Agile BioFoundry

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

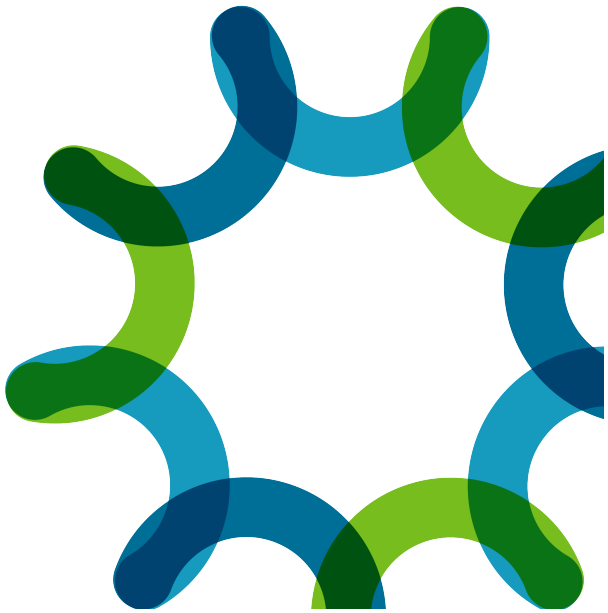
BIOENERGY TECHNOLOGIES OFFICE

U.S. DOE Agile BioFoundry: Organization and Capabilities

Nathan J. Hillson
njhillson@lbl.gov

Principal Investigator, DOE Agile BioFoundry

ABF Industry Day
Emeryville, CA
Octobober 4, 2019



Goal Statement

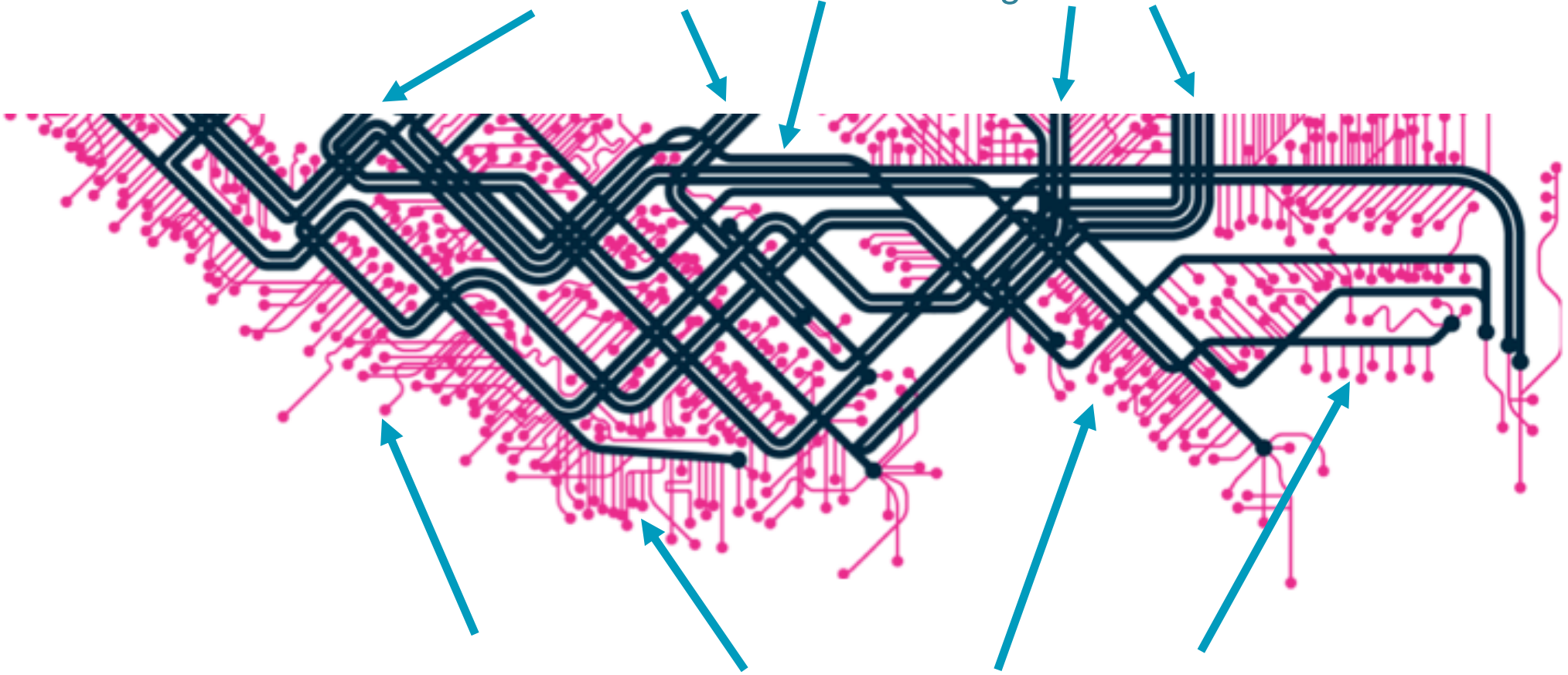
- **Goal**: Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry that will productionize synthetic biology.

- **Outcomes**: 10X improvement in Design-Build-Test-Learn cycle efficiency, new host organisms, new IP and manufacturing technologies effectively translated to U.S. industry ensuring market transformation.

- **Relevance**: Public infrastructure investment that increases U.S. industrial competitiveness and enables new opportunities for private sector growth and jobs.

