



U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2017 Project Peer Review

Agile BioFoundry
(WBS 2.5.3.104-12)

March 6, 2017, 3:15-4:15 PM
Conversion Technologies

Nathan J. Hillson
Lawrence Berkeley National Lab



Goal Statement

- **Goal:** Enable a biorefinery to achieve a positive return on investment through a 50% reduction in time-to-scale up compared to the average of ~10 years by establishing a distributed Agile BioFoundry that will productionize synthetic biology.
- **Outcomes:** 10X improvement in Design-Build-Test-Learn cycle efficiency, new host organisms, new IP and manufacturing technologies effectively translated to U.S. industry ensuring market transformation.
- **Relevance:** Public infrastructure investment that increases U.S. industrial competitiveness and enables new opportunities for private sector growth and jobs.



Quad Chart Overview

Timeline

- Start: October 1, 2016
- End: September 30, 2019
- 10% complete

Budget

| | FY 16 Costs (Pilot/ Visioning Projects) | FY17 | Total Planned Funding (FY17- Project End Date) |
|---------------|---|-----------------------|---|
| DOE Funded | \$3M (Pilot) \$500k (Visioning) | \$20M \$10M | \$40M \$30M |

Barriers

- Technical:
 - *Ct-H*. Efficient Catalytic Upgrading of Sugars/Aromatics, Gaseous and Bio-Oil Intermediates to Fuels and Chemicals
 - *Im-D*. Cost of Production
 - *At-C*. Data Availability across the Supply Chain
- MYPP targets addressed:
 - Provide enabling capabilities in synthetic biology for industrially relevant, optimized chassis organisms and Design-Build-Test-Learn cycles for fuel & chemical production which reduces time-to-scale up by at least 50% compared to the average of ~10 years
 - Develop a suite of conversion technologies that produce both fuels and high value chemicals to enable a biorefinery to achieve a positive return on investment

Partners

- LBNL (25.1%); NREL (19.4%); PNNL (16.6%); SNL (15.8%); LANL (6.4%); ORNL (5.4%); ANL (5.2%); INL (3.5%); Ames (2.6%)

1 - Project Overview

The Opportunity

The U.S. has ~**billion tons** of renewable **biomass** available annually that is a strategic national resource for the bioeconomy



U.S. **bioeconomy** is estimated at ~**\$250B/yr** and expected to grow significantly over the next decade

Mobilizing and valorizing this resource through **biomanufacturing** could **rapidly expand** the U.S. **bioeconomy**

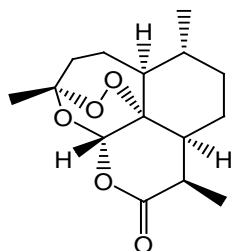
Biomanufacturing remains **nascent** in terms of robustness, scale and standardization

The Challenge: Cost and Time to Market

| Molecule | Company | Cost | Time |
|-----------------------|-------------------------|--------|----------|
| 1,3-Propanediol (PDO) | DuPont - Tate & Lyle | \$120M | 15 years |



Artemisinin



| | | |
|-----------------------------------|-------|----------|
| UC Berkeley, Amyris, Sanofi | \$50M | 10 years |
|-----------------------------------|-------|----------|

Possible savings of *billions* of dollars by reducing development time of products

Public Infrastructure Investment Enables Private Industry

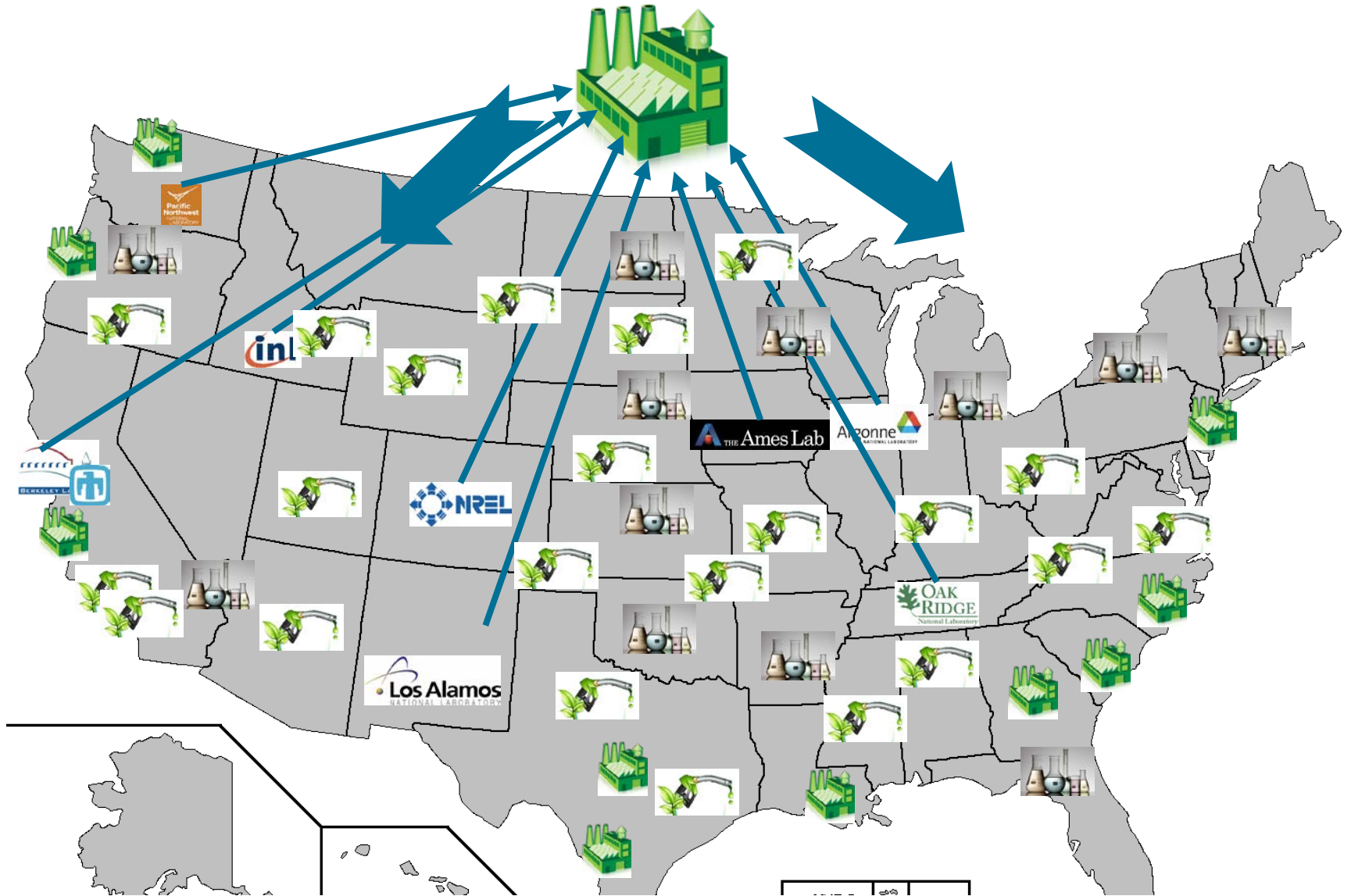
Public investment in biomanufacturing infrastructure

