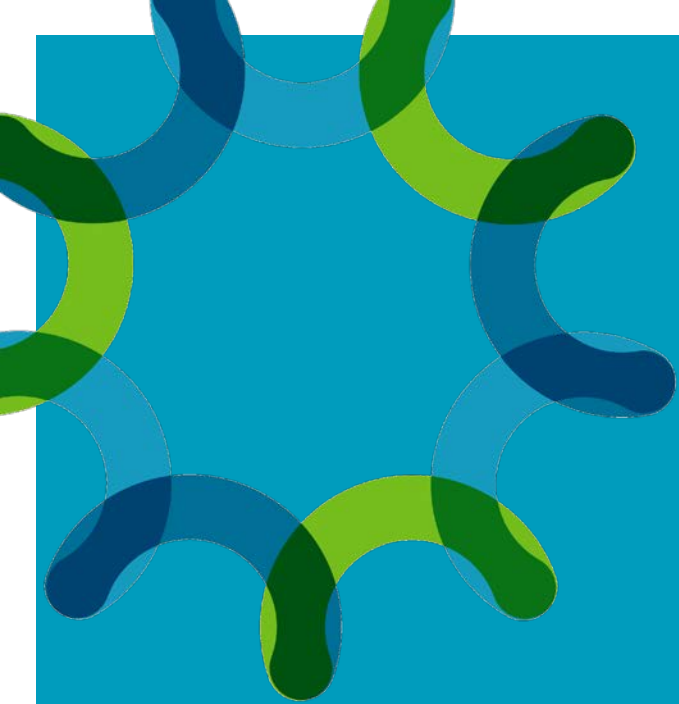
- **ABF is an integrated, geographically distributed multi-Lab team**
  - Effective communications are essential
- **Internal**
  - Bi-weekly Executive Committee meetings
  - Bi-weekly ABF Task Lead meetings
  - Weekly to monthly demonstration project/beachhead meetings
  - **Weekly software and automation infrastructure user meetings / webinars**
  - Monthly activity summary including **DBTL cycle reports** to BETO
  - Monthly Host Onboarding and Development Task team meetings
  - Monthly Learn team meetings - activities and milestone planning
  - Monthly Industry Outreach and Engagement Task team meetings
  - Quarterly progress / milestone completion reports to BETO
  - **Software infrastructure (e.g. ICE, DIVA, EDD, LabKey, AgileBioCyc, Jupyter, github/bitbucket, etc.)**
  - Google Platforms – file storage and sharing
  - Annual Learn Summit
  - Annual ABF All-Hands Meeting
- **External**
  - ABF website (agilebiofoundry.org) and social media (@agilebiofoundry)
  - Presentations, posters, booths at domestic and international scientific / technical conferences
  - Publications
  - Quarterly Industry Advisory Board meetings and Industry Listening Days
  - Annual Global BioFoundry Alliance meeting, and monthly webinar series

# Technical Risks and Mitigation Plans

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

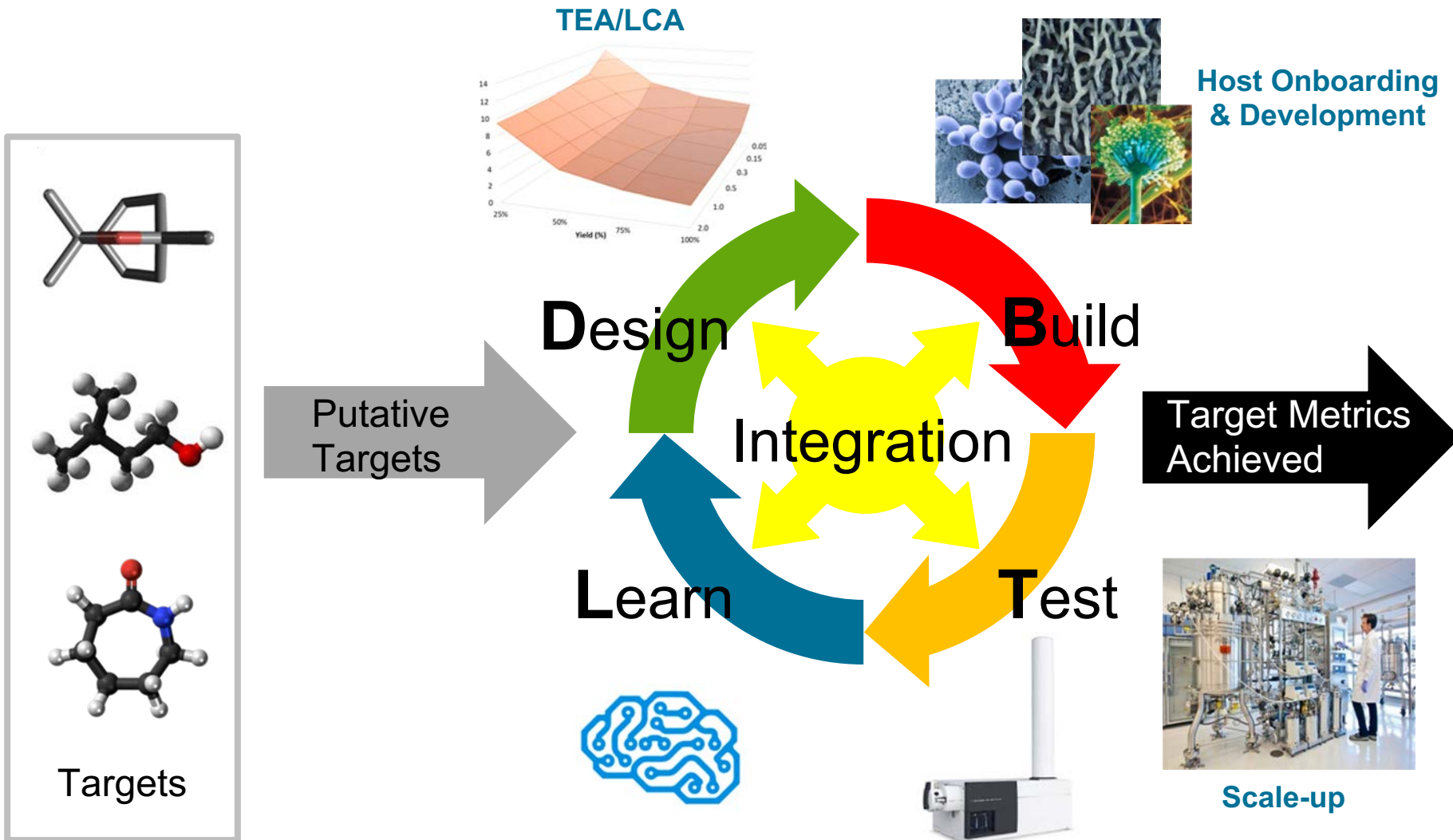
# Collaboration with Related Projects and Advisory Boards

- **Other BETO consortia / projects and BETO State Of Technology (SOT)**
  - Other BETO projects could leverage Agile BioFoundry capabilities:
    - Methods, workflows, instrumentation, software, expertise
    - Accumulated enzyme/pathway/host/process Learnings and data
  - BEEPS FOA: DNAda software (supporting DNA construction) collaborations
  - CCPC (BPMS): Bayesian inference of metabolic kinetics collaborations
  - Improve genetic tools for SOT organisms to accelerate & increase DBTL cycle efficiency
- **Other DOE programs**
  - Energy I-Corp: real-time data for in-line process control and predictive scale-up studies
  - BRCs and EFRCs:
    - Target/host suggestions for ABF; technology off-ramping into ABF
    - Shared technical challenges collaboratively addressed (e.g. DNAda/EDD)
    - Provide compelling examples of DOE teams working together
    - Enhance technology transfer and commercialization efforts
- **Global Biofoundries Alliance:** Software and Metrology/Standards working groups
- **ABF Industry Advisory Board:** Provides guidance relevant to DBTL infrastructure



## 2 - Approach

# The Agile BioFoundry Approach





# What makes ABF different than other BETO-funded metabolic engineering projects?

- The ABF has highly collaborative teams that work together to:
  - Move target / host pairs and strategic beachheads through the pipeline
  - **Build the tools and infrastructure to do so**
  - More closely mirror industry in terms of breaking effort into domains (e.g. Test team)
- Strategic focus on analysis of and routes through beachhead molecules
  - Requires innovation in TEA/LCA analysis (e.g. via exemplar molecules)
  - Goal is to maximize flux through, as opposed to accumulation of, the beachhead molecule
- **Learn component**
- **Infrastructure to support scale / throughput / depth of analysis / Learn**
- Integrated whole that might be separated in other projects
  - Including Integrated Analysis (TEA/LCA), Host Onboarding and Development, Scale-up

# Changes made in light of 2019 Peer Review

- **DBTL cycle specification and efficiency metrics:**
  - **Specification:** a concrete DBTL cycle specification is now in place
  - **Metrics capture:** we are increasingly automatically capturing efficiency metrics in our workflow-supporting software infrastructure
  - **Software usability:** we are engaging a software firm to improve the user interface and user experience of our software to reduce friction and improve efficiency metrics reporting accuracy and completeness
  - **Quantitative evaluation:** through the Platonic approach, we will be quantitatively estimating efficiency improvements made and identifying the most opportune unit operations(s) to further improve

# Top 2 potential challenges

- **Leverage past collaboration learnings with future collaborators**
  - Only portions of past collaborative data or learning methods that do not reveal the underlying primary data may be available
- **Predictive scale-up, and method transferability/reproducibility**
  - Our lack of ability to predict how a process will scale, or how well a method can be transferred across facilities, may limit the impact of our research and development efforts

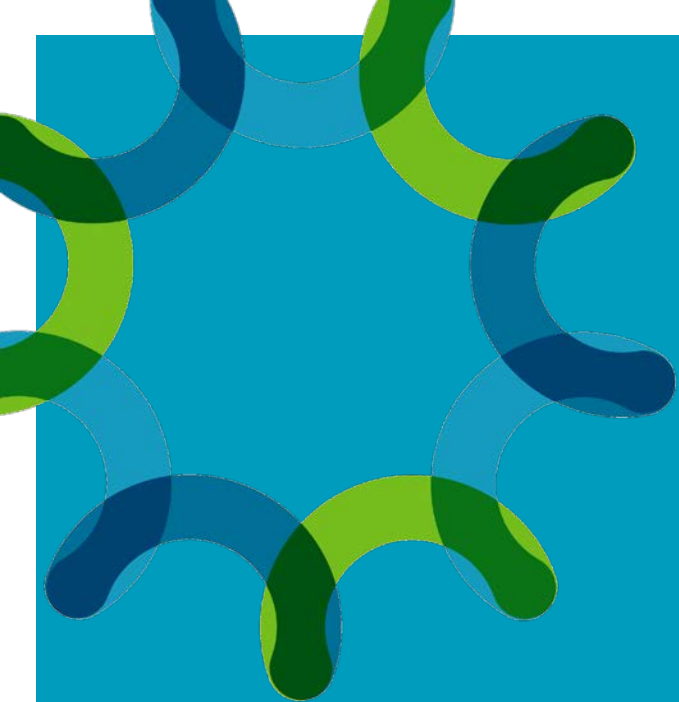
# Go/no-go decision points

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

Target Molecule/Tool	Original Host	Transfer Host (s)	Efficiency gain
3HP	<i>A. pseudoterreus</i>	<i>A. niger</i> ; <i>R. toruloides</i>	9X; 6X
Muconate	<i>P. putida</i>	<i>C. glutamicum</i>	In progress
Beta ketoadipate	<i>P. putida</i>	<i>C. glutamicum</i>	In progress
Muconate biosensor	<i>P. putida</i>	<i>C. glutamicum</i>	6X
Microfluidic screening	<i>P. putida</i>	<i>C. glutamicum</i> ; <i>Rhodobacter</i>	2X; 2X
Integration tools	<i>P. putida</i>	<i>C. necator</i>	10X
Fungal transporters	<i>A. pseudoterreus</i>	<i>R. toruloides</i>	9X