Public Infrastructure Investment Enables Private Industry

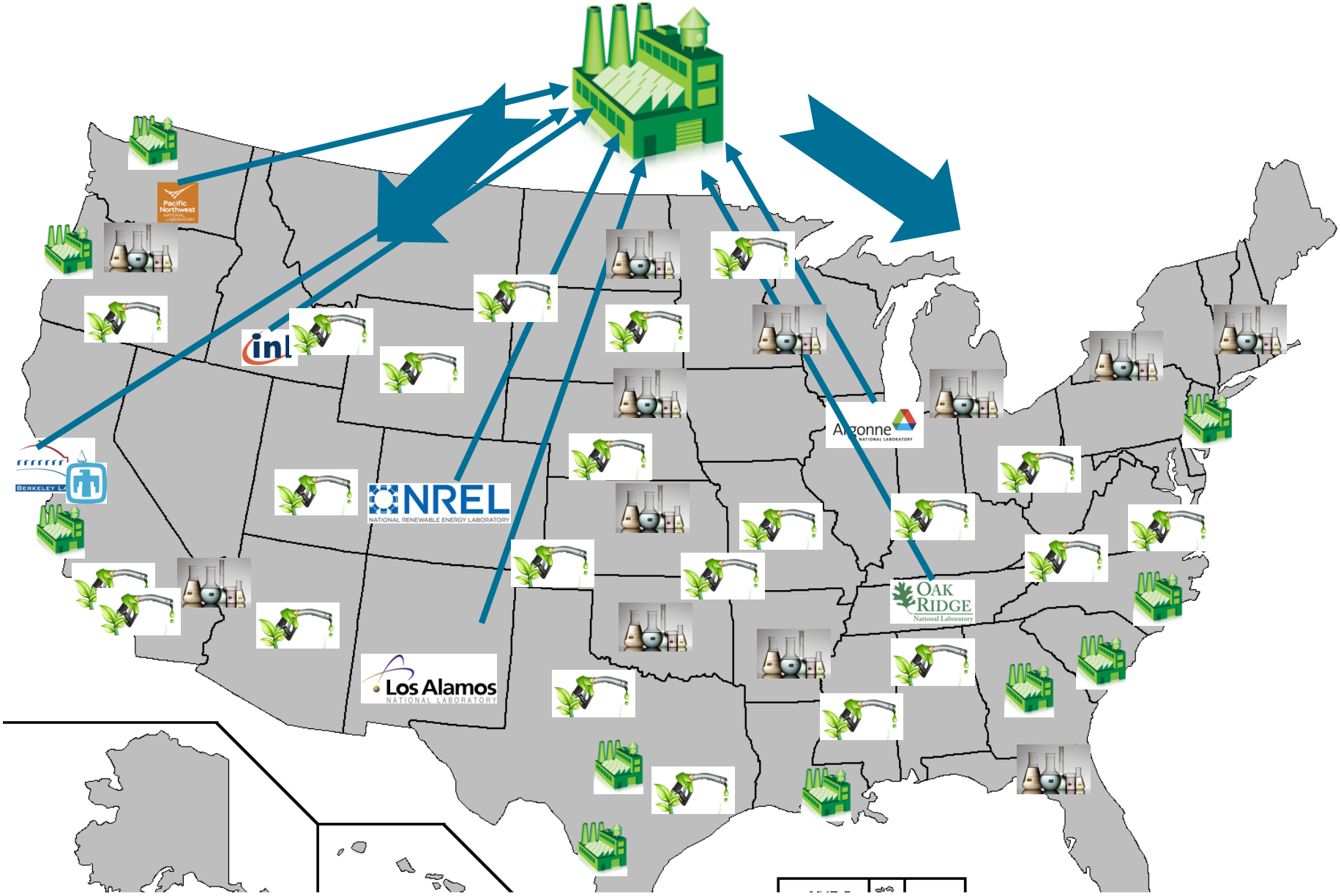
Public investment in biomanufacturing infrastructure