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



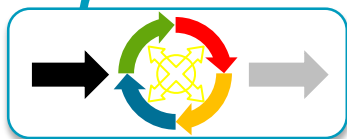
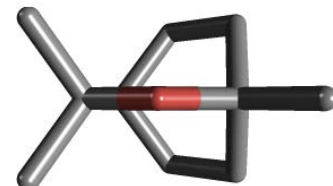
Adapted from Lyft

A Distributed Agile BioFoundry

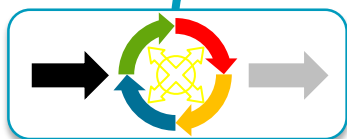


Agile BioFoundry Will Reduce Time-to-Scale up

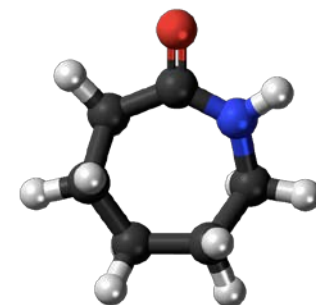
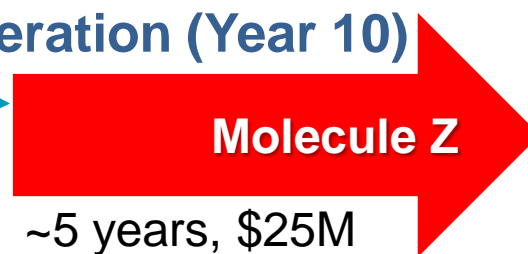
1st Generation (Years 1-3)



2nd Generation (Year 5)



3rd Generation (Year 10)



Time and cost for commercialization

2 – Approach (Management)

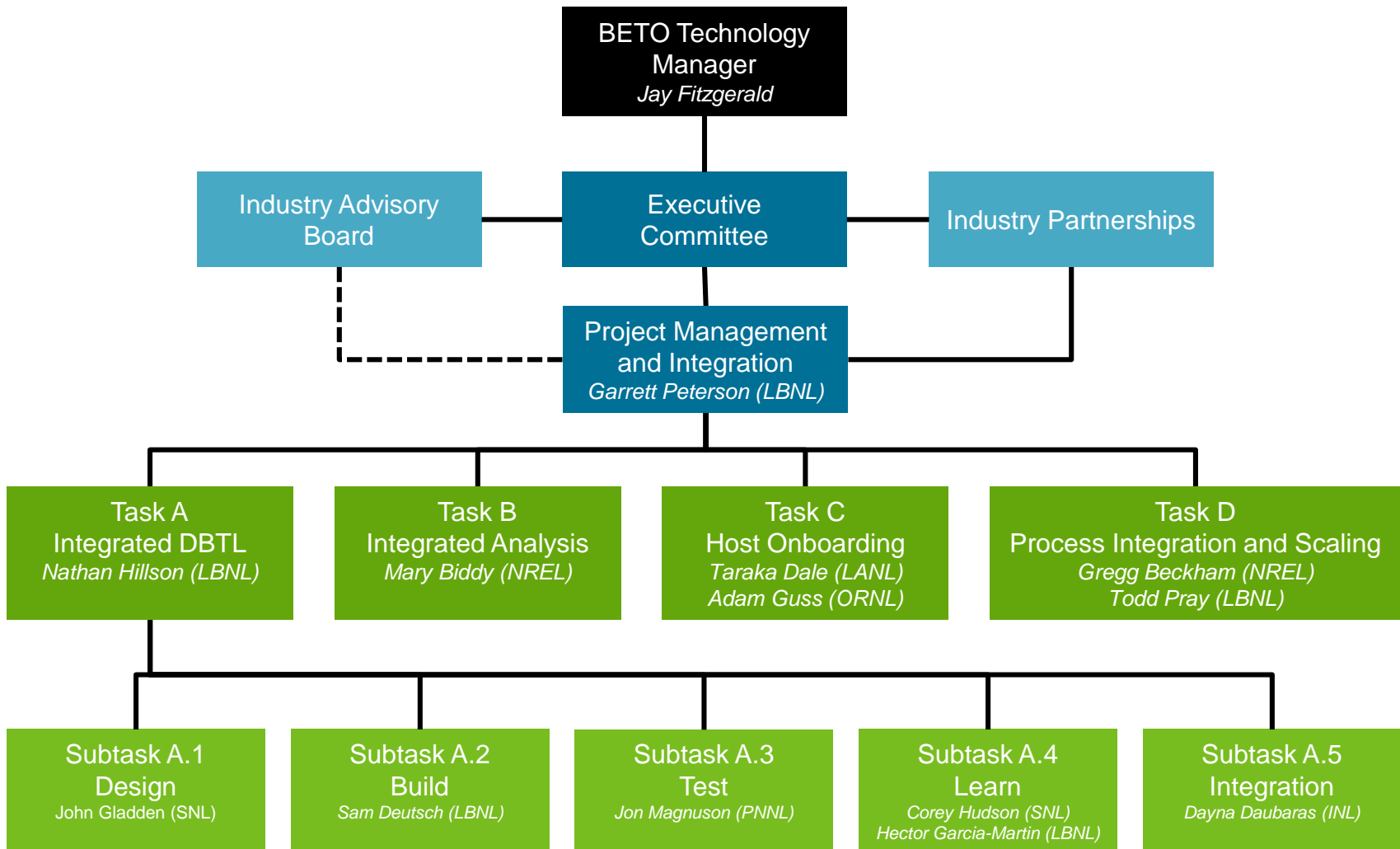
Agile BioFoundry Project Structure

- Funding: planned at \$40M over 3 years
 - Year 1: \$20M planned, **\$10M funded**
 - Years 2 & 3: \$10M/year
 - Potential funding to work with industry
- Nine National Lab consortium
- Industrial Advisory Board actively engaged
- Six tasks for overall project
 - 4 research tasks, 2 management tasks
- One milestone per quarter
 - Annual SMART milestone
 - 18 month Go/No-Go decision point
- Quarterly progress reporting to BETO

Six Tasks for Overall Project

- **Task A: Integrated Design-Build-Test-Learn** (*Nathan Hillson* - lead)
 - Integrate design-build-test-learn cycle with process automation and sample tracking.
- **Task B: Integrated Analysis** (*Mary Bidy* - lead)
 - Evaluate proposed molecules; develop, update, and improve existing process designs and LCA.
- **Task C: Host Onboarding** (*Taraka Dale/Adam Guss* – co-leads)
 - Evaluate possible host organisms to determine which on-boarding criteria are not yet met, and fill these gaps through tool development and data collection.
- **Task D: Process Integration and Scale-up** (*Gregg Beckham/Todd Pray* – co-leads)
 - Standardize, produce, ship, and store hydrolysates; compare clean sugar processes with hydrolysates; test and scale fermentation to improve titer, rate, and yield; provide integrated, bench-scale data for TEA and LCA; scale fermentation to produce data for Learn.
- **Task E: Industry Engagement** (*Babs Marrone* - lead)
 - Identify barriers to industry adoption of synthetic biology technologies, expand number and diversity of industry partnerships, and establish a set of metrics for determining impact of project technologies on industry.
- **Task F: Management** (*Blake Simmons* - lead)
 - Manage project management, develop internal and external communications, provide deliverables to BETO, and make some capital equipment purchases.