# Economic/technical metrics

- **DBTL and tool/target transfer efficiency:**
  - **Efficiency:** unit operations or objectives achieved, per time (wall/clock), per resource (human/instrument)
  - **Platonic DBTL cycle:** efficiency estimated from underlying unit operations

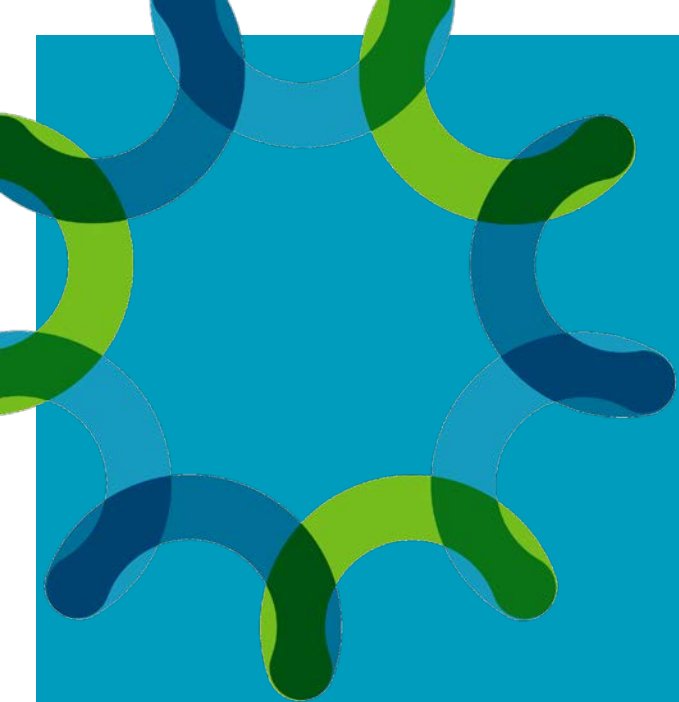


# 3 - Impact

# Impact Highlights

- **Impact on state of technology/industry if successful:**
  - Accelerated biomanufacturing commercialization
  - No need to re-establish metabolic routes and hosts
  - Likelihood assessments / demonstrations of process transfer
  - Increased access to broadly enabling DBTL infrastructure
- **How disseminating results:**
  - 250+ citations across 50 publications to date (since FY17)
  - 67 citations across 17 publications since FY20
  - 5.91 impact factor
  - 6 records of invention, 7 software disclosures, 16 patent applications, 5 licenses
  - Reducing barriers to commercialization
- **Memoranda of Understanding (pending):**
  - NSF and DOE
  - Global Biofoundries Alliance



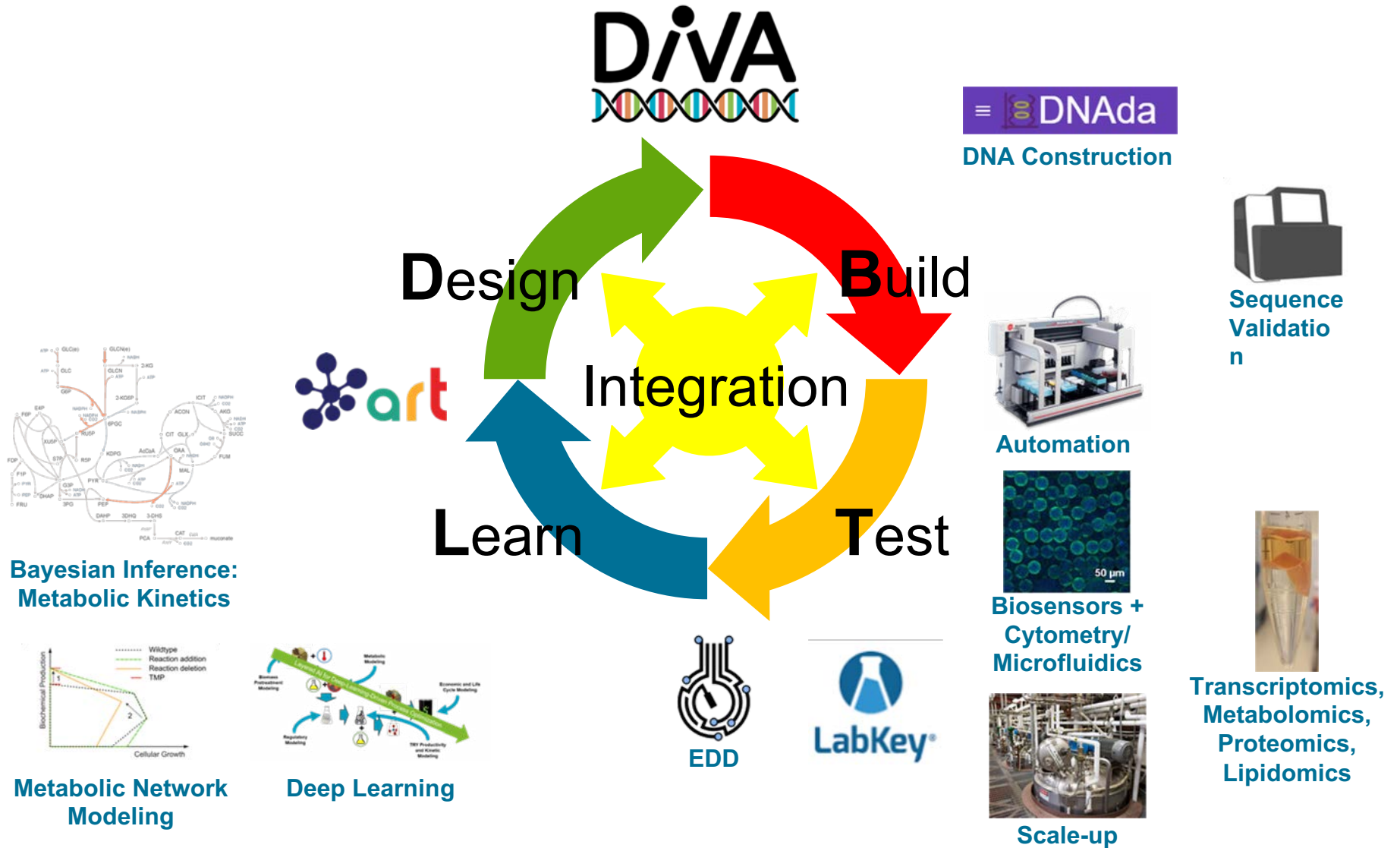


# 4 - Progress and Outcomes

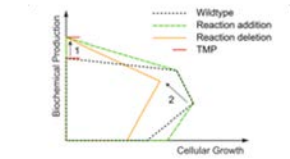
# Progress made towards goals

- **Acceleration of biomanufacturing commercialization:**
  - Collaboration: Industrial and academic collaboration projects will be presented the over the next two days. As leading indicators, progress therein is very promising (e.g., Lygos - 20X increase in isobutyrate titers)! Through the outcomes of these collaborations over time, the ABF endeavors to definitively establish end-to-end impacts on time from bioprocess conception to scale-up and commercialization
  - **Internal:** The ABF, as assessed through target/tool transfer and **DBTL efficiency metrics**, along with established beachheads and hosts, is itself making good progress towards this goal
- **Broadly enabling DBTL infrastructure**
  - The following slides will offer concise highlights thereof
- **Assessments and demonstrations of bioprocess transferability**
  - Target and Host Engineering, and the Host Onboarding and Development ABF presentations are later this afternoon
  - These subsequent presentations will further detail our progress

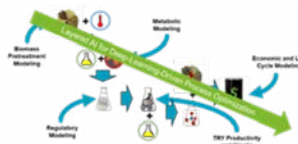
# DBTL infrastructure



Bayesian Inference: Metabolic Kinetics



Metabolic Network Modeling



Deep Learning

**DiVA**  
DNA Construction

Automation  
Sequence Validation

Biosensors + Cytometry/ Microfluidics

Scale-up

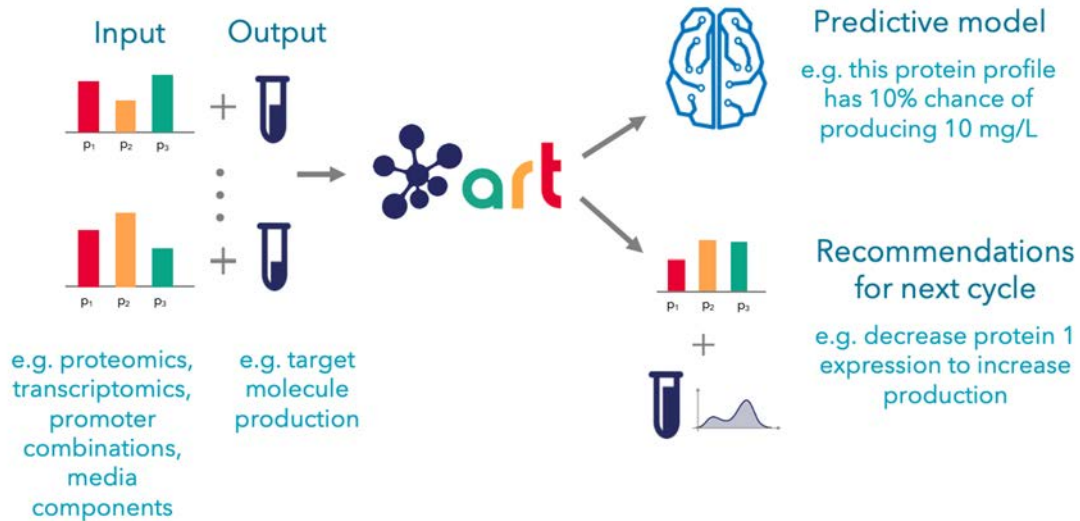
Transcriptomics, Metabolomics, Proteomics, Lipidomics

EDD  
LabKey

# Learn/Design Highlight – ART Software

## Automated Recommendation Tool

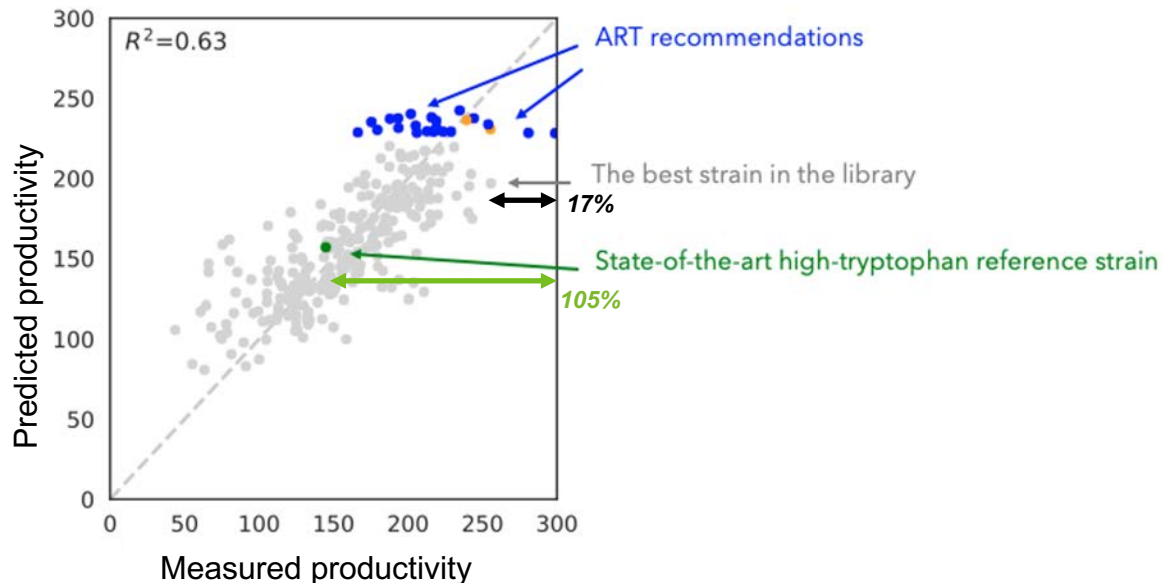
- Machine learning tool **specifically adapted to synthetic biology's needs**: small data sets, uncertainty quantification.
- Builds a **predictive model from data** and uses that model to **recommend new designs** for next cycle.
- Can be used via Web-UI@[art.agilebiofoundry.org](http://art.agilebiofoundry.org) or Jupyter notebooks



- ART has been used to successfully **guide the bioengineering process**.