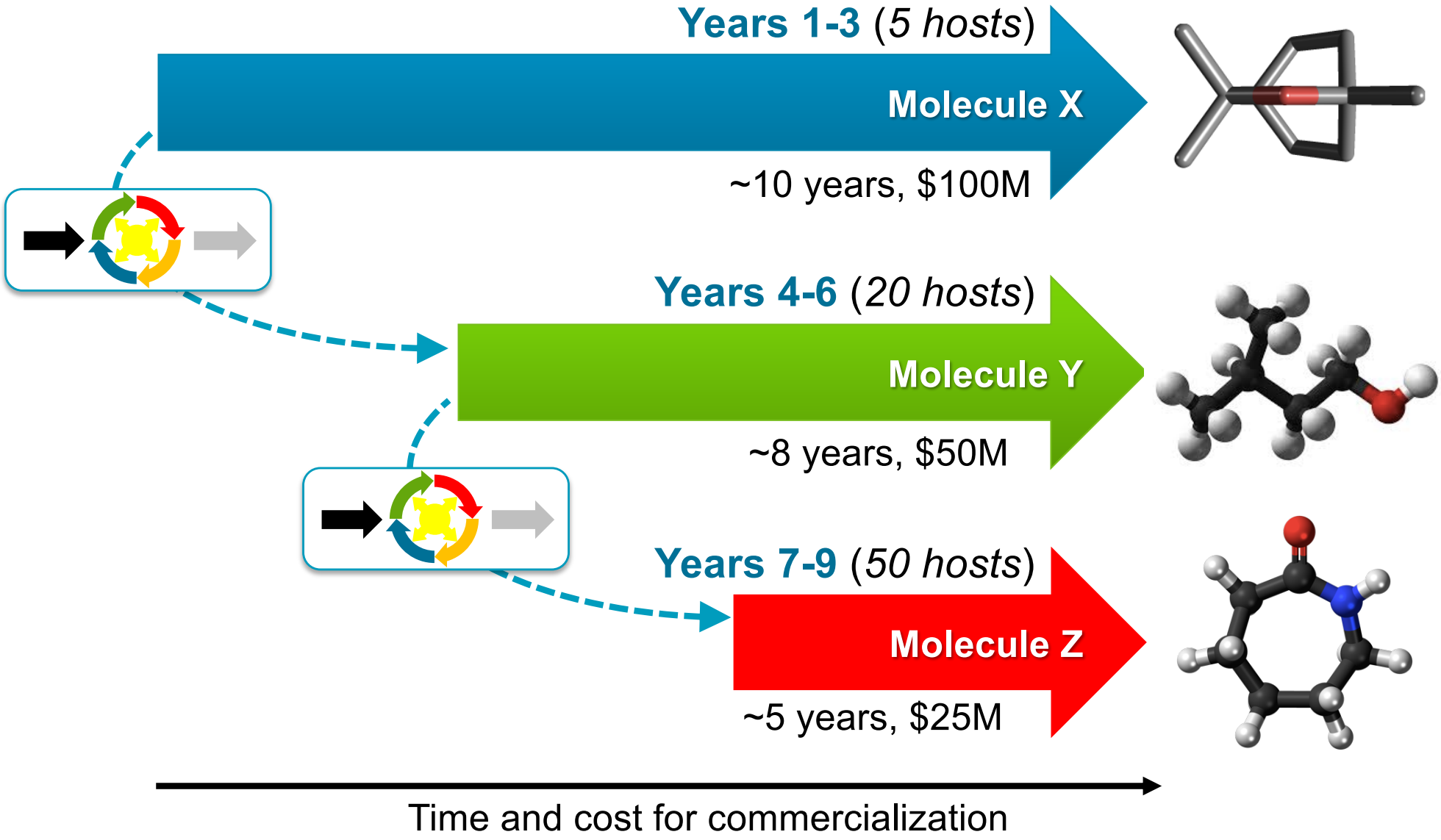
Private investment in product development, scaling, and tailoring to unique pathways and products

Adapted from Lyft

A Distributed Agile BioFoundry



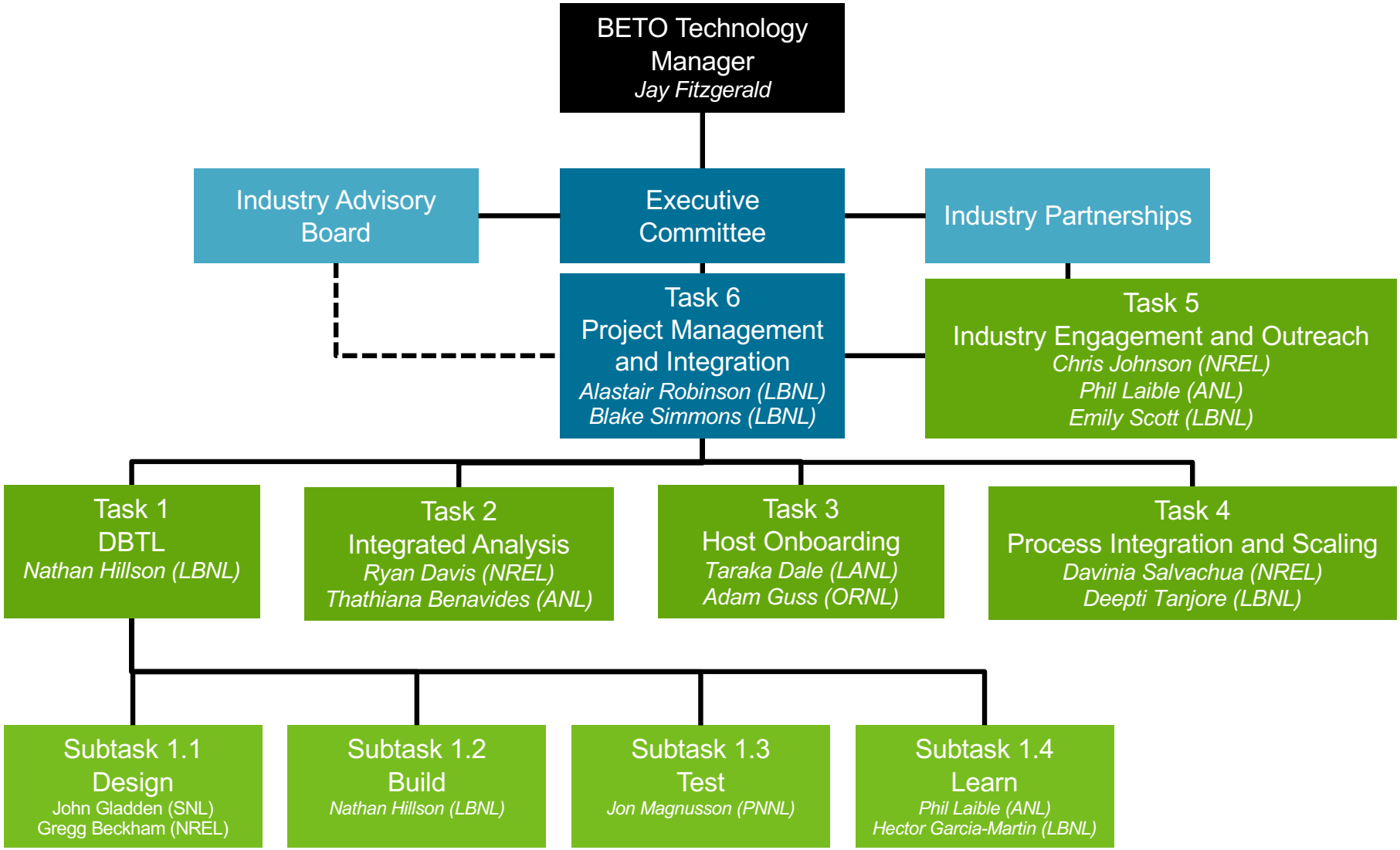
Agile BioFoundry Will Reduce Time-to-Scale up