Project Management – Org Chart



Project Management – Roles and Responsibilities

- **Executive Committee**

- Composition: representative from each lab, PI, Program Manager, BETO Technology Manager, industry/management task leads, others as needed
- Strategic direction and oversight
- Support collaboration between institutions
- ABF policy guidance
- Conflict resolution and performance management

- **Project Management and Integration Team**

- Composition: Program Manager, task (co-)leads, others as needed
- Progress tracking
- Target/Host selection
- Technical challenge identification/resolution

- **Agile BioFoundry Program Manager**

- Garrett Peterson – full time effort
- Manage overall project
- Collaboration and progress tracking tools
- Ensure adequate communication between labs for integration of whole consortium
- Primary communications and operations point-of-contact
- BETO reporting

The National Lab Network



Brent Shanks,
Igor Slowing,
Marit Nilson-Hamilton,
Cynthia Jenks



Argonne
NATIONAL LABORATORY
Phil Laible,
Jennifer Dunn,
Felix Odom,
Cristina Negri



Dayna Daubaras,
Dave Thompson,
Erin Searcy



Taraka Dale,
Babs Marrone,
Shawn Starkenberg



Nathan Hillson,
Blake Simmons,
Garrett Peterson,
Katy Christiansen



Gregg Beckham,
Mary Bidy,
Adam Bratis,
Eric Payne



Adam Guss,
Tony Palumbo,
Brian Davison



Jon Magnuson,
Mark Butcher,
Malin Young,
Corinne Drennan



Sandia
National
Laboratories
John Gladden,
Anup Singh,
Corey Hudson,
Ryan Davis

Jay Fitzgerald, Ian Rowe, Beau Hoffman,
Kevin Craig, Jonathan Male



**Energy Efficiency &
Renewable Energy**

BIOENERGY TECHNOLOGIES OFFICE

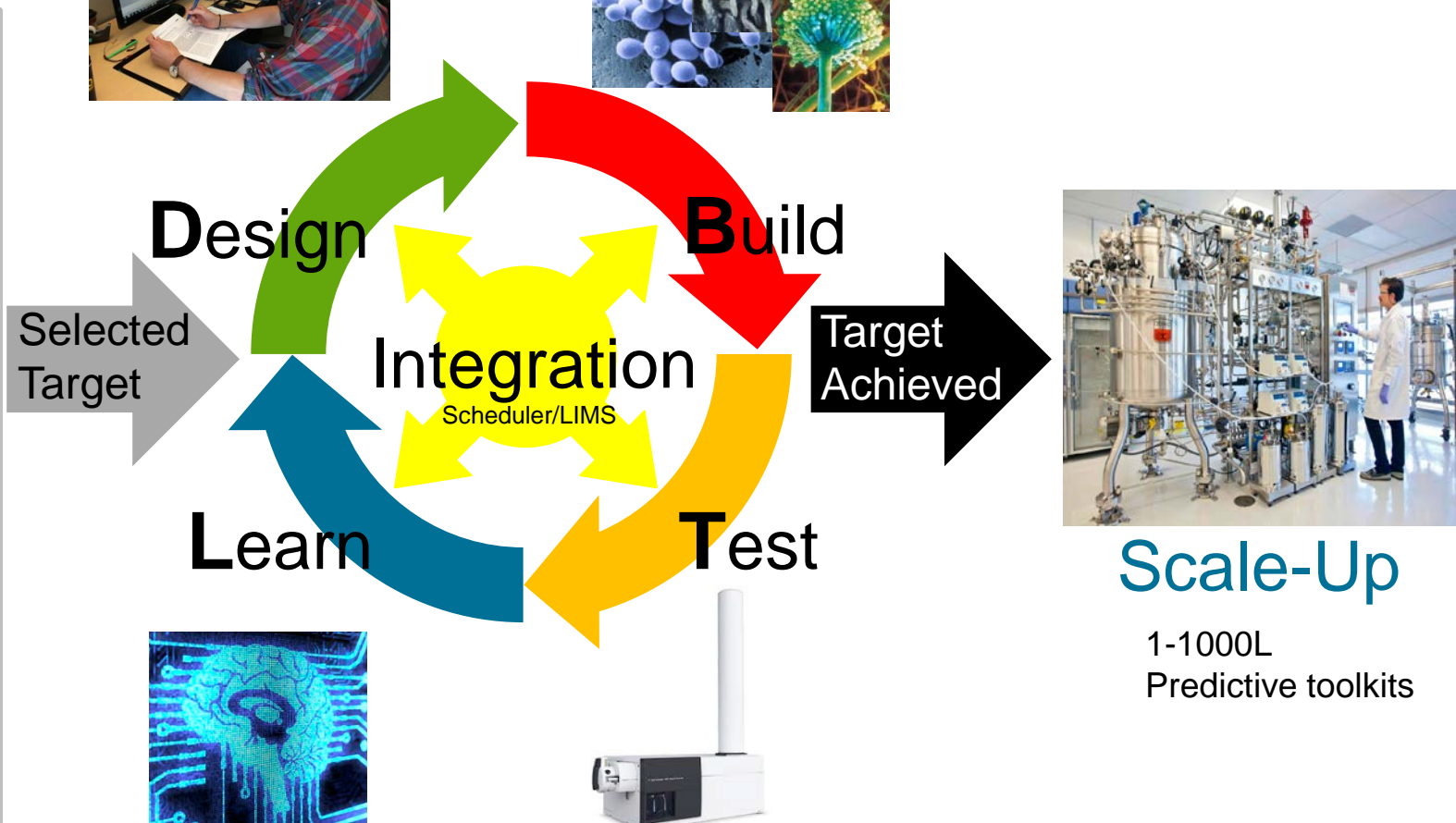
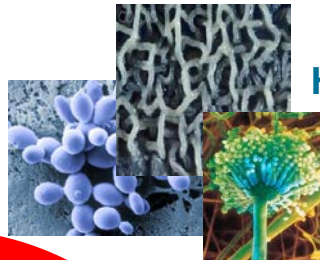
2 – Approach (Technical)