Radivojević, et al.  
*Nature communications* 11.1 (2020): 1-14.

Zhang, et al.  
*Nature communications* 11.1 (2020): 1-13.



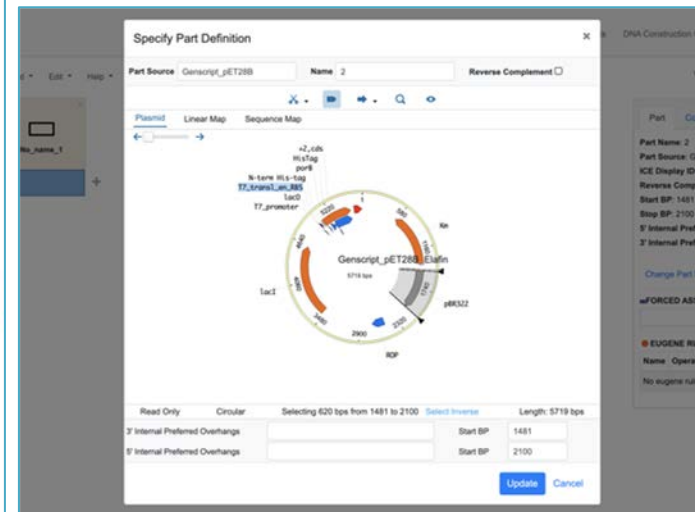
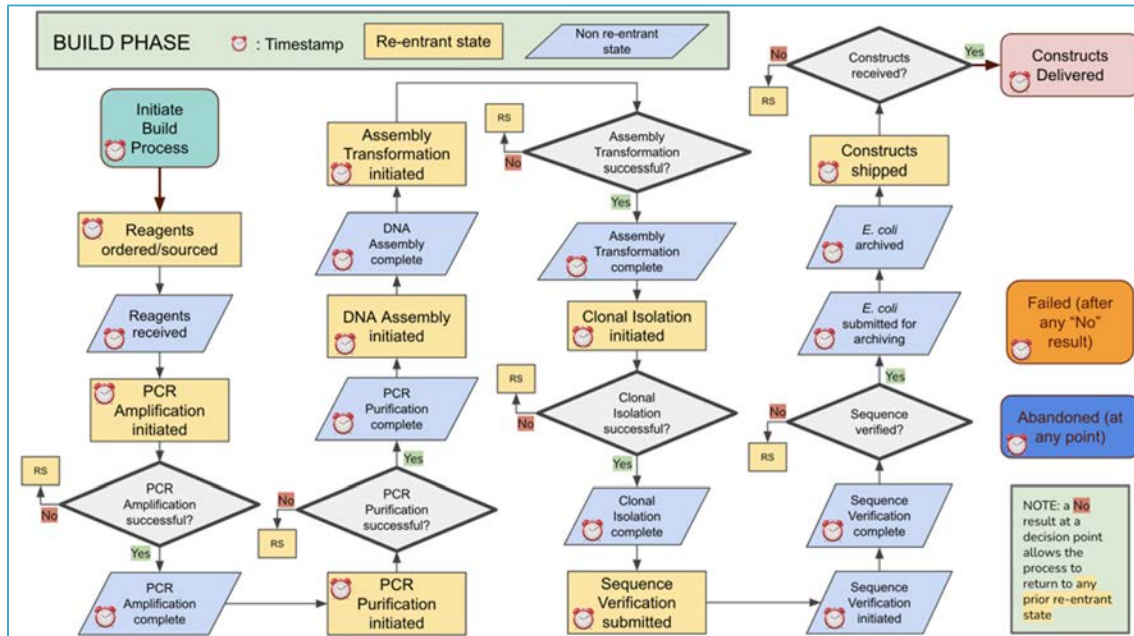
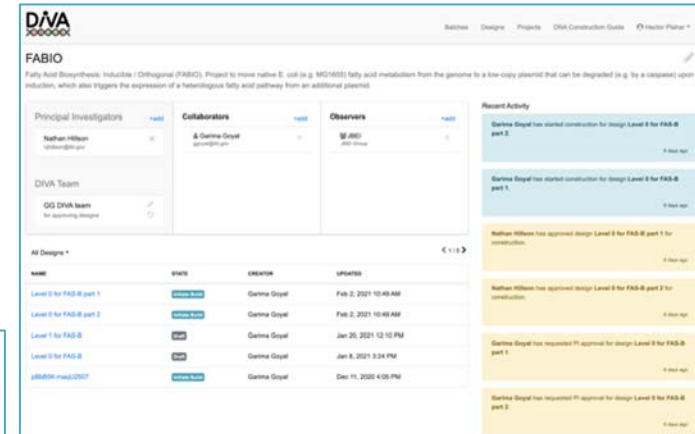
# Design/Build Highlight – DiVA Software



- **Design Implementation Validation Automation**
  - Software platform integrating tools for Designing and Building DNA constructs

## Recent improvements

- Design batching for increased Build efficiency (FY20Q3\_DBTL\_R4)
- Design and Build cycle time metric capture (FY20Q3\_DBTL\_R4)
- User interface redesign and client web framework modernization
- Support for “empty” parts in a design
- Support for custom DiVA Teams
- Improved OpenVectorEditor integration for part and construct visualization



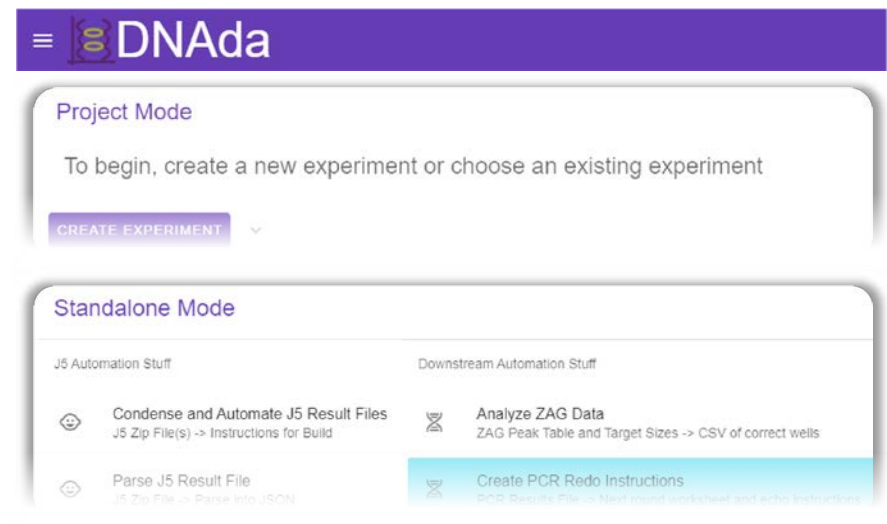
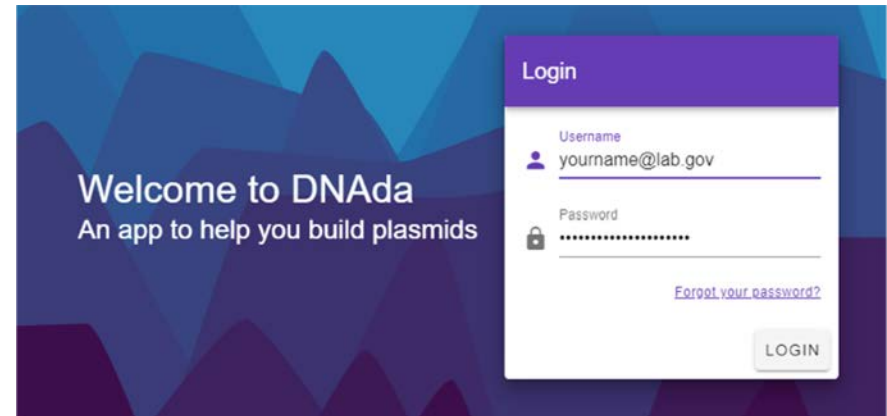
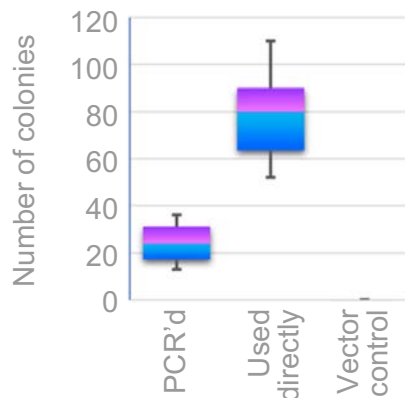
# Build Highlight – DNA Construction

## • Informatics

- Collaborations with BETO- and BRC- supported projects on software interfacing dry and wet-labs

## • Process improvement

- Throughput increase from automated (e.g., 4x for *E. coli* transformation) and non-automated methods (e.g., large scale DNA purification) - 32 days from synthesis to delivery of 100+ constructs
- Progress on plasmid DNA copy-control strain for accelerating Build of large constructs
- Higher efficiency and reduced hands-on time for cloning synthetic DNA w/o adapters (skipping PCR/purification)

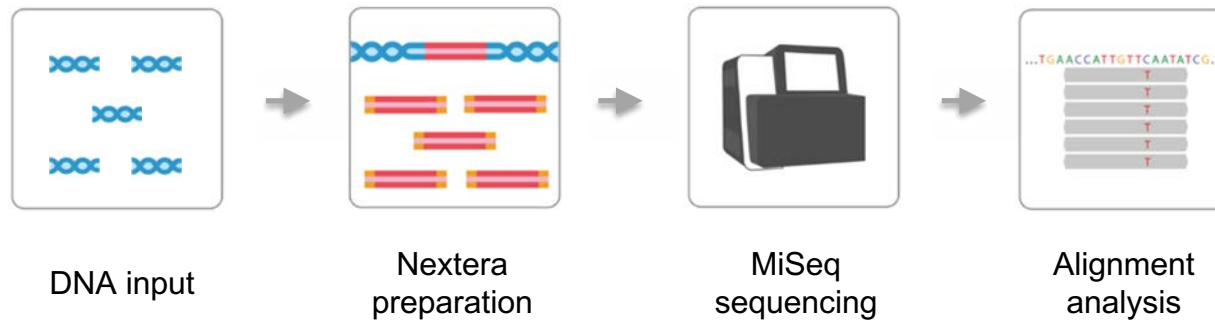




# Build Highlight – DNA sequence validation

## • Overview

- \$8 per sample (full amplicon/plasmid coverage, no Sanger-method oligos required)
- Sample types: boiled cell culture, purified plasmid, or PCR amplicon
- Up to 1536 purified DNA samples, or 384 cell cultures per MiSeq run



## • In progress

- Automating plasmid minipreps on the Biomek to facilitate higher-throughput MiSeq runs