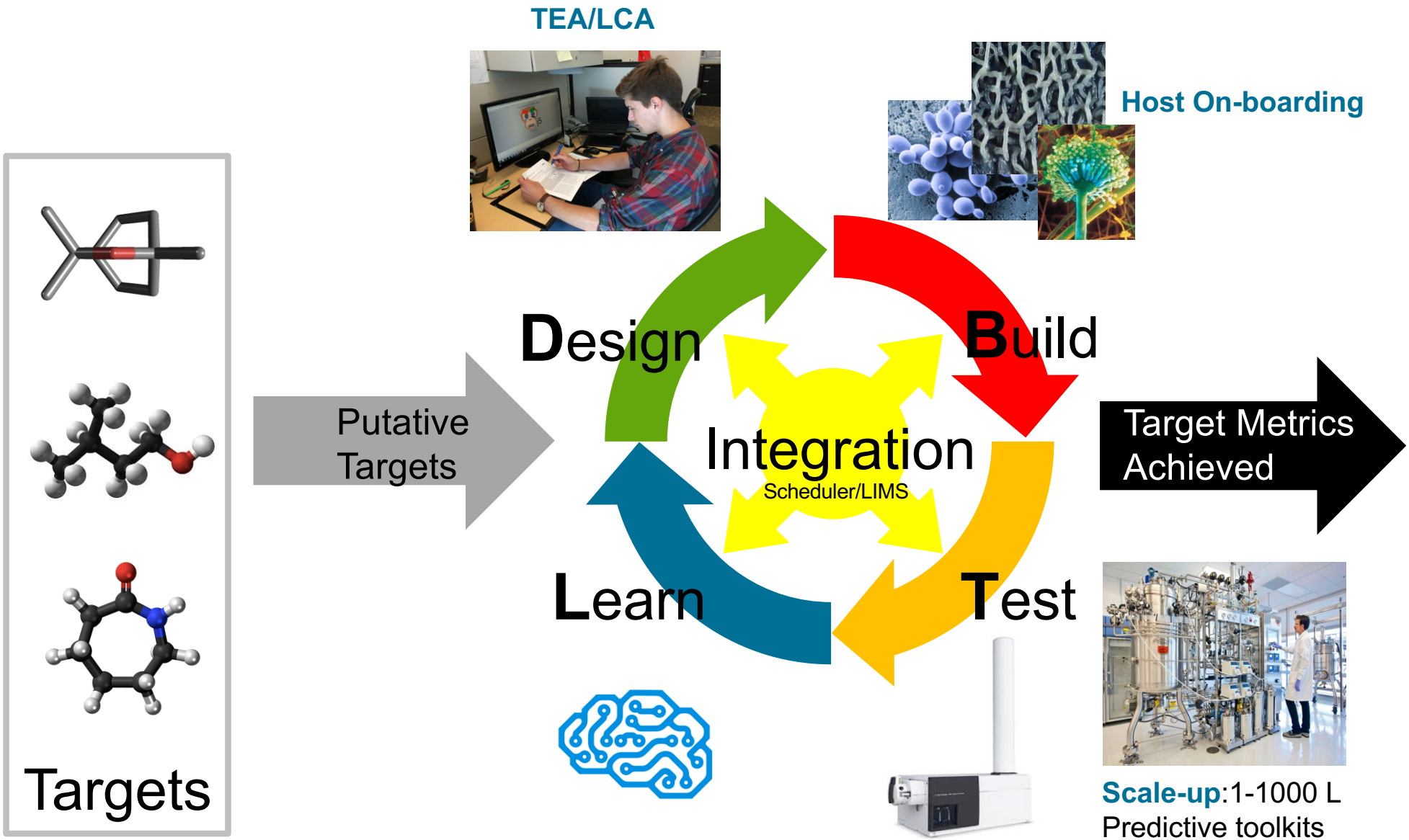
Six Tasks for Overall Project

- Task 1: Design-Build-Test-Learn (*Nathan Hillson* - lead)
  - Integrate design-build-test-learn cycle with process automation and sample tracking.
- Task 2: Integrated Analysis (*Ryan Davis/Thathiana Benavides* – co-leads)
  - Evaluate proposed molecules; develop, update, and improve existing process designs and LCA.
- Task 3: 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 4: Process Scale-up (*Davinia Salvachua/Deepti Tanjore* – 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 5: Industry Engagement (*Emily Scott/Chris Johnson/Phil Laible* – co-leads)
  - 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 6: Management (*Blake Simmons* - lead)
  - Manage project management, develop internal and external communications, provide deliverables to BETO, and make some capital equipment purchases.