The Agile BioFoundry Approach

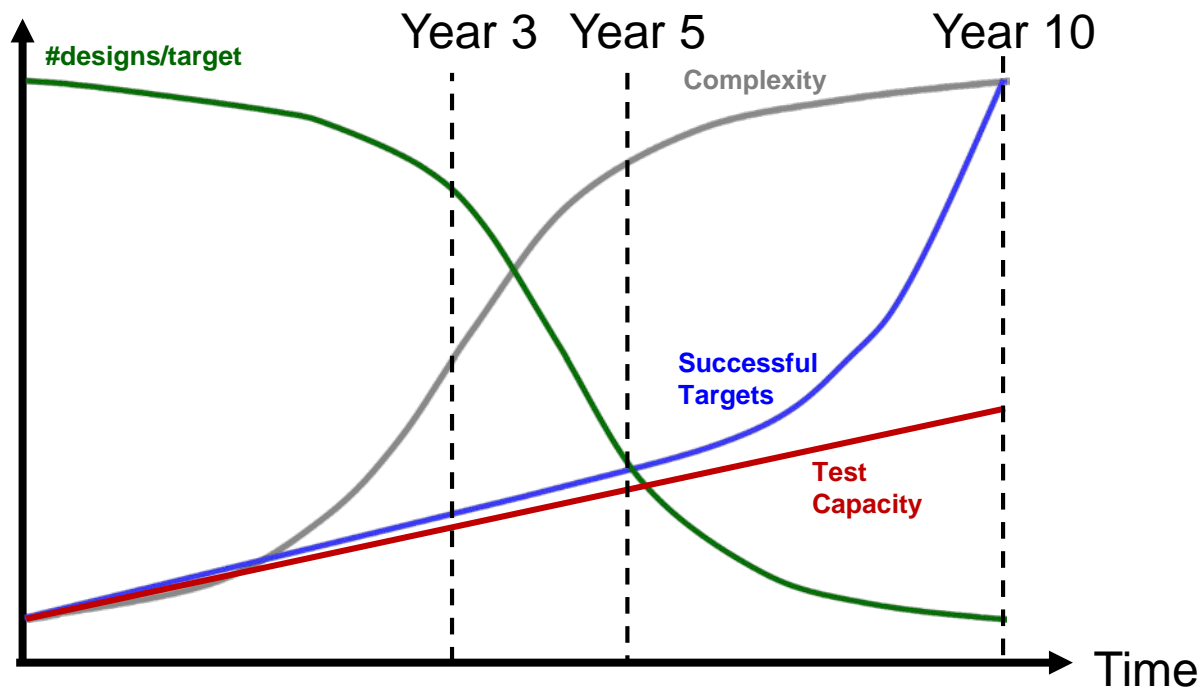
TEA/LCA



Host On-boarding



Projected Agile BioFoundry Evolution



| | FY17 | FY18 | FY19 | 5 years |
|--|----------|----------|----------|------------|
| Hosts in operation | 5 | 7 | 10 | > 20 |
| Concurrent target/host combinations | 5 | 15 | 30 | > 100 |
| DBTL cycle time | 9 months | 8 months | 6 months | < 3 months |
| Strain samples analyzed per year | 35,000 | 50,000 | 75,000 | > 100,000 |

Technical Risks and Mitigation Plans

| Risk | Classification | Description | Mitigation Plan | Probability | Risk Severity |
|---|----------------|--|---|-------------|---------------|
| Initial designs have low performance | scope | Initial biosynthetic pathway designs do not function or have very low productivity | Generate several initial variant pathway designs for each target. Use Test and Learn to identify bottlenecks and redesign | M | L |
| Small-scale cultivations are not predictive | scope | Productivity of bench-scale cultivations do not predict performance in bioreactors | Use small scale results to prioritize screening in bioreactors. Explore predictability of mid-scale cultivations | M | L |
| Designs do not work in selected host | scope | Target pathways do not function in selected host | Use another host. Use Test to understand lack of function to inform future designs | M | L |

3 – Technical Accomplishments/ Progress/Results

How we got here

- Initial internal investments at National Labs to build capabilities
- BETO funded two foundational projects in FY16:
 1. Agile BioFoundry Pilot project
 - Besides piloting the R&D effort, the team learned valuable lessons about operating cohesively across labs
 2. Agile BioFoundry Visioning project
 - Developed a vision for the Agile BioFoundry National Lab consortium with industry input and feedback
 - Convened internal and external stakeholder engagement meetings
 - Developed a Vision Document that outlines the activities and goals for the Agile BioFoundry
- Expansion of the consortium during FY17 planning
 - Working meeting in August 2016 to plan

Target/host selection process FY17 Q1

Objective – Identify 15 target molecules and their production hosts for FY17-19

Strategy - Establish and execute a target/host selection process that is:

- Transparent
- Documented
- Democratic (across staffing levels and participating national labs)
- Technical (assessments such as Technoeconomic/Market Analysis)
- Responsive (to stakeholders such as DOE BETO/Industry)

Target/host selection implementation

Nomination



Staff across labs nominate target/host pairs and provide a minimal set of requisite information for each

49 target/host pairs

Socialization



Each lab openly/collaboratively allocates 100 points across nominees. At least 50 points allocated to other labs' nominees. Top 20 scoring target/host pairs move forward.

22 move forward

Technical Assessment



Each target/host pair scored from 1 to 10 in 12 technical categories: TEA/Market, LCA, Strategic, Scientific Novelty, DOE Relevance, Designability, Buildability, Hostability, Testability, Scalability, Industry Relevance, BioSafety

264+ scores

DOE BETO Feedback



DOE BETO evaluates technical assessment, suggests preferences for (or against) particular target/hosts pairs and which technical categories should be weighed more heavily