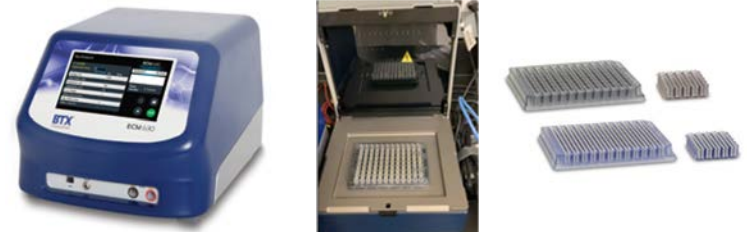
- Developing sequencing statistics web app (SSGUI) to streamline analysis for users
- Coordination with microbial strain archivists to buffer demand for more frequent/consistent sequencing cycles
- Evaluating alternatives to Nextera library kits



# Build/Test Highlight – Automation

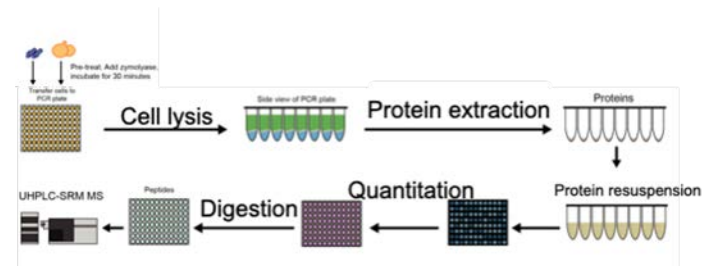
- **Build: *Rhodospiridium toruloides* & *Pseudomonas putida* transformation**

- New 96-well plate based methods created
- 400% throughput improvement
- Created and validated method with live samples (>5 cycles of improvements completed)



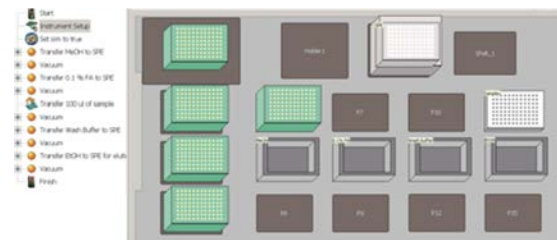
- **Test: Proteomics Sample Preparation**

- Modularized the sample preparation workflow and established modules on multiple biomek liquid handlers
- ~50% faster and more flexible



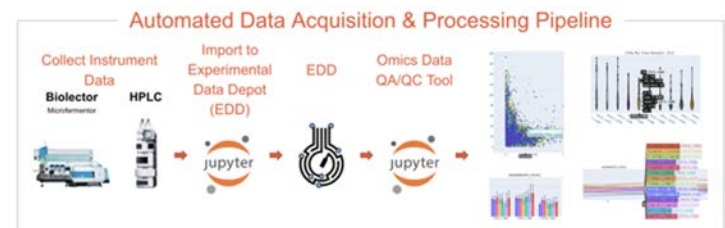
- **Test: Analytical Sample Prep (Solid Phase Extraction)**

- 96-well plate based method on Biomek FX
- Facilitates cleaner samples
- 400% improvement in throughput over manual method in same time



- **Test: Omics Data QA/QC Tool**

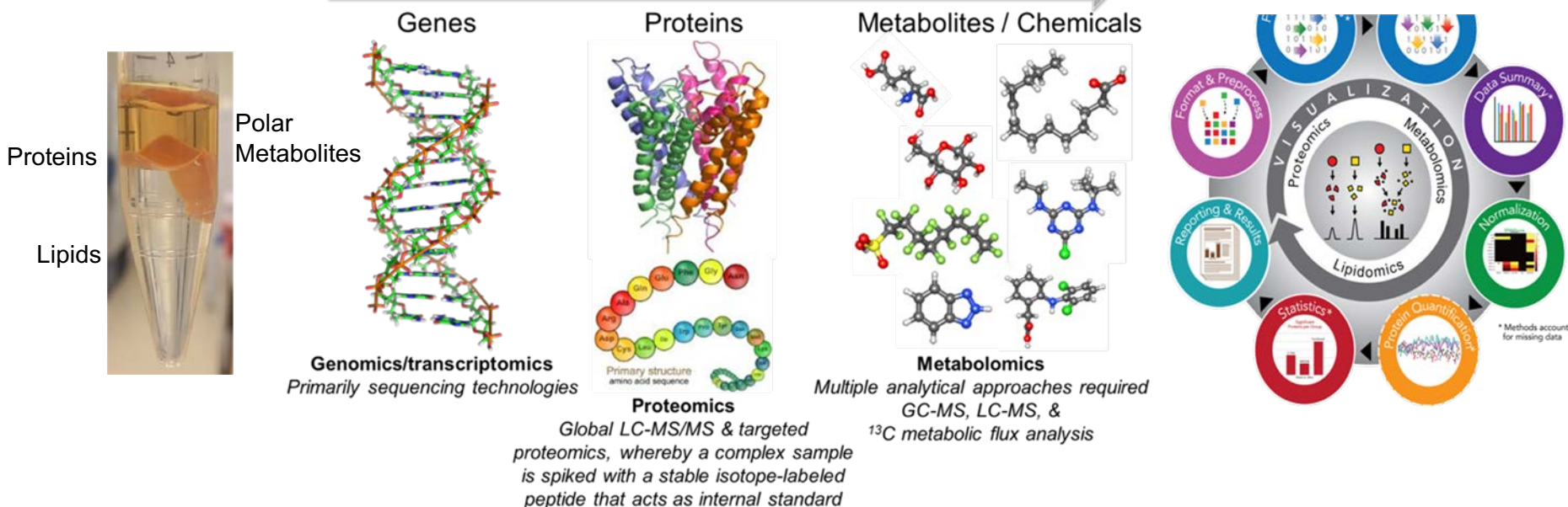
- Analysis tool that calculates the repeatability, precision, and quality of experimental data uploaded to the Experiment Data Depot (EDD)
- Supports transfer of high-quality data to Learn activities
- Expanded to HPLC, GC, and bioreactor data types in the EDD



# Test Highlight – Multi-omics Analysis

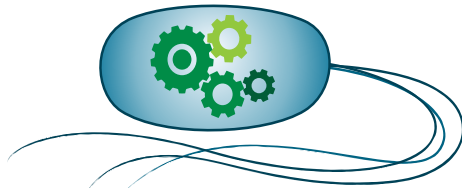
- **New ABF targeted proteomic method increases throughput of protein quantification by 4 times**
- Gao, Yuqian, et al. “High-Throughput Large-Scale Targeted Proteomics Assays for Quantifying Pathway Proteins in *Pseudomonas putida* KT2440” *Front. Bioeng Biotechnol.* 2020 Dec 2;8:603488. doi: 10.3389/fbioe.2020.603488.

## Single-sample Metabolite, Protein and Lipid Extraction



Host	Proteomics (global + targeted)	Metabolomics/Lipidomics (Intra/Extracellular)
<i>P. putida</i>	> 780 datasets	> 520 datasets
<i>A. pseudoterreus</i>	> 590 datasets	> 550 datasets
<i>A. niger</i>	> 150 datasets	> 150 datasets
<i>R. toruloides</i>	> 660 datasets	> 1000 datasets

# Test Highlight – Biosensors and Cytometry



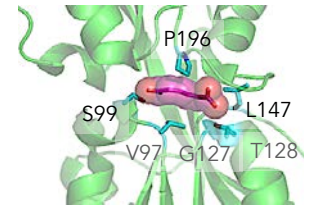
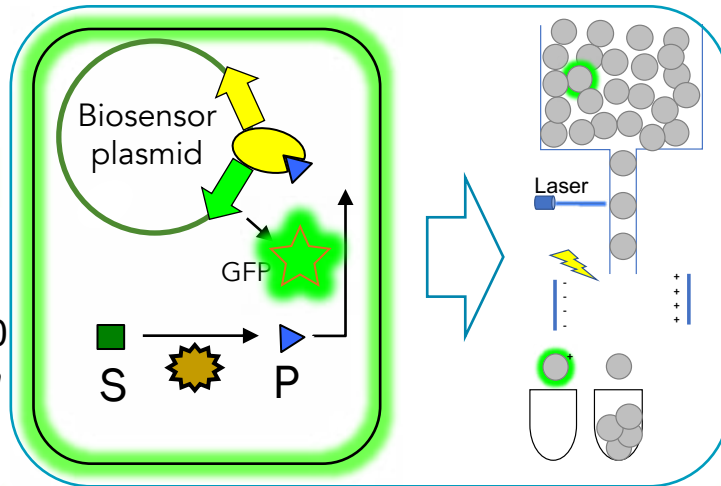
## Organisms

Established in

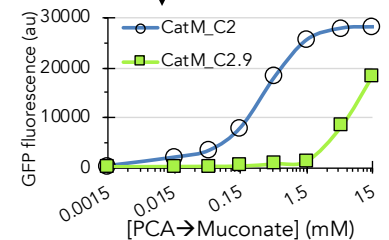
- *Pseudomonas putida* KT2440
- *Corynebacterium glutamicum*
- *Acinetobacter baylyi* ADP1

In progress

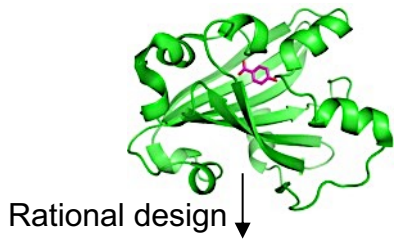
- Anaerobes



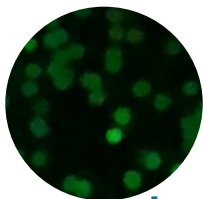
Custom design



**Sensors with variable sensitivities**



Rational design

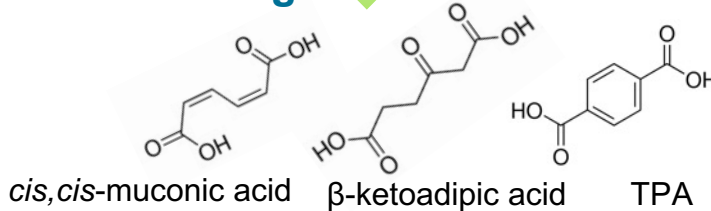


## Enzyme engineering

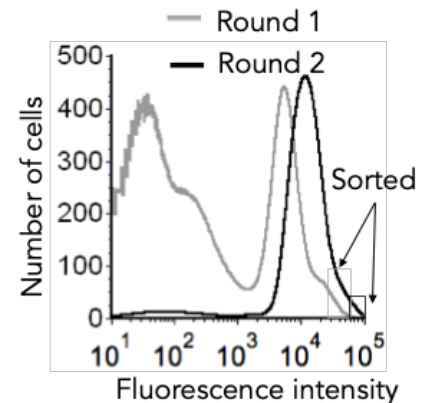
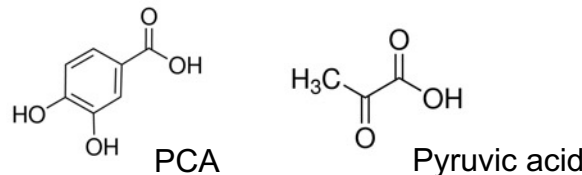
Selection based on fluorescence