Org Chart


BETO Technology Manager
Jay Fitzgerald
Industry Advisory Board
Executive Committee
Industry Partnerships
Task 6
Project Management and Integration
Alastair Robinson (LBNL)
Blake Simmons (LBNL)
Task 5
Industry Engagement and Outreach
Chris Johnson (NREL)
Phil Laible (ANL)
Emily Scott (LBNL)
Task 1
DBTL
Nathan Hillson (LBNL)
Task 2
Integrated Analysis
Ryan Davis (NREL)
Thathiana Benavides (ANL)
Task 3
Host Onboarding
Taraka Dale (LANL)
Adam Guss (ORNL)
Task 4
Process Integration and Scaling
Davinia Salvachua (NREL)
Deepti Tanjore (LBNL)
Subtask 1.1
Design
John Gladden (SNL)
Gregg Beckham (NREL)
Subtask 1.2
Build
Nathan Hillson (LBNL)
Subtask 1.3
Test
Jon Magnusson (PNNL)
Subtask 1.4
Learn
Phil Laible (ANL)
Hector Garcia-Martin (LBNL)

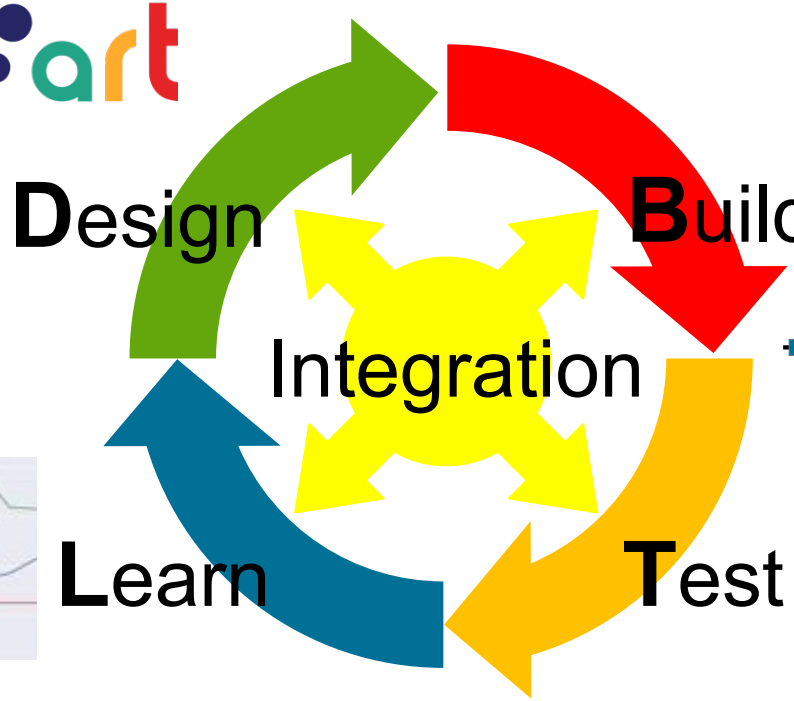
The Agile BioFoundry Approach



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

Agile BioFoundry

Highlights – DBTL infrastructure



DiVA

art

Sequence Validation

EASy

Biosensors

Transcriptomics, Metabolomics, Proteomics, & Lipidomics

Data Quality Assessment

EDD

LabKey

Bayesian Inference: Metabolic Kinetics

Kinetic Learning

Metabolic Modeling

Deep Learning

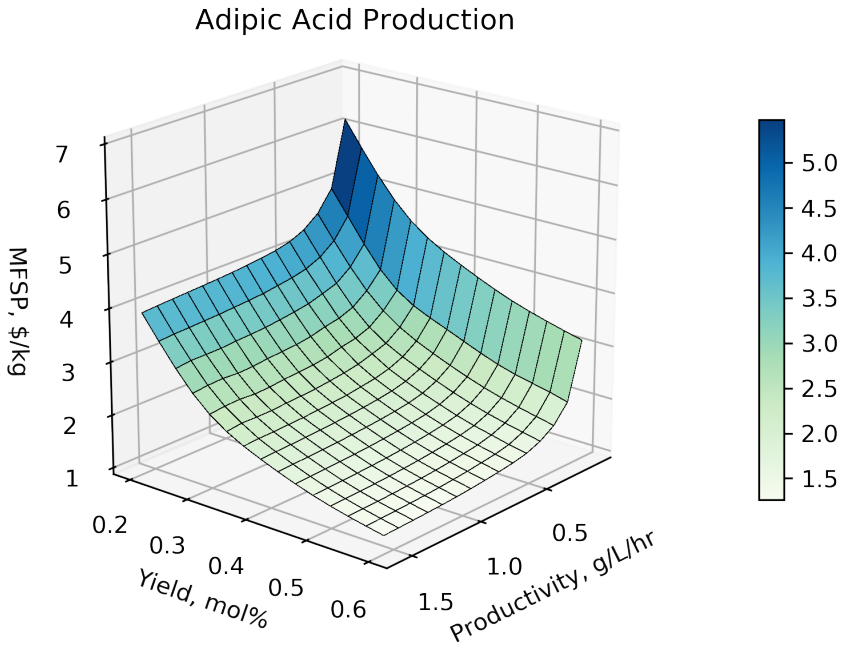
U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy
BIOENERGY TECHNOLOGIES OFFICE