TEA/Market of particular interest

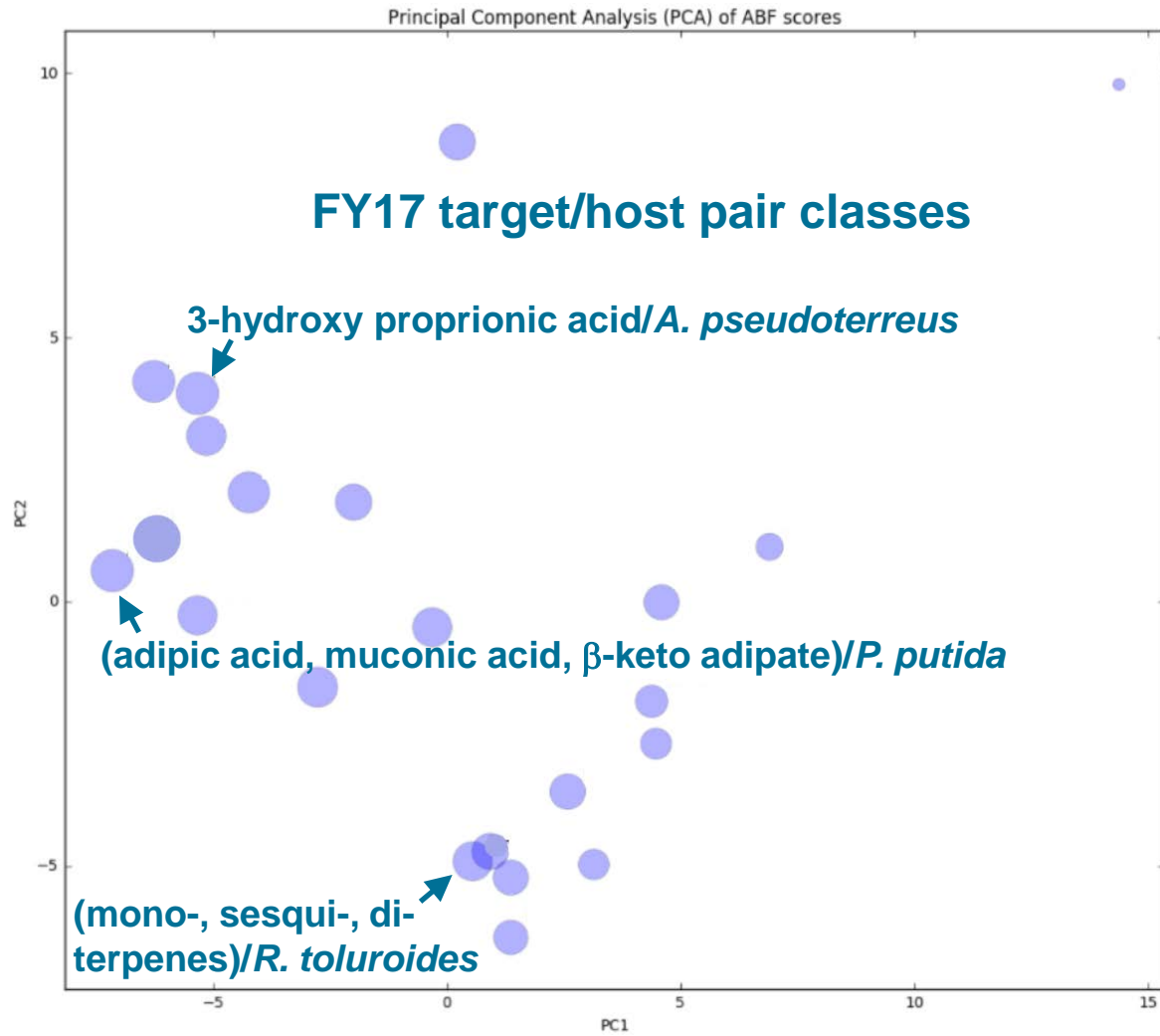
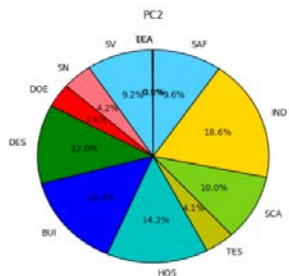
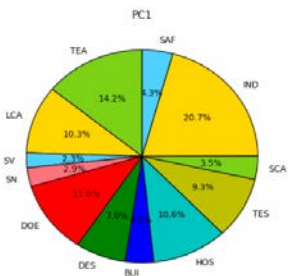
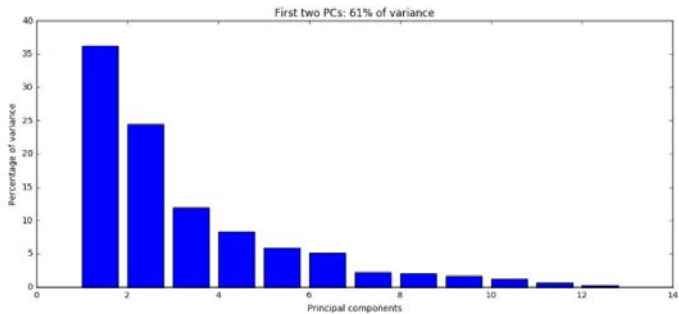
Portfolio Building



Following technical assessment, identify up to 15 target/host pairs for FY17-19. Build a balanced portfolio of 3 classes of target/host pairs to pursue in FY17 in context of operational resources

15 FY17-19 pairs
3 FY17 pair classes

Analysis of Technical Assessment Data



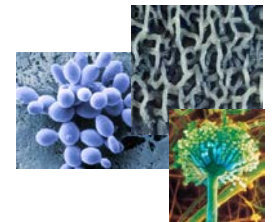
4 – Relevance

Enabling biorefineries to achieve positive returns on investment

- Directly supports BETO's mission and goals:
 - “*Develop and demonstrate transformative and revolutionary bioenergy technologies for a sustainable nation*”
 - “*Encourage the creation of a new domestic bioenergy and bioproduct industry*”
- Addresses BETO's Conversion FY20 and FY21 R&D Milestones:
 - “*Provide **enabling capabilities in synthetic biology** for **industrially relevant, optimized chassis organisms** and **Design-Build-Test-Learn cycles** for fuel & chemical production which **reduces time-to-scale up by at least 50% compared to the average of ~10 years**”*
 - “*Develop a suite of conversion technologies that produce both fuels and high value chemicals to enable a **biorefinery to achieve a positive return on investment***”

Relevant Outcomes

- 50% reduction in time-to-scale up compared to the average of ~10 years
- 10X improvement in Design-Build-Test-Learn cycle efficiency
- Public infrastructure investment that increases U.S. industrial competitiveness and enables new opportunities for private sector growth and jobs
- New IP and manufacturing technologies effectively translated to U.S. industry ensuring market transformation
- New industrially relevant, optimized chassis organisms for fuel and chemical production



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 molecules/hosts to demonstrate broad utility

5 – Future Work

Project Milestones

Quarterly milestones and progress reporting, along with annual SMART milestones and Go/No-Go decisions

- **FY17 Annual SMART milestone**

- Demonstrate the Agile BioFoundry process by successfully completing one or more Design, Build, Test, Learn cycles for 5 molecules in their designated onboarded hosts, hitting baseline titers of 100 mg/L in mock or DMR-EH hydrolysate for at least 2 molecules.

- **Go/No-Go Decision, Q2 FY18**

- Demonstrate process integration and scaling in 2 L bioreactors in DMR-EH hydrolysate using a target molecule introduced into the BioFoundry in FY17 with a target titer of at least 1 g/L.

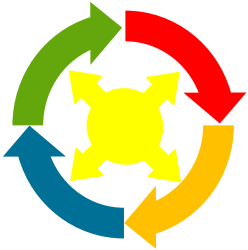
- **FY18 Annual SMART milestone**

- From a set of 10 target molecules, demonstrate successful production of 40% with titers for FY18 target molecules of at least 100 mg/L in mock or DMR-EH hydrolysate, and titers for FY17 target molecules of at least 500 mg/L in DMR-EH hydrolysate.

Modified FY17 Project Plan

- Budget uncertainties posed by 7 month Continuing Resolution (CR)
 - BETO spending to the lower of House and Senate marks for appropriations
- ABF planning for annualized spending of ~\$10M until CR is resolved in April
- Focus on delivering functional Integrated DBTL cycle for 3 target/host combinations
 - Reduce number of target/host combinations from 5 to 3
 - Reduce long-term tasks such as Host Onboarding
 - Delay capital expenditures and new hires not yet in process
 - Reduce amount of planned carryover (e.g. normally ~20% of project costs) to 0%
 - Reduce material and travel expenditures
- Opportunities that would be (re-)enabled with additional funding
 - Larger number of target/host combinations
 - Better demonstrate broad utility of Agile BioFoundry platform
 - Get up to speed more quickly
 - Improved throughput and capacity
 - New analytical instruments and bioreactors
 - Logistics tracking software
 - Improved knowledge dissemination and peer networking
 - Travel to Agile BioFoundry annual meeting

Active Consortium Efforts



DBTL

- Finalize FY17 target/host Designs, submit for Build
- Prepare Test capabilities for selected targets/hosts
- Develop and refine Learn models