Established sensors

## Target chemicals



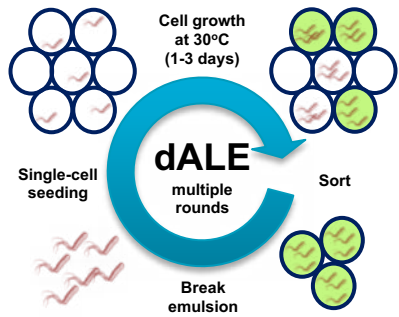
## Beachheads



## Strain evolution

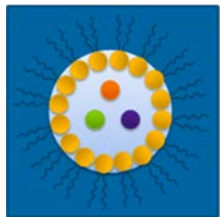
Sorting heterogeneous population

# Test Highlight – Biosensors and Microfluidics



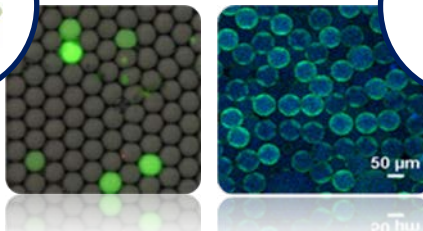
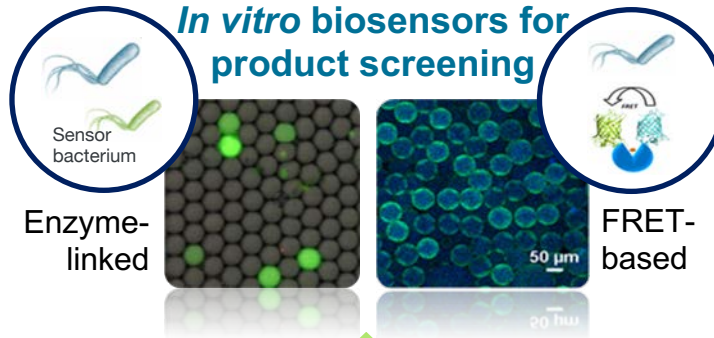
## Droplet-based adaptive laboratory evolution (dALE)

- Enriches for fast growers
- Avoids over-growth of non-productive cells
- Maximizes strain diversification



## Biologically friendly droplets

- Aqueous droplets in fluorinated oil
- Volume: **1-30 pL**
- Droplets/run: **~10,000,000**



## High-speed LC-Mass Spectrometry

- Analysis time: **~1 min/sample**
- Monitor four single ions
- Simultaneous UV/vis



## Droplet-based microfluidics

- Mixing and encapsulation
- Strain evolution
- Enzyme evolution



## Laboratory automation

- Liquid handlers allow parallel strain testing
- Efficient validation in multi-well plates or shake flasks



# Test Highlight – Scale-up

## LBNL

- RoboLector + Biolector
- 12 X 250 mL Sartorius Ambr®
- 4 X 2 L Sartorius Biostat B
- 1 X 50 L ABEC
- 2 X 300 L ABECs
- Thermofisher Gallery for micronutrient analysis

## NREL

- RoboLector + BioLector Pro
- 2 X 250 mL Applikon my-control
- 36 X 500 mL Sartorius BioStat-Q
- 6 X 3 L Applikon single-wall
- 5 X 10 L 320 Eppendorf BioFlo
- 1 X 30 L, 2 X 160 L, 2 X 1450 L, 4 X 9000 L

## PNNL

- 3 x Sixfors (6 x 0.5 L each)
- 1 x 2-10 L Sartorius Biostat
- 2 x 30 L Sartorius
- 120 L Sartorius

## SNL

- Biolector Pro



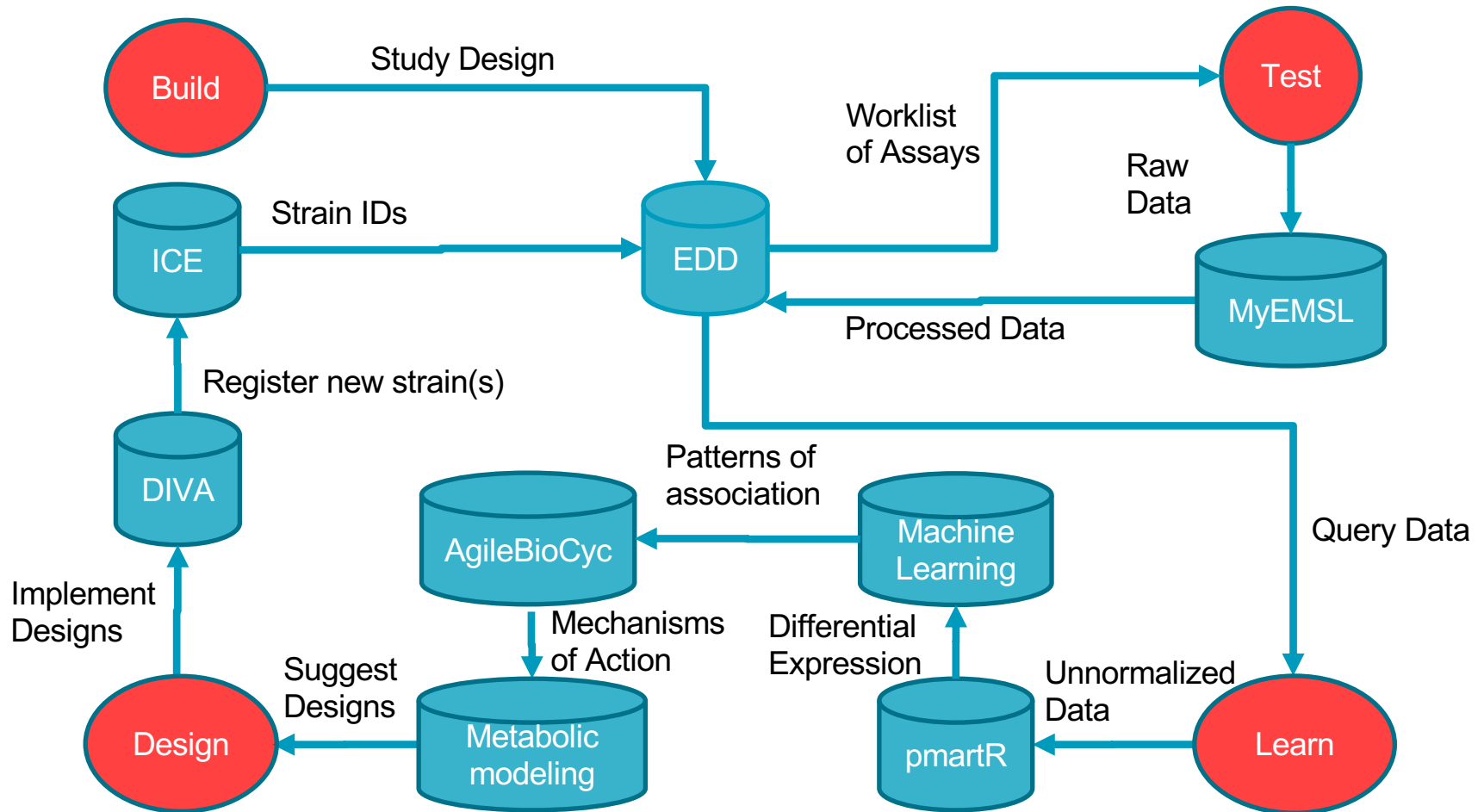
12 X 250 mL bioreactor



4 X 9000 L bioreactors

# Test/Learn Highlight – ABF Data Flows

- Raw, processed, assay, and sample data and metadata from Test activities
  - May additionally be channeled into the ABF data ecosystem via MyEMSL

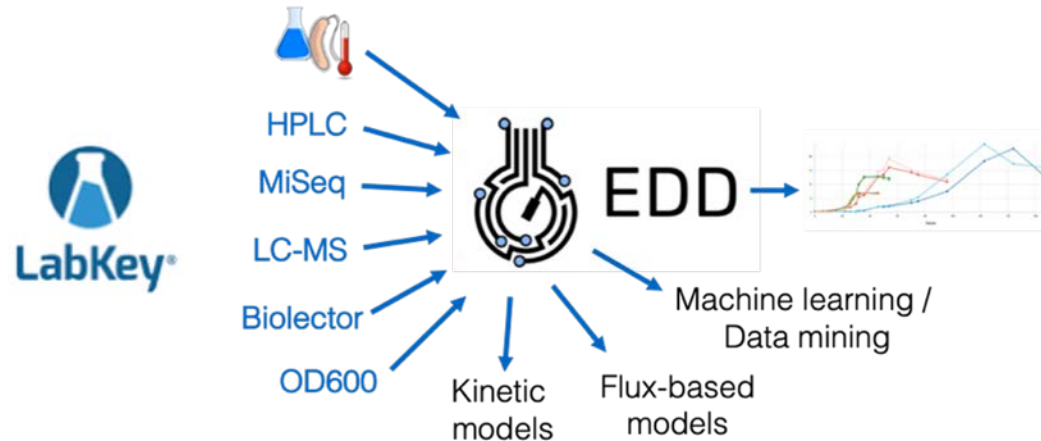


# Test/Learn Highlight – EDD Software



- **Experiment Data Depot**

- Software platform repository for actionable biological datasets and metadata



- **Recent improvements**

- Internal updates
  - Updated script builds to Webpack 4
  - Improved organization of code modules
  - Automated building, testing, and deploying
  - Added scripts to generate deployment configuration
- Added features
  - Account approval and management tools
  - Improved import workflow
  - Support / requirement for UniProt, PubChem IDs
  - Speed improvements by working in background
  - Include Assay metadata in building worklists
  - JSON Web Token login option in REST APIs
- In-Progress features
  - Replaced in-house table implementation with library
  - Further improved import workflow
  - Replicate support & improved graphing
  - Support for AWS S3 -- removing upload size limits
  - Campaigns to group related Studies (i.e., DBTL cycles)
  - Link to Protocols from protocols.io
- New EDD-related paper:
  - Roy *et al.* (2021) *Frontiers in Bioeng Biotech* (see additional slides for full reference)

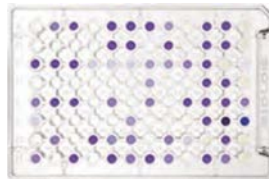
# Learn Highlight – Metabolic network modeling

## Metabolic network reconstruction, modeling, and validation

BiGG Models



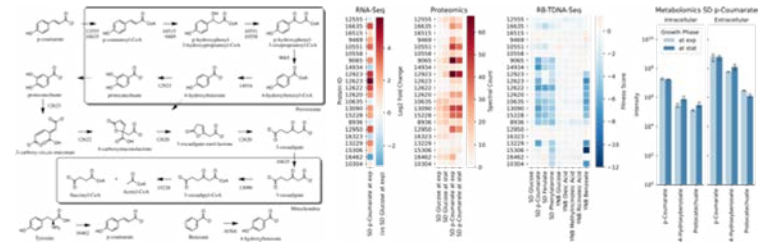
- Reconstruction of metabolic networks using high-quality metabolic models and databases
- AgileBioCyc at <https://cyc.agilebiofoundry.org>



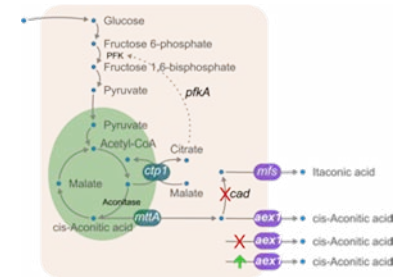
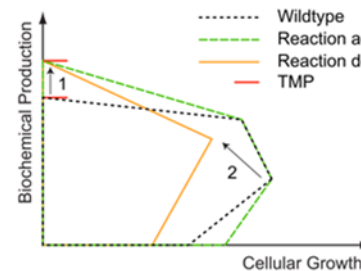
		Experimental data	
		Growth	Essential
In silico	Essential	FN	FP
	Growth		

- Curation and refinement of metabolic networks using multi-omics and high-throughput data
- The genome-scale metabolic network model of *R. toruloides* and associated data available at [https://github.com/AgileBioFoundry/Rt\\_IFO0880](https://github.com/AgileBioFoundry/Rt_IFO0880)