Agile BioFoundry

Highlights – Integrated Analysis

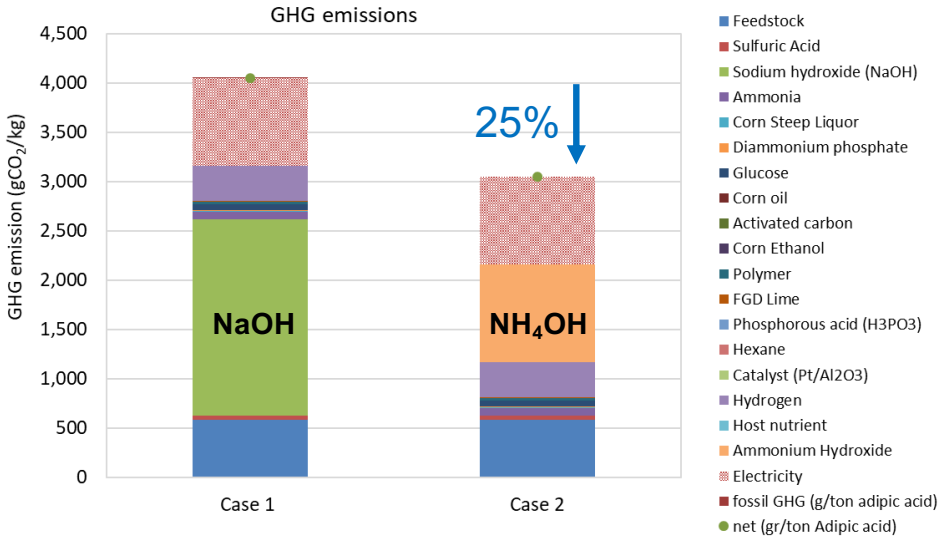
Example of outlining key drivers in both cost and sustainability

Quantify "economic gradient" of targeting yield or productivity



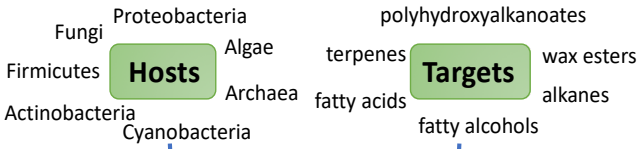
Key Outcome and Link to R&D:
Bounding analysis to show help identify critical R&D targets. ABF adopted 0.5 g/L/hr productivity target.

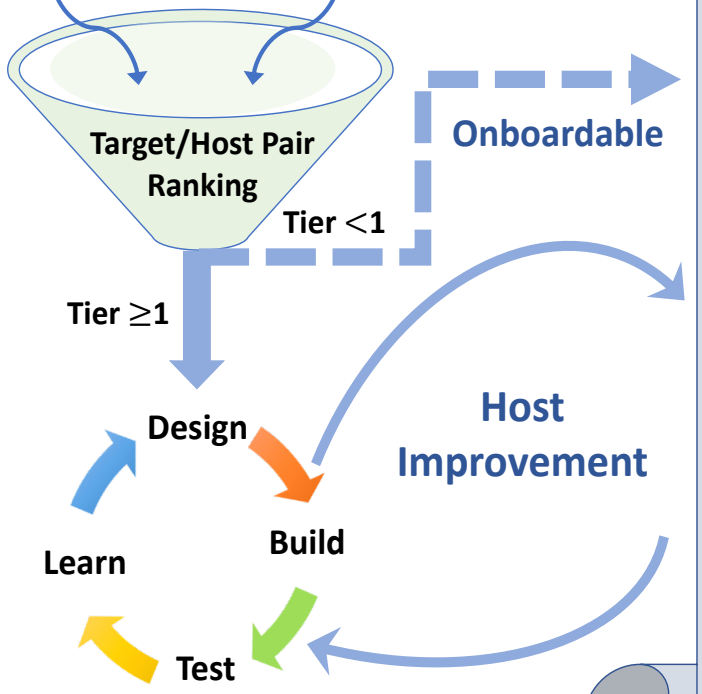
Decreasing GHG emission by using less GHG intensive chemicals



Key Outcome and Link to R&D:
ABF is testing the use of other neutralizing agents to see impact of performance.

Highlights – Host Onboarding





Tier System Criteria - "Hostability"

Tier 1

Annotated genome; growth conditions; growth kinetics and simple growth models; antibiotic susceptibility; selectable markers; transformation methods; plasmids/vectors; basic expression parts; biosafety/biosecurity information

Tier 2

Substrate utilization panel; toxicity profiles; bioreactor growth; counter-selectable markers; genome integration system; chromosomal safe sites/landing pads; induction systems; panel of constitutive promoters, RBSs, terminators; models of promoters and RBSs/Kozak sequences; genome-scale models; pan genome analysis; transcriptomic, proteomic, metabolomic datasets

Tier 3

Biosensors; cellular stress monitoring; CRISPR/CAS, Lambda Red, Cre-*lox* systems; advanced genomic integration platforms; gene expression tuning; high throughout protein engineering platform; lipidomic and glycomic datasets; centralized omics databases; multi-omic data integration and analysis; protein localization; protein degradation tags; protein interactome datasets; ^{13}C-MFA experiments and model; kinetic model; population balance model

Tier 4

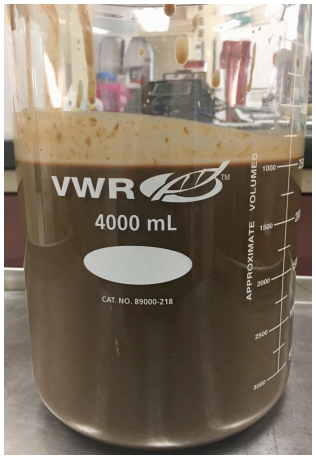
Culture scalability; saturated deletion/loss of function libraries; genomic overexpression platform; adaptive laboratory evolution/cell sorted libraries; baseline strains for maximal flux to metabolic nodes; cellular state sensors and dynamically regulated production strains; signaling model, gene regulation model, multi-scale model; predictive cellular model