Rotary drum filter

Process Scale & Integration

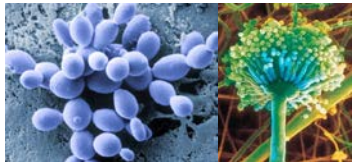
- Produce corn stover DMR-EH hydrolysate for fermentation
 - Process optimization
 - Hydrolysate characterization

Host Onboarding

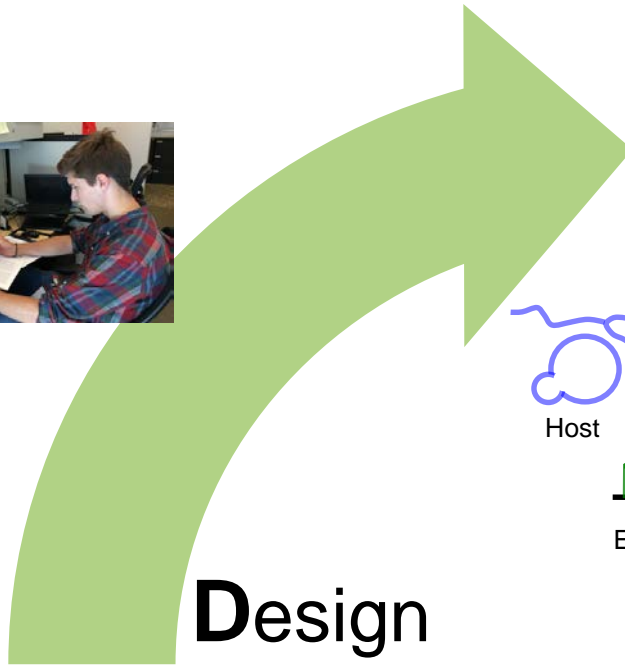
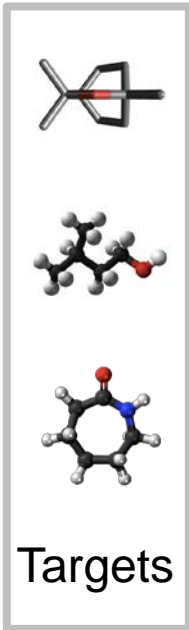
- Characterization of promising “new” Host organisms for future years

Industry Engagement

- Webinars and one-on-one interviews with Industrial Advisory Board to strengthen partnerships and develop metrics on industry impact

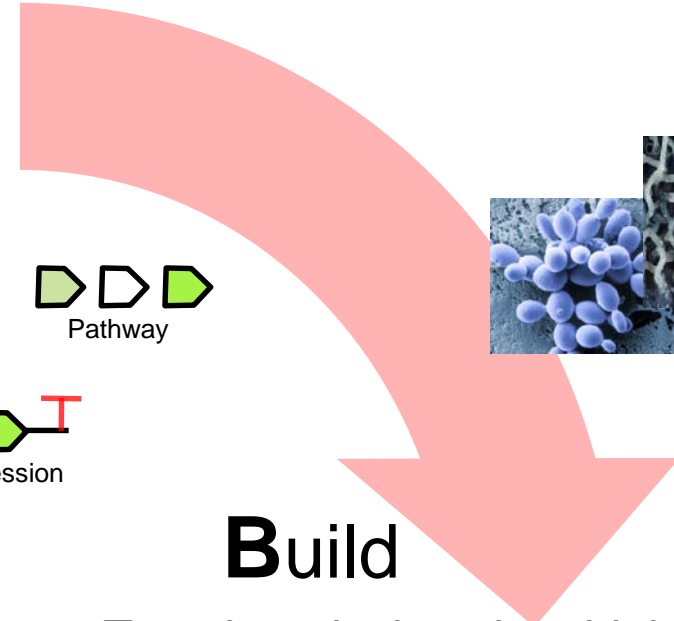
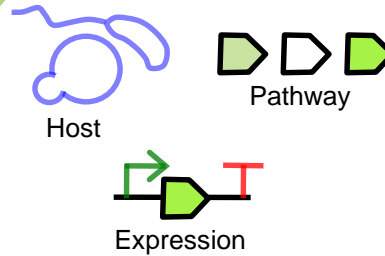


DBTL: Design & Build



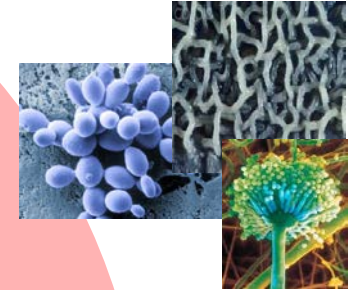
Design

- Utilize and optimize integrated design toolchain software architecture with push notifications
- Determine optimal DNA construction strategy for targets/hosts



Build

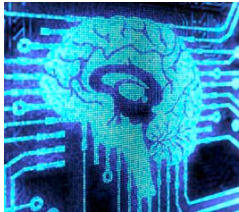
- Translate designs into biological reality, including DNA construction, transformation into hosts, and forward genetic screens
- Small-scale cultivation of engineered strains



DBTL: Test & Learn

Learn

- Statistical and mechanistic modeling
- Machine learning applied to multi-omic Test data to predict performance and improve Design process
- Mechanistic modeling for flux analysis and pathway kinetics



Test

- Culture newly built organisms and assess performance to identify specific improvements in subsequent DBTL cycles
- Transcriptomic, proteomic, and metabolomic analysis based on the needs of Design and Learn
- Provide data for refinement of TEA and LCA



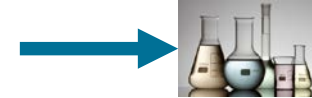
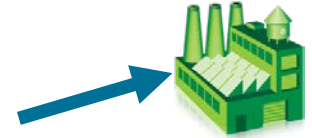
Growing the Agile BioFoundry

Nine
National
Labs
+
Industrial
Advisory
Board



| FY17 | FY18 | FY19 |
|--|---|--|
| 3 host/target combinations 9 month DBTL cycle Demonstrate Foundry platform success through execution of DBTL | +2 hosts +10 host/target combinations 8 month DBTL cycle Demonstrate scale-up and increased titers, rates, and yields. | +3 hosts +15 host/target combinations 6 month DBTL cycle Publicly available tools IP packages prepared Industrially relevant, optimized hosts |

Industry
Partnerships



Summary

- **Goal:** Enable a biorefinery to achieve a positive return on investment through a 50% reduction in time-to-scale up compared to the average of ~10 years by establishing a distributed Agile BioFoundry that will productionize synthetic biology.
- **Outcomes:** 10X improvement in Design-Build-Test-Learn cycle efficiency, new host organisms, new IP and manufacturing technologies effectively translated to U.S. industry ensuring market transformation.
- **Relevance:** Public infrastructure investment that increases U.S. industrial competitiveness and enables new opportunities for private sector growth and jobs.



Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

- (Presentation) Hillson, N.J. "Foundry vision and proof of concept". Invited talk, Agile Biomanufacturing Industry Listening Workshop, Berkeley, CA, March 15, 2016.
- (Presentation) Hillson, N.J. "FutureBio foundry: A biological foundry to support US Biomanufacturing". Invited talk, INDO-US Workshop On "Cell Factories", Mumbai, India, March 19, 2016.
- (Presentation) Hillson, N.J. "Introduction to the FutureBio Foundry". Invited talk, University of Edinburgh, Edinburgh, Scotland, UK, April 1, 2016.
- (Presentation) Hillson, N.J. "Introduction to the FutureBio Foundry". Invited talk, Center for BioSustainability, Copenhagen, Denmark, April 5, 2016.
- (Presentation) Nathan J. Hillson. "Advanced Biomanufacturing: The SynBio Foundry". Invited talk, Public Meeting of the Biomass Research and Development Technical Advisory Committee, Washington, DC, June 13, 2016.
- (Presentation) Hillson, N.J. "SynBio Foundry vision and proof of concept". Invited talk, Advanced Biomanufacturing Industry Listening Workshop, Washington, DC, June 14, 2016.
- (Presentation) Hillson, N.J. "Advancing Biomanufacturing: The Agile BioFoundry". Invited talk, SynBioBeta Activate!, Edinburgh, Scotland, July 7, 2016.
- (Presentation) Hillson, N.J. "Enabling the Bioeconomy: The Agile BioFoundry". Invited talk, JBEI Targets Council Workshop, Emeryville, CA, July 28, 2016.
- (Presentation) Hillson, N.J. "Enabling the Bioeconomy: The Agile BioFoundry". Invited talk, Mitsubishi Chemical Visit to JBEI, Emeryville, CA, August 12, 2016.
- (Presentation) Hillson, N.J. "Enabling the Bioeconomy: The Agile BioFoundry". Invited talk, Total Visit to JBEI, Emeryville, CA, September 28, 2016.
- (Presentation) Simmons, B., "Enabling the Bioeconomy: the Agile BioFoundry". Invited talk, Bioenergy 2016, Washington, DC. July 12-14, 2016.
- (Presentation) Simmons, B., "Advancing the Bioeconomy through Biomanufacturing". Invited talk, ABLCNext, San Francisco, CA, November 2-4, 2016.
- (Presentation) Hillson, N.J. "Enabling the Bioeconomy: The Agile BioFoundry". Invited talk, Synthetic Biology Institute/Agilent 11th Technical Exchange Workshop, Berkeley, CA, October 10, 2016.
- (Presentation) Hillson, N.J. "Data sovereignty implications of distributed physical/informatic biomanufacturing infrastructure". Invited talk, National Academies workshop – Safeguarding the Bioeconomy, San Francisco, CA, October 21, 2016.
- (Presentation) Fitzgerald, J. "DOE's Agile BioFoundry: An Applied Synthetic Biology Engine for Biomanufacturing". Invited talk, NSF-sponsored workshop on Bioprivileged Molecules, Arlington, VA, January 5, 2017.
- (Presentation) Hillson, N.J. "Overview of the Agile BioFoundry ". Invited talk, U.S. Department of Energy-Imperial College London-National University of Singapore Foundry Technical Meeting: Global Network for Foundry Development, National University of Singapore, Singapore, January 25, 2017.
- (Presentation) Simmons, B., "Building the Low Carbon Bioeconomy". Invited talk, TEDxTU 2017, New Orleans, LA, February 13, 2017.
- (Presentation) Hillson, N.J. "Strategy Update: Biomanufacturing". Invited talk, Berkeley Lab Biosciences Expert Advisory Committee, Berkeley, CA, Feb 23, 2017.

FY17 Milestones

- Q1
 - Identify up to 15 possible target bioproduct or beachhead precursor molecules and their production hosts for FY17-19
 - Standup a clearly defined management structure and establish work flow and task collaboration infrastructure between the partner labs
 - Complete data management plan, including handoffs between labs
- Q2
 - Select at least up to 5 initial target molecules and their production hosts and finalize their design
- Q3
 - Produce 150 L of concentrated DMR-EH hydrolysate with a full characterization, and distribute to all partners that require hydrolysate
 - Deliver report detailing the research and tool gaps required for onboarding at least six industrially relevant host organisms into the BioFoundry
 - Compile information from a cohort of at least 25 industry stakeholders
- **Annual SMART milestone**
 - Demonstrate the Agile BioFoundry process by successfully completing one or more Design, Build, Test, Learn cycles for 5 molecules in their designated onboarded hosts, hitting baseline titers of 100 mg/L in mock or DMR-EH hydrolysate for at least 2 molecules

FY18 Milestones

- Q1
 - Increase the DBTL throughput capacity two fold by initiating the DBTL cycle for an additional 5 molecules in FY18
 - Initiate host onboarding for any additional FY18 hosts
- Q2
 - **Go/No-Go Decision:** Demonstrate process integration and scaling in 2 L bioreactors in DMR-EH hydrolysate using a target molecule introduced into the BioFoundry in FY17 with a target titer of at least 1 g/L
- Q3
 - Compile suggested targets/pathways/hosts provided by industry collaborators. Report TEA and LCA target cases on 5 selected FY18 molecules to identify the primary cost drivers and R&D barriers
- **Annual SMART Milestone**
 - From a set of 10 target molecules, demonstrate successful production of 40% with titers for FY18 target molecules of at least 100 mg/L in mock or DMR-EH hydrolysate, and titers for FY17 target molecules of at least 500 mg/L in DMR-EH hydrolysate

FY19 Milestones

- Q1
 - Using input from industry collaborators, select up to 5 additional molecules for FY19, for a further >30% expansion in total DBTL throughput capacity to up to 15 molecules
 - Initiate host onboarding for any additional FY19 hosts
- Q2
 - Report TEA and LCA target cases on 5 selected FY19 molecules to identify the primary cost drivers and R&D barriers
 - Complete host onboarding for up to five FY19 production hosts
- Q3
 - Complete multiple rounds of the DBTL cycle, demonstrating successful production of at least 50% of the 15 molecules with target titers of 1 to 10 g/L
- **Annual SMART Milestone**
 - From a set of 15 target molecules, assess production in at least 10 L bioreactors of the top 3 target molecules produced in their on-boarded hosts, with target titers of at least 10 g/L, rate of 100 mg/L/h, and 40% theoretical yield in DMR-EH hydrolysate

Pilot Project Technical Accomplishments

Goal

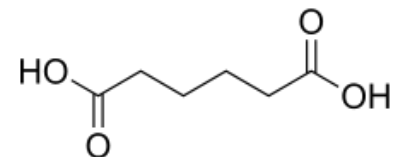
Standup, demonstrate, and optimize Agile BioFoundry core concept, develop workflows, and establish inter-laboratory logistics and infrastructure

Four Partner Labs

LBNL, NREL, PNNL, SNL

Target: adipic acid

- 2.5 billion tons produced annually
- No biochemical routes in industry
- Chose two hosts and two biosynthetic pathways



Accomplishments

- Demonstrated establishment of Agile BioFoundry core concept through distributed inter-laboratory Design-Build-Test-Learn cycle to produce adipic acid in two host organisms
- Documented and disseminated Lessons Learned for improvement of expanded Agile BioFoundry