## Multi-omics data integration and computational strain design



- Analysis and visualization of multi-omics data using metabolic models and maps



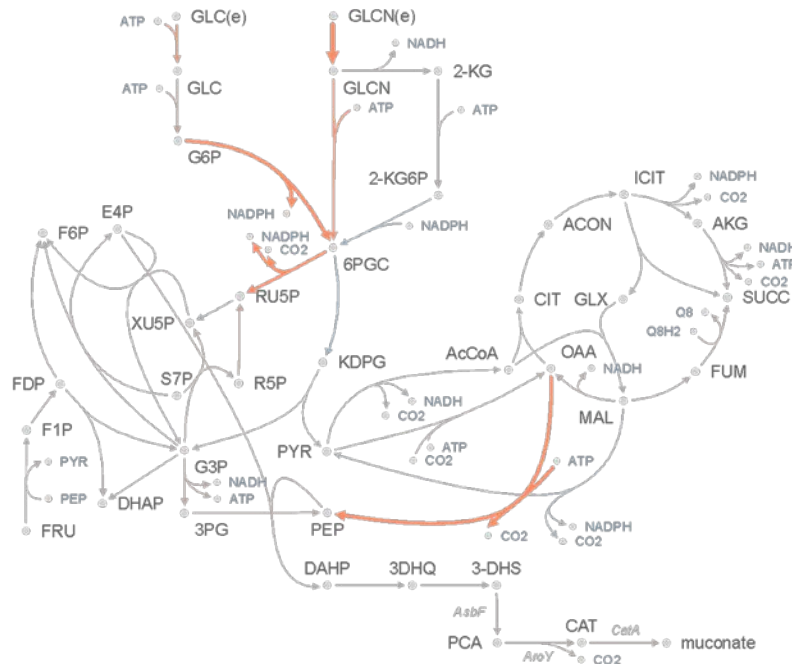
- Computational strain design approaches using genome-scale metabolic models at [https://github.com/AgileBioFoundry/Strain\\_Design](https://github.com/AgileBioFoundry/Strain_Design)



# Learn Highlight – Bayesian inference of metabolic kinetics from multi-omics data

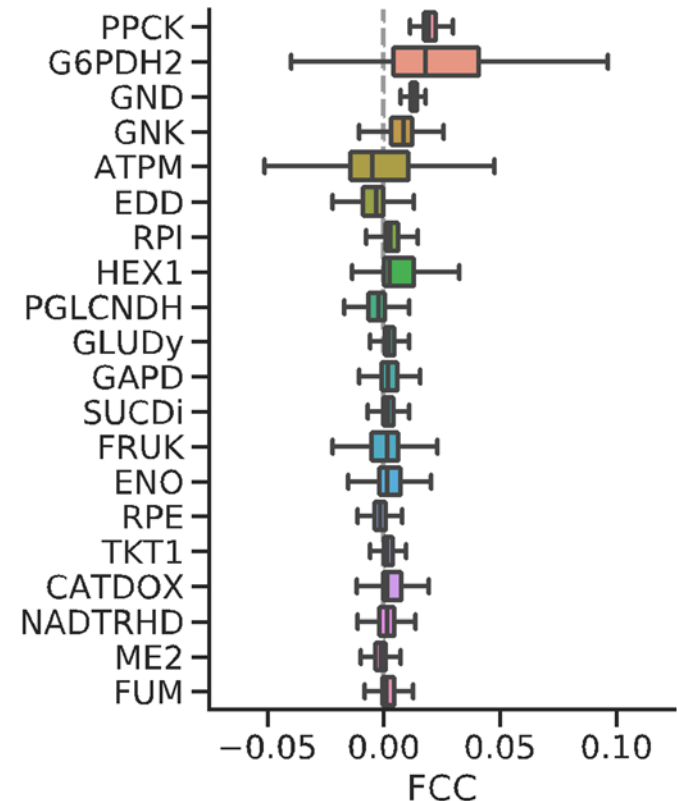


- Infer probabilistic relationship between variables we can control (enzyme expression; media composition) and those we cannot (intracellular fluxes and metabolomics)
- Method applied to *P. putida* media and strain experiment
- Revealed several core-carbon enzymes that might lead to higher muconate flux



*Red arrows indicate overexpression is predicted to result in higher muconate flux*

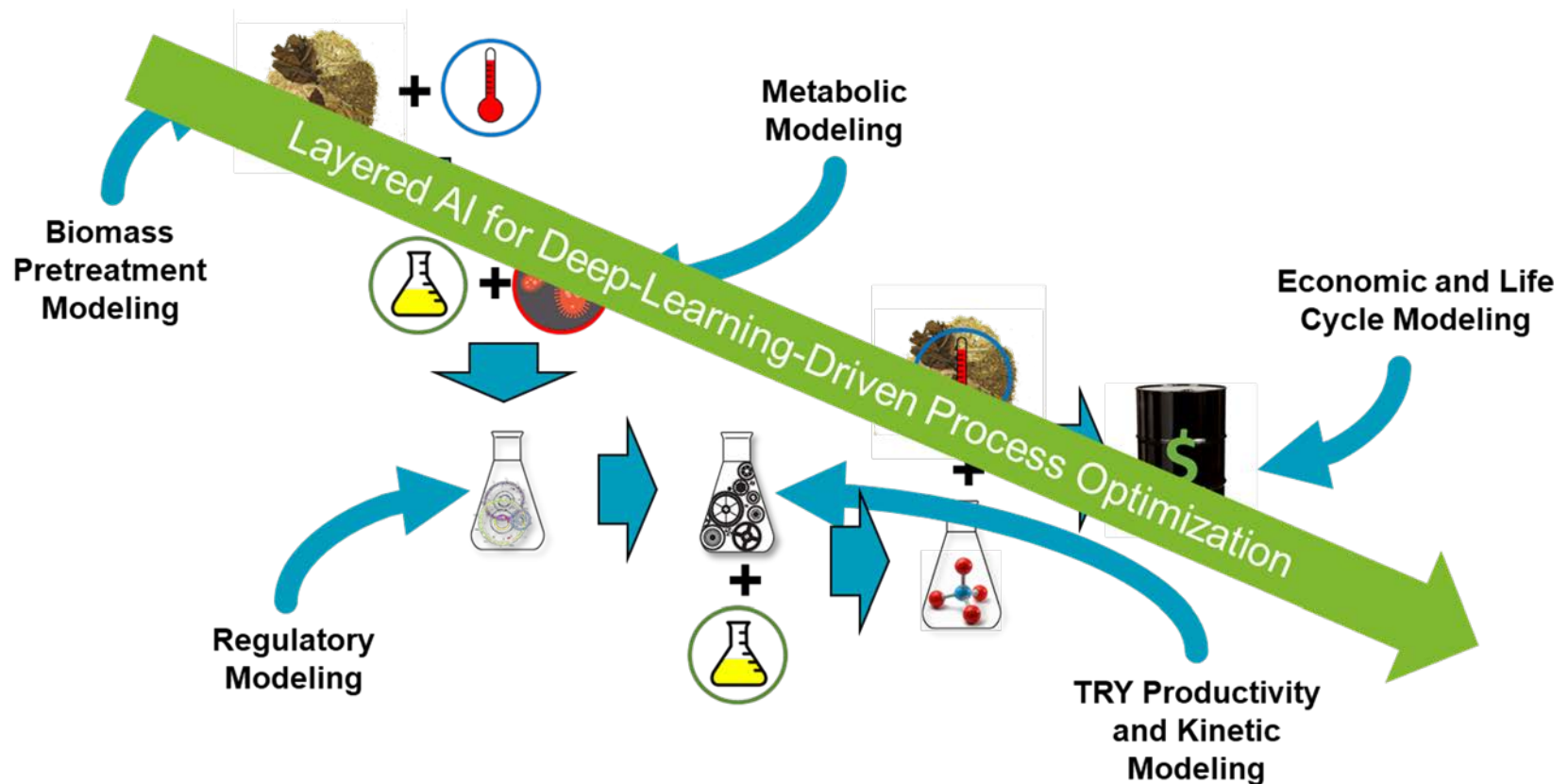
- The resulting model offers predictions that are more mechanistic than black-box approaches



# Learn Highlight – Deep Learning

## • Integrated AI subsystems for Deep Learning in Biomanufacturing

- An ecosystem of learn models for continuous data collection and integration
- Outcome: An integrated layering of modules where output of one is input of next
- Ongoing: Required complexity and inter-lab coordination being established



# Status of key milestones

- **Completed in FY20 (representative):**

- Q1: DBTL cycle defined with specific required unit operations comprised
- Q1: 4 DBTL unit operations formally specified
- Q3: Opportunities for DBTL efficiency improvements identified, development initiated for these in at least 3 workflows and improvements quantified
- Q3: Power analysis of -omics datasets for two organisms completed to determine number of replicates needed for Learn
- Q3: Opportunities for DBTL task automation identified and prioritized, and development initiated of automation workflows for 2 identified priorities
- Q4: Reproducibility of 3 Test unit operations quantified through comparison of results for on-site vs. off-site sample analysis for 3 or more variables

- **On track for completion in FY21 (representative):**

- Q2: (Go/No-Go) 5 metabolic pathways and/or tools transferred between hosts, with 2X improvements in second host, with metrics defined for each case
- Q3: Efficiency gains in DBTL workflows assessed
- Q3: Biosensors developed for two beachheads or energy/redox indicators
- Q4: 1-2 DBTL automation workflows finalized that improve efficiency by => 2X
- Q4: Cross-validated 20% improvement in predictive power demonstrated for two or more ABF Learn methodologies, for multiple vs. single data modalities

# Risk mitigations - all on-track / ongoing

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

# Summary - DBTL Infrastructure

- **Goal:** Design, implement, operationalize, and maintain Design-Build-Test-Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF Tasks and enables the overall ABF goal
- **Outcomes:** 10X improvement in Design-Build-Test-Learn cycle efficiency, new IP and manufacturing technologies demonstrated and ready for translation to U.S. industry
- **Relevance:** Public infrastructure investment that supports the ABF and other BETO projects, and that can be leveraged by U.S. industry
- **Risks:** Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from



# Quad Chart Overview

## Timeline

- Start: October 1, 2019
- End: September 30, 2022

	FY20	FY21	FY22	Total Active
DOE Funding	\$2.3M	\$4.8M	\$5.4M	\$12.5 M

## Project Partners

- LBNL (34%), SNL (21%), PNNL (19%), NREL (11%), ANL (4%), LANL (7%), ORNL (5%)

## Barriers addressed

- *Ct-L*. Decreasing Development Time for Industrially Relevant Microorganisms
- *Ct-D*. Advanced Bioprocess Development

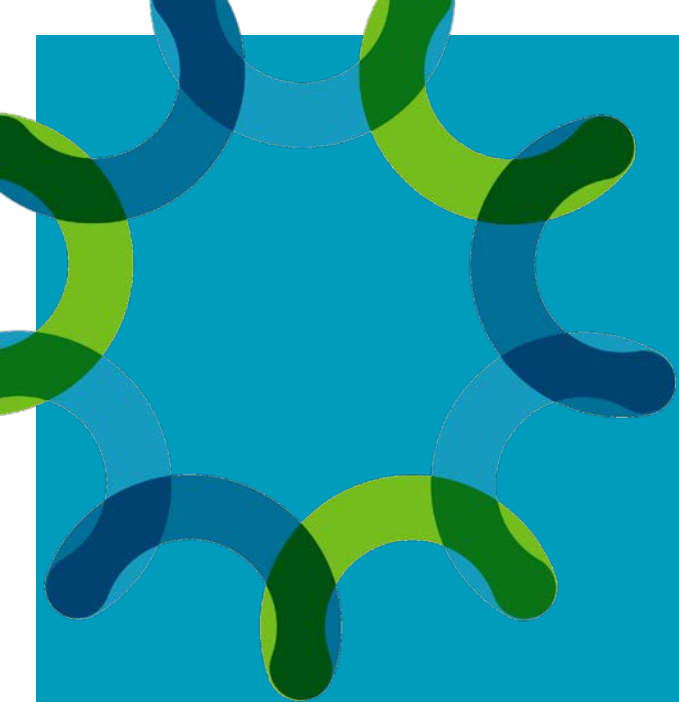
## Project Goal

Design, implement, operationalize, and maintain Design-Build-Test-Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF Tasks and enables the overall ABF goal