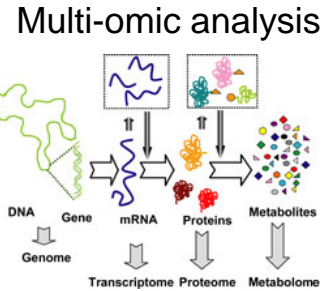
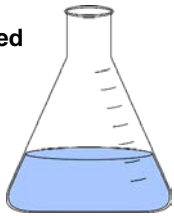
Pilot Project: Establishing DBTL Workflows

Design/Build

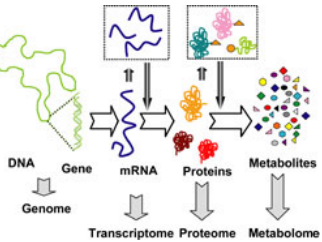
Test

Learn

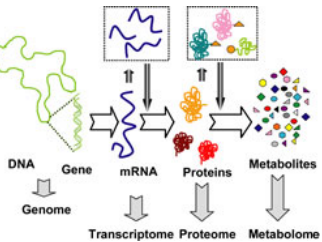
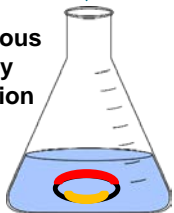
Unmodified parent strain



Host genome modification

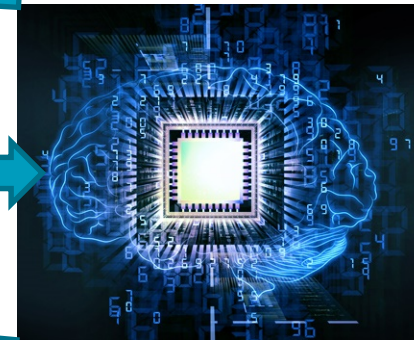


Heterologous pathway introduction



Transcriptomics

- Identify transcriptional bottlenecks
- Global expression/host response
- Promoter mining



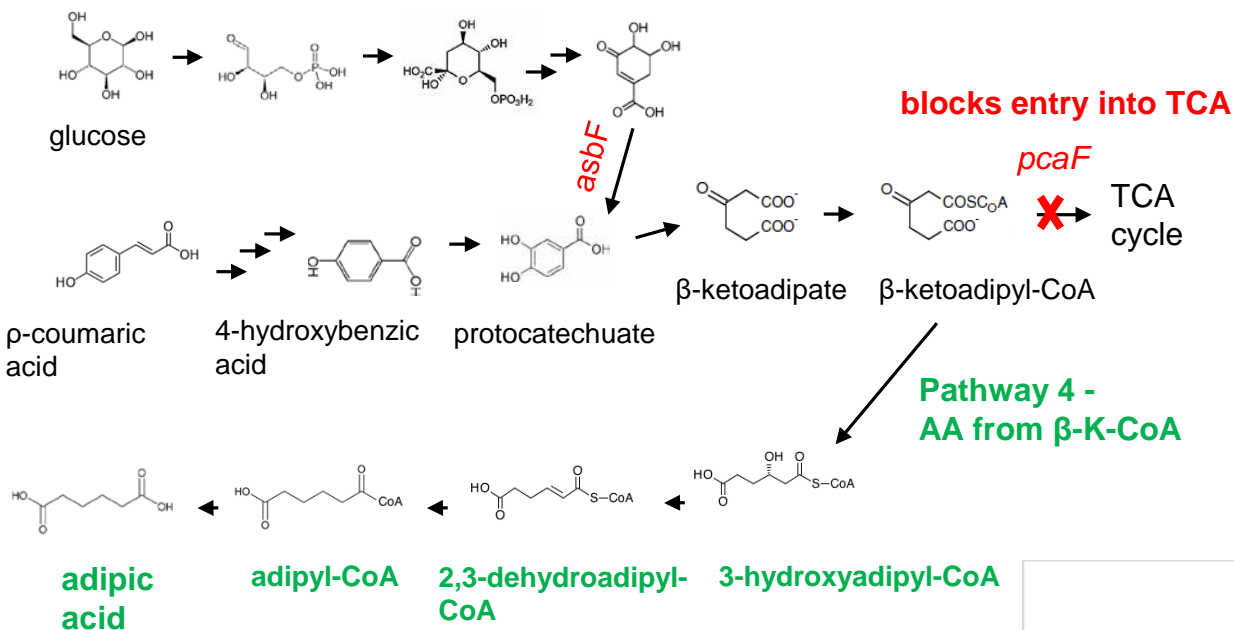
Proteomics

- Identify translation bottlenecks
- Global translation/host response

Metabolomics

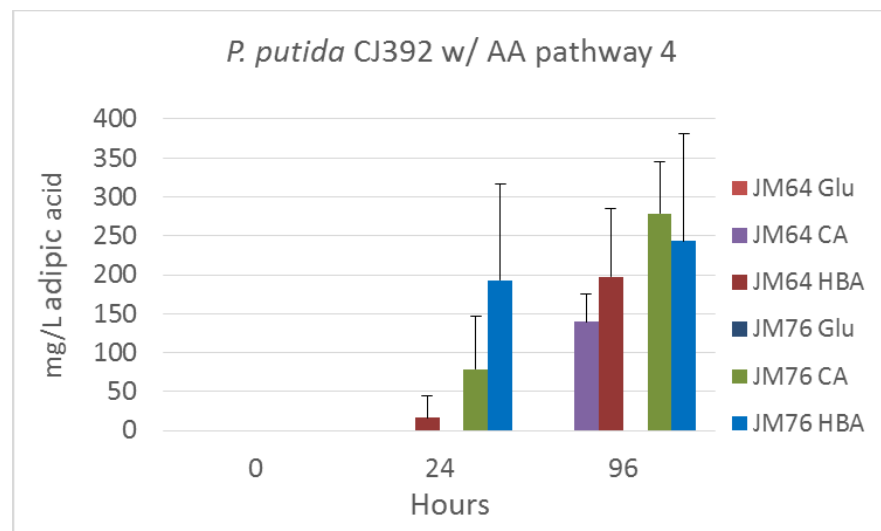
- Identify metabolic bottlenecks (flux analysis)
- Global metabolite profiles/host response

P. putida: Incorporating Adipic Acid Pathways



- Glu = glucose
- HBA = 4-hydroxybenzoic acid
- CA – p-coumaric acid

- Created three pathway variants that draw from β -ketoacidipyl-CoA and one from β -ketoacidipate.
- Pathway #4 produced ~250mg/L from aromatic substrates
- Need to optimize AA detection method
- Working on optimization to produce AA from sugars



Why a Distributed Effort?

- Unites unique and complementary capabilities towards a common goal
 - Technoeconomic and life cycle analysis
 - Biological computer-aided design and manufacture platforms
 - Application-focused repositories of genes, proteins, pathways, and organisms
 - Characterization of genomes, transcriptomes, proteomes, and metabolomes
 - High-performance computing, statistical/mechanistic modeling
 - Equipment and expertise for integration and scale-up
- Leverages broad range of hosts and targets in the National Lab portfolio
- Equipment/expertise co-location considered to ensure functional workflows