## End of Project Milestone

- 5X efficiency improvement in DBTL engineering cycle

## Funding Mechanism AOP



# Additional Slides

# Responses to Previous Reviewers' Comments

- **C:** The DBTL infrastructure is the core of the ABF and supports all other tasks. Having this infrastructure in place and streamlined is critical for reducing both DBTL cycle time as well as overall project timelines. This is dependent on having a good software platform to maintain data and enable Learn activities, and the team has built or acquired a number of tools. These include DNA construct design and assembly, various types of metabolic modeling, deep learning algorithms, and LIMS for data storage and sharing. New Build tools are NextGen sequencing to verify construct accuracy, and a novel method for gene evolution based on duplication and recombination. Sample processing for omics analysis has also been streamlined. The DBTL cycle time has been improved, but is still too long. Now that all the computational and experimental tools are in place, effort should be spent on developing a streamlined workflow. Also, the current ABF projects may be too early to really gain full benefit from DBTL. As a test case, it would be useful to apply this to a mid-stage project with an organism with well established genetic tools.
- **R:** We agree that our DBTL cycle time (as of Peer Review 2019) is too long. We are now quantitatively defining what constitutes a DBTL cycle (vs. mini-DBTL), beyond the qualitative definitions provided at Peer Review. We will be working towards increasing the coverage and granularity of our cycle time metrics capture, and use the resulting data to prioritize our DBTL workflow streamlining efforts. We agree that some ABF projects may be too early stage to benefit from DBTL (which might otherwise be better served by mini-DBTL); finding the transition point (in terms of project maturity needed in order to benefit from DBTL) is a good idea.



# Responses to Previous Reviewers' Comments (cont.)

- **C:** Development of the DBTL infrastructure to realize efficiency gains in project execution and delivery is at the heart of the ABF engine design room. The software, tools, processes and other assets combine to optimize the project development cycle and can greatly enhance productivity and success at the ABF and, if made available, to external stakeholders such as industry and academia. It will be important to identify the appropriate business model to achieve this.
- **R:** The ABF's philosophy is to use methods, instruments, software, etc. that are accessible (and develop those that will be accessible) to industry and academia, either through commercial vendors or through licensing from the ABF itself (via the National Labs). This enables our industrial and academic collaborators to practice these same methods, instrumentation, and software, behind their own corporate or institutional firewalls without persistent reliance on the ABF. There are established licensing models and mechanisms (e.g. exclusive in a field of use, non-exclusive, freely open-source) that enable this, with the general broad objective to maximize impact and market transformation (which determines the licensing mechanism). For the ABF in particular, the non-exclusive (including freely open-source) mechanisms are strongly preferred (so that multiple companies and academic groups can benefit from them) with the exception of exclusive licenses to technology platform companies that will make the technologies broadly accessible. Part of the sustainable business model for the ABF, then, is to incentivize its collaborators to opt for non-exclusive licensing options in Collaborative Research and Development Agreements (CRADAs), and we plan to explore these options in the next phase of the ABF.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Geographic distribution of the different steps poses logistical challenges, requiring strains and samples to be shipped around different sites. This could slow down the cycle.
- **R:** We are aware of the distributed logistical challenges. While these do slow down DBTL to an extent, it is a rather minor (at least currently) contributor to overall DBTL cycle time.
  
- **C:** Weakness: It would be helpful if they provided more quantitative benchmark for progress and success. These are a little vague; as a consequence, it is difficult to evaluate whether the team is on track to achieve their final goals.
- **R:** Not clear what the Reviewer is referring to specifically. We did agree elsewhere that more closely connecting sub-tasks with the overall ABF goal is important, and we also mentioned that that we used simplified milestone language in the presentations, which resulted in not sharing all the quantitative benchmark details with the reviewers.
  
- **C:** Weakness: Specific relevance of the current work toward the 10x efficiency improvement is not quantitatively explained. The goals of the ABF should be focused on the performance period, especially for peer review. Descriptions for individual projects should include explanation of how their contributions quantitatively feed into the program-level goal(s) for the period.
- **R:** We don't have this quantitative data yet. Until we have a clear definition of DBTL and granular performance metrics captured (next 3-year AOP objectives), this won't be possible. Discussed elsewhere how we will use this data to prioritize and inform subsequent core technology development. It is a fair point that presenting 9-year ABF objectives during a 3-year Peer Review can make assessments less straightforward.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: More data/results are required to determine how much non-intuitive learning has been enabled to date. It will be important to keep a scoreboard on such successes so they can be highlighted in future reviews.
- **R:** Agreed. We won't have the results from evaluating the unintuitive predictions until the end of FY19. It should be noted, though, that we did not ask the Reviewers to assess the value of the unintuitive predictions (can't be done until we have the data!), but rather we asked them to assess if the predictions were unintuitive (or not) independent of their future successes or failings.
  
- **C:** Weakness: Many different tools are being developed, but no clear plan on how they will be put together in a workflow. It would be good to have a case study to showcase all the tools, taking a strain through a full cycle.
- **R:** This would have needed to have been done in the Target/Host presentations. There was no time to do this in the DBTL infrastructure presentation. If there had been more time, a case study would have been nice and effective.
  
- **C:** Weakness: Quantitative benchmarks would be helpful for evaluating success, be it partial or complete. Also, it would help to more clearly define what is meant by non-intuitive predictions. The basic idea is clear; however, the metric for success here is somewhat murky. In particular, non-intuitive for some may be intuitive for others. Clearly, rigorously defining this metric will be challenging (and the team recognizes it). Nonetheless, the team should continue to work towards refining this metric, given its central role in justifying the Agile Biofoundry.
- **R:** Quantitative benchmarks addressed elsewhere. We did define what we meant by unintuitive in the overview presentation - namely a prediction or design choice that a skilled metabolic engineer would not have come up with without access to the deep/wide Test data and Learning methods employed at the ABF. This comment/response is very similar to that addressed above regarding how to quantitatively assess the value offered by any particular Test dataset or Learn methodology.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Management of individual projects appears to follow typical collaboration dynamics (regular meetings, etc...). Although they are working fine now, they may not scale as ABF's portfolio grows (especially with the CRADAs). It may become important to institute additional project management tools and/or layers of project management. The overall approach to the organization of the core technology development efforts was not presented. By presenting only highlights, the team did not communicate a clear, comprehensive picture of the approach as a whole, so it is hard to assess the planned capability. The team should articulate the entire tech-dev effort, explain how it is broken down into individual components, provide rationales/justifications for the areas where it is placing its biggest bets, and explain how they all quantitatively add up to achieve each of the high-level objectives for the ABF. In this way, it will be possible to regularly, holistically review the portfolio to ensure that all component efforts are still relevant going forward. Similarly, the team did not communicate a clear, comprehensive picture of the current state of technology development as a whole. As a result, it is not possible to evaluate the current state (nor development progress) of the ABF capability as a whole, and review is limited to highlights. This is due, in part, to omissions from the presentations of important activities that the team is certainly perusing. For example, regarding "Build," Target-Host-pair presentations mention strain construction as a bottleneck, yet there was no coverage of this critical component of ABF, nor its plans going forward. Strategically, in order to attain the 10x efficiency goal, there must exist a sub-goal/sub-plan for strain construction efficiency. (I am assuming that this was simply not communicated.) Similarly, there must be an assessment of what ABF's current status is toward these sub-goals and intermediate milestones. The same kinds of omissions are also assumed for the other aspects of "DBTL."
- **R:** This is an important point - how will the ABF scale its CRADA project management along with operations? We will need to discuss this internally with DOE BETO. Our current approach appears to be working well (lead lab PIs doing project management of CRADA projects, whereas Alastair is doing it for core ABF activities), but this may or may not scale. In the first three year AOP cycle for the ABF, we have identified many places around the DBTL cycle that present as opportunities for further efficiency gains. To date, we have largely been pursuing an all-the-above approach to core technology development. With increasingly precise and granular DBTL performance metric data capture, we can more holistically and strategically approach core technology development prioritization and staging. Since the presentations were limited by time, we were not able to present all of the work that we have been doing. This includes "Build", which other than the sequence validation component, was not discussed or presented.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: A lot of great software tools have been developed, but there are no examples showing how the software tools have facilitated the Learn function. Some were covered in the host presentations, but it is still too soon to tell how much the modeling helped identify targets. Much of the Test function is performed in small-scale plates. It is not clear how well this translates to actual fermentation conditions. Cycle time is still too long. It is understandable that the Build function will take a long time for novel hosts. However, in many cases Test is very long too. Since they have all culturing, analytical capability, and omics within the ABF, Test should only take a few weeks.
- **R:** The structure of the talks, which split the presentations of the tools themselves from their applications / predictions did not make it easy for the Reviewers to connect the tools with their unintuitive predictions. Agree that most Test activities are at small scale, with a minority taking place in bioreactors; in the next 3-year AOP for the ABF, we will be working more on scale-up and scale-down, and the addition of Ambr250 and BioLector instrumentation will help us very much in this regard. Cycle time is addressed elsewhere. A given Test unit operation clock time may be fast, but the wall time of an overall Test phase depends on the different types and numbers of unit operations, along with how resources - personnel and instrumentation - are allocated and prioritized. For multi-omics analysis, several weeks is probably not a realistic expectation.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: A comprehensive picture of the entire DBTL capability is not presented, so it is hard to review. (See comment under “Approach.”) It would help to better understand impact to explain who/which projects are using these tools, and how much. For example, Diva was only mentioned by one other project, which gives the (false?) impression that there is only one user. Similar questions can be applied to EASy, EDD, or microfluidics, etc.. It is not clear how are all of these tools combined into a DBTL cycle. Ad-hoc is ok, but it should be stated explicitly if so. Regarding newer AI/Learn efforts, many were described superficially, so the approaches are hard to evaluate, especially since there are no validated predictions yet. For the purpose of review, it would help to present these innovations at a deeper level, perhaps by the ABF’s ML experts. An overarching concern is that these methods can often require large numbers of experiments, even if the amount of data from each individual experiment is large (e.g. metabolomics). It will be important to show that findings from any new ML approach are substantially better than what would be generated via “old” ML (regression/clustering). The layered AI approach has even greater technical risk, as it would require substantial data for each step.
- **R:** It is a good point that we do not have not collected comprehensive data about which target/host projects are using which tools / infrastructure. During the Peer Review presentation, there was not time to go into depth on any one tool or capability. We are assessing how much data is required for each Learn approach. For example, for kinetic learning, we have used simulated data to help us know how fast the methods will begin to converge.

# Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Motivations/justifications for particular directions (e.g. specific software features, new analytical capabilities) is not provided, so it is hard to evaluate whether the collective set of directions make sense as whole. As a hypothetical example, maybe more emphasis should be placed on "Build" or on collaboration software, and less on sequence verification-- not enough contextualization is provided to judge. Another example would be to assess why "Test" times are currently so long, and to address this in future plans. The dynamic that the leadership should aim to avoid is one in which the shape of the portfolio starts to be determined by inertia rather than by careful, strategic assessment and re-assessment. For some technical directions (e.g. biosensors), it will be important to quantify performance requirements (dynamic range, S/N) that are needed in order to have relevance, and then to evaluate progress against these requirements. One alternative might be to institute a scientific advisory board (domain experts; could include some members of IAB), to perform deep-dive portfolio review.
- **R:** The Reviewers were presented with highlights, and not a comprehensive and detailed survey of all of the work we are doing. Just because something wasn't presented (at all or in sufficient detail) doesn't imply that it is not being done. "Build" scope and "Test" times are addressed elsewhere. DBTL core technology development prioritization mentioned elsewhere. We will be discussing with DOE BETO the addition of a under-NDA Scientific Advisory Board that would complement the IAB.

# Publications, Patents, Presentations, Awards, and Commercialization

- **50 publications, 126 presentations to date**
  - 16 publications and 20 presentations since FY20
    - The following slides provide explicit lists thereof
- **2020 R&D 100 Award**
  - Awarded to Smart Microbial Cell Technology for rapid optimization of biocatalysts
  - Special Recognition (Silver Medal) for Market Disruptor in the Services category
- **36 patents, records of invention, software disclosures, & licenses**
  - The following slides list these intellectual property assets



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

- **(Publication)** Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. “Engineered *Pseudomonas putida* KT2440 co-utilizes galactose and glucose.” *Biotechnol Biofuels* 12, 295 (2019).
- **(Publication)** Christopher B. Eiben, Tristan de Rond, Clayton Bloszies, Jennifer Gin, Jennifer Chiniquy, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Oliver Fiehn, Jay D. Keasling. "Mevalonate Pathway Promiscuity Enables Noncanonical Terpene Production", *ACS Synth. Biol.* (2019).
- **(Publication)** Yan Chen, Deepwanita Banerjee, Aindrila Mukhopadhyay, Christopher J. Petzold. “Systems and synthetic biology tools for advanced bioproduction hosts”, *Curr. Op. Biotechnol.* (2020).
- **(Publication)** Jacquelyn M. Blake-Hedges, Jose Henrique Pereira, Pablo Cruz-Morales, Mitchell G. Thompson, Jesus F. Barajas, Jeffrey Chen, Rohith N. Krishna, Leanne Jade G. Chan, Danika Nimlos, Catalina Alonso-Martinez, Edward E. K. Baidoo, Yan Chen, Jennifer W. Gin, Leonard Katz, Christopher J. Petzold, Paul D. Adams, Jay D. Keasling. “Structural Mechanism of Regioselectivity in an Unusual Bacterial Acyl-CoA Dehydrogenase”, *J. Am. Chem. Soc.* (2019).

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

- **(Publication)** Thompson, Mitchell G., Allison N. Pearson, Jesus F. Barajas, Pablo Cruz-Morales, Nima Sedaghatian, Zak Costello, Megan E. Garber et al. "Identification, characterization, and application of a highly sensitive lactam biosensor from *Pseudomonas putida*." ACS Synthetic Biology (2019).
- **(Publication)** Geiselman GM, Zhuang X, Kirby J, Tran-Gyamfi MB, Prael JP, Sundstrom ER, Gao Y, Munoz Munoz N, Nicora CD, Clay DM, Papa G, Burnum-Johnson KE, Magnuson JK, Tanjore D, Skerker JM, Gladden JM. "Production of ent-kaurene from lignocellulosic hydrolysate in *Rhodospiridium toruloides*." Microb Cell Fact. 19(1):24. (2020).
- **(Publication)** Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachúa, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson\*, Gregg T. Beckham\*, "Engineering glucose metabolism for enhanced muconic acid production in *Pseudomonas putida* KT2440," Metabolic Eng. (2020).

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

- **(Publication)** Chen, Y.; Guenther, J.; Gin, Jennifer; Chan, Leanne J.; Costello, Z.; Ogorzalek, T.; Tran, Huu; Blake-Hedges, J.; Keasling, J. D.; Adams, P.; Garcia Martin, H.; Hillson, N.; Petzold, C. "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" *Journal of Proteome Research* (2019)
- **(Publication)** Isabel Pardo, Ramesh K. Jha, Ryan E. Bermel, Felicia Bratti, Molly Gaddis, Emily McIntyre, William Michener, Ellen L. Neidle, Taraka Dale, Gregg T. Beckham, Christopher W. Johnson. "Gene amplification, laboratory evolution, and biosensor screening reveal MuckK as a terephthalic acid transporter in *Acinetobacter baylyi* ADP1." *Metabolic Engineering*, (2020), Vol 62, 260-274
- **(Publication)** Radivojević, T., Costello, Z., Workman, K., & Martin, H. G. "A machine learning Automated Recommendation Tool for synthetic biology." *Nature Communications*, 11(1), 1-14.(2020).
- **(Publication)** Zhang, J., S. D. Petersen, T. Radivojevic, A. Ramirez, Andrés Pérez-Manríquez, E.Abeliuk, B. J. Sánchez et al. "Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism." *Nature Communications* 11, no. 1 (2020): 1-13.

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

- **(Publication)** Ernst Oberortner, Robert Evans, Xianwei Meng, Sangeeta Nath, Hector Plahar, Lisa Simirenko, Angela Tarver, Samuel Deutsch, Nathan J. Hillson, and Jan-Fang Cheng. "An Integrated Computer-Aided Design and Manufacturing Workflow for Synthetic Biology". In: Chandran S., George K. (eds) DNA Cloning and Assembly. Methods in Molecular Biology, vol 2205. (2020).
- **(Publication)** Gledon Doçi, Lukas Fuchs, Yash Kharbanda, Paul Schickling, Valentin Zulkower, Nathan Hillson, Ernst Oberortner, Neil Swainston, Johannes Kabisch. "DNA Scanner: a web application for comparing DNA synthesis feasibility, price, and turnaround time across vendors". OUP Synthetic Biology, ysa011 (2020).
- **(Publication)** 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. "Multiomics Data Collection, Visualization, and Utilization for Guiding Metabolic Engineering". Frontiers in Bioengineering and Biotechnology 9, 45 (2021).

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

- **(Publication)** Chris Lawson, Jose Manuel Martí, Tijana Radivojevic, Sai Vamshi R. Jonnalagadda, Reinhard Gentz, Nathan J. Hillson, Sean Peisert, Joonhoon Kim, Blake A. Simmons, Christopher J. Petzold, Steven W. Singer, Aindrila Mukhopadhyay, Deepti Tanjore, Josh Dunn, and Hector Garcia Martin. "Machine learning for metabolic engineering: A review" *Metabolic Engineering* (2020)
- **(Publication)** Riley LA and Guss AM\*. "Approaches to genetic tool development for rapid domestication of non-model microorganisms". *Biotechnol* 14:30 (2021)

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

- **(Presentation)** Nathan J. Hillson “U.S. DOE Agile BioFoundry: Organization and Capabilities”, Invited Talk, ABF Industry Day 2019, Emeryville, CA October 4, 2019
- **(Presentation)** Garcia Martin, H. “Machine Learning, Synthetic Biology and Automation: Engineering Life for the Benefit of Society”. NERSC data seminar, Berkeley CA, November 1st, 2019
- **(Presentation)** Benavides PT, Davis R, Klein, B. “Economic and environmental assessment of biological conversions of Agile BioFoundry (ABF) bio-derived chemicals”. 2nd Bioenergy Sustainability Conference 2020, Virtual meeting, October 15th, 2020
- **(Poster)** Tijana Radivojevic, Zak Costello, Kenneth Workman, Soren Petersen, Jie Zhang, Andres Ramirez, Andres Perez, Eduardo Abeliuk, Benjamin Sanchez, Yu Chen, Mike Fero, Jens Nielsen, Jay Keasling, Michael K. Jensen, Hector Garcia Martin, “ART: A machine learning Automated Recommendation Tool for synthetic biology”, BRC Workshop on AI and ML for Biosystems Design, Washington, DC, February 27, 2020

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

- **(Presentation)** Garcia Martin, H. “ART: a machine learning Automated Recommendation Tool for guiding synthetic biology”. AI4Synbio Symposium, Arlington VA, November 8th, 2019.
- **(Presentation)** Garcia Martin, H. “Opportunities in the intersection of:Artificial Intelligence & Synthetic Biology & Automation”. Army Science Planning and Strategy Meeting, Burlington MA, November 13th, 2019.
- **(Presentation)** “ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- **(Presentation)** Garcia Martin, H. “Opportunities in the intersection of machine learning, synthetic biology, and automation”. ABLC 2020, Virtual meeting, July 10th, 2020.
- **(Presentation)** Garcia Martin, H. “Leveraging machine learning and automation to make synthetic biology predictable”. SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- **(Panel)** Garcia Martin, H. “Sustainable Living Systems”. LA Life Summit, Virtual meeting, October 15th, 2020.



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

- **(Presentation)** T. Radivojevic, “Automatic Recommendation Tool”, Invited Talk, Agile BioFoundry Learn Summit 2020, Argonne/Lemont, IL, March 4, 2020
- **(Presentation)** T. Radivojevic, “Using ART to improve tryptophan production”, Invited Talk, Agile BioFoundry Learn Summit 2020, Argonne/Lemont, IL, March 4, 2020
- **(Presentation)** T. Radivojevic, “Guiding synthetic biology via machine learning”, Invited Talk, Biofuels & Bioproducts Division Meeting, JBEI, Emeryville, CA, March 11, 2020
- **(Presentation)** T. Radivojevic, “ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- **(Presentation)** Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020

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

- **(Presentation)** 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
- **(Presentation)** Garcia Martin, H. "Opportunities in the intersection of machine learning, synthetic biology, and automation". ABLC 2020, Virtual meeting, July 10th, 2020.
- **(Presentation)** Garcia Martin, H. "Leveraging machine learning and automation to make synthetic biology predictable". SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- **(Presentation)** Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020
- **(Presentation)** 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

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

- **License partners**

- University of Georgia
- Kiverdi, Inc.
- LanzaTech, Inc.
- Visolis, Inc.
- Danimer Scientific

- **Patent Applications**

- 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

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

- Patent Applications (cont.)

- A Generative Model for Protein Sequences for the Purpose of Protein Design or Phenotypic Inference
- Predicting Metabolic Pathway Dynamics from Time Series Multiomics Data Using Machine Learning Techniques
- Use of Statistical Learn Approaches to Predict Next Generation Sequencing Subsequence Depth of Coverage
- Mutant transporters for bacterial uptake of terephthalic acid
- Method and strain for sugar conversion
- Engineered Microorganisms for the Production of Intermediates and Final Products (1<sup>st</sup>)
- Engineered Microorganisms for the Production of Intermediates and Final Products (2<sup>nd</sup>)
- Production of organic acids from *Aspergillus pseudo-terreus* cadA deletion strain (1<sup>st</sup>)
- Production of organic acids from *Aspergillus pseudo-terreus* cadA deletion strain (2<sup>nd</sup>)

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

- **Patent Applications (cont.)**

- Genetically engineering an industrial filamentous fungus *Aspergillus niger* for 3-hydroxypropionic acid production
- A specific exporter responsible for aconitic acid high production in *Aspergillus pseudo terreus*

- **Records of Invention**

- Bioproduction of limonene from syngas
- Mutant transporters for bacterial update of terephthalic acid
- Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids
- A genetic circuit to reduce cell-to-cell production heterogeneity
- High yield conversion of D-xylose to D-arabitol in *R. toruloides*
- Manipulation of tRNA thiolation gene *ncs2* for enhanced production of fatty-acyl-CoA derived chemicals in *R. toruloides*

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

## • 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