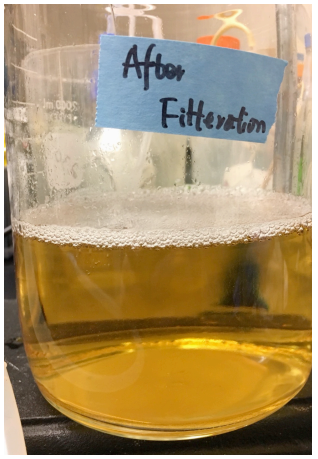
- Tier 1 represents the basic tools needed for DBTL
- Hosts that do not meet Tier 1 require further development prior to usage
- Tools increase in sophistication as an organism moves up Tiers
- Not all tools in all Tiers are required for all organisms

Highlights – Process Integration/Scale-up

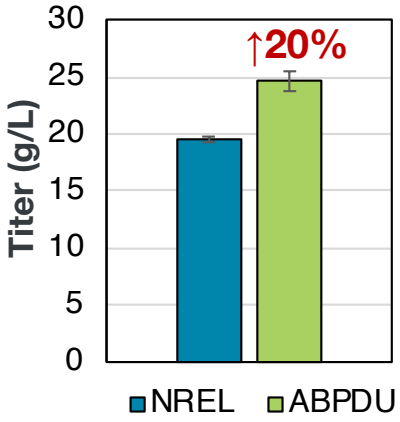
Hydrolysate production

Two batches to date implementing process improvements at pilot scale



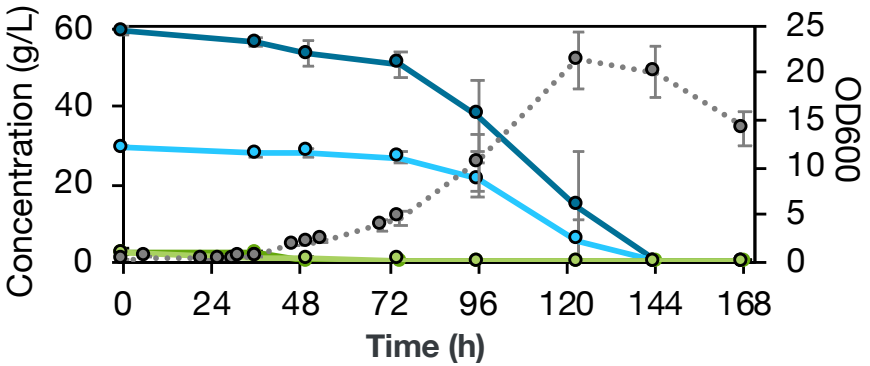


Pan-scale muconate Test/Learn



Round Robin study for muconate

Bioprocess development

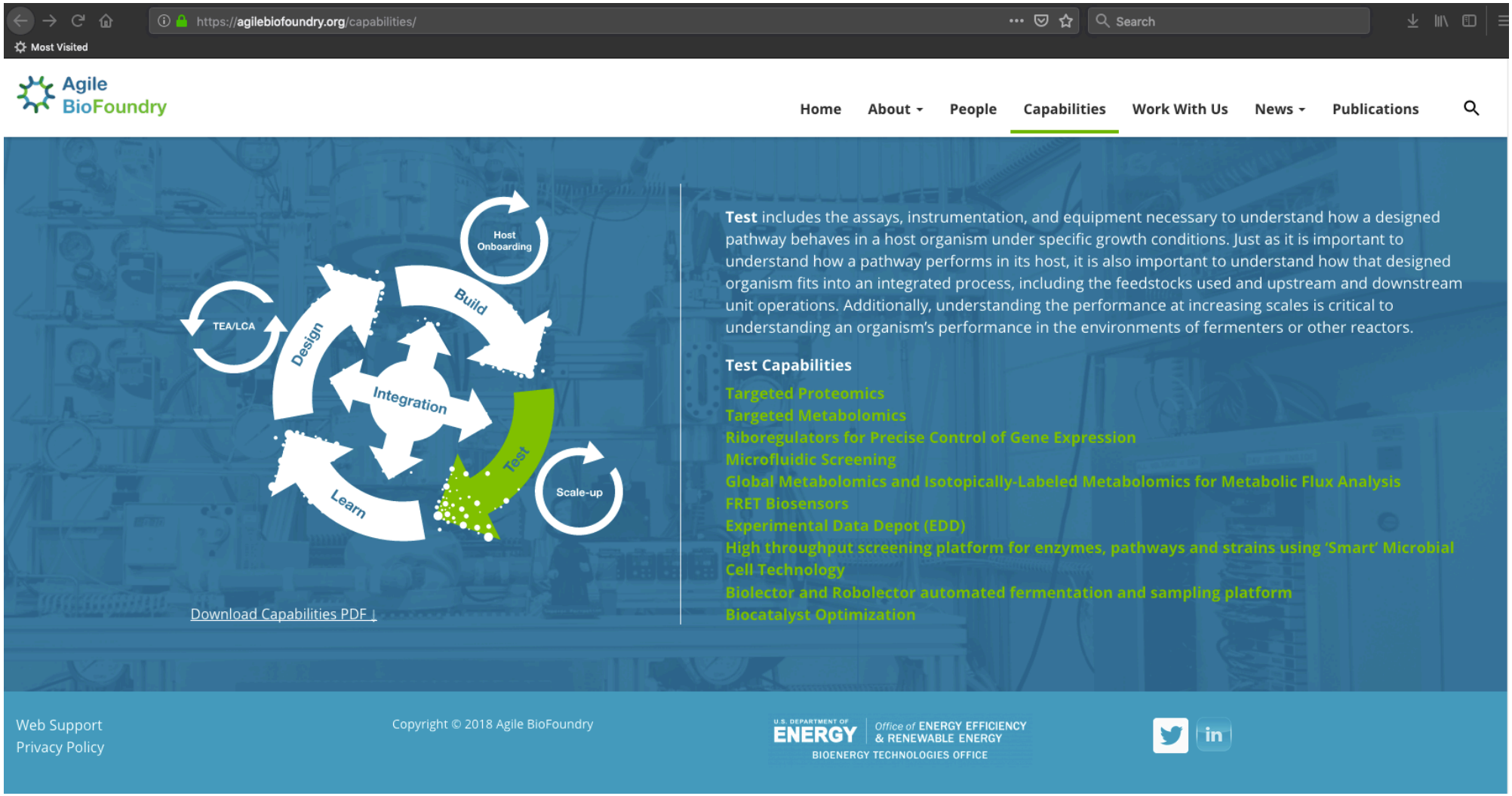


U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy
BIOENERGY TECHNOLOGIES OFFICE

Agile BioFoundry

Highlights – Industry Outreach

- **Revamped ABF website**
  - Capabilities section; ***template non-negotiable CRADA***; 2019 BETO Peer Review slides/posters

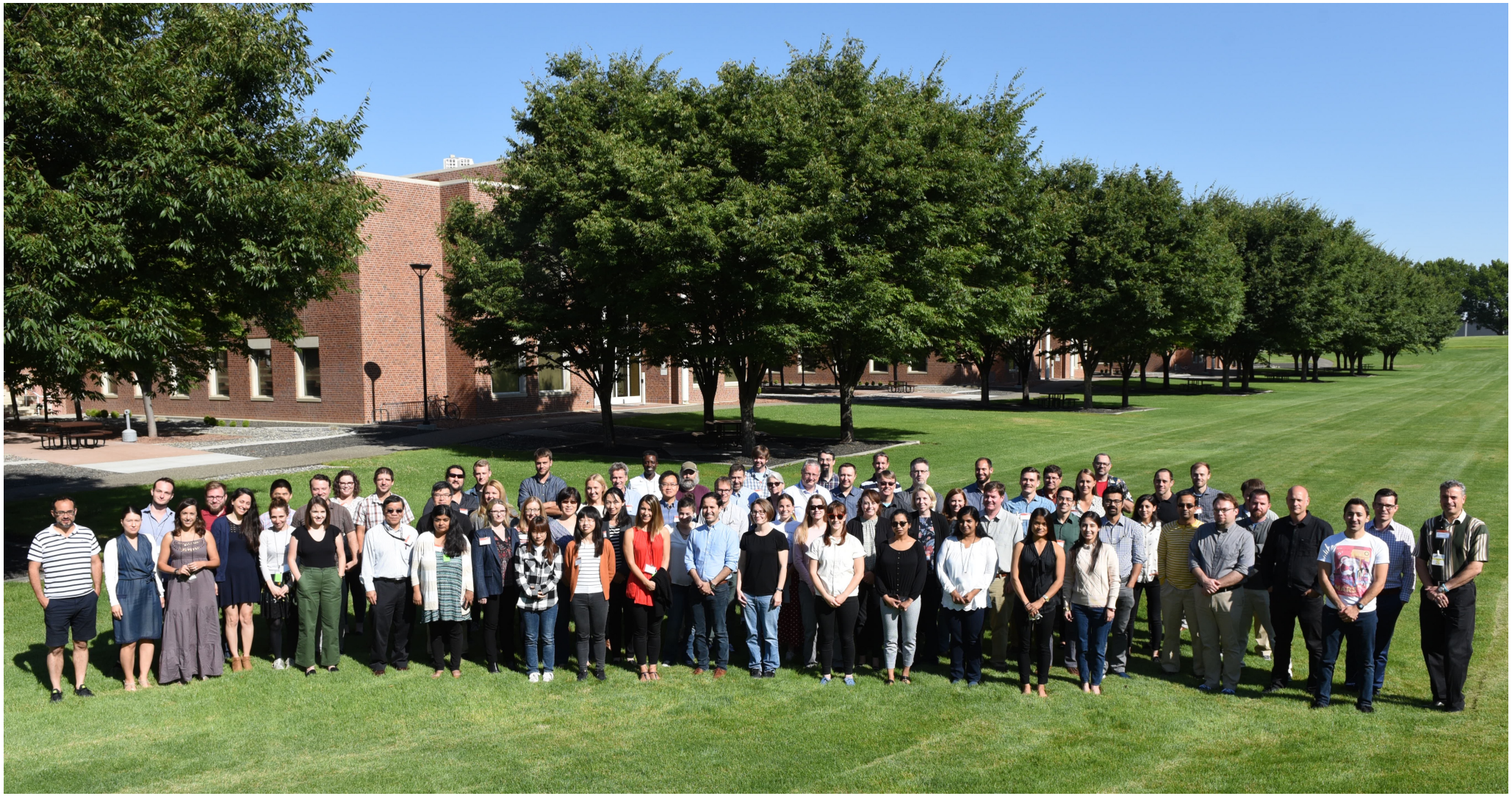


U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy
BIOENERGY TECHNOLOGIES OFFICE

Agile BioFoundry

Highlights – Management

- **ABF Annual Meeting: July 30-31, 2019 in Richland, WA**



FY17 ABF DFO

- **Goal**: to accelerate innovation and adoption of new biomanufacturing approaches that will foster growth of the bioeconomy.
- **Approach**: oversee a $5M directed funding opportunity (DFO) for industry partners to utilize the ABF to develop novel microbial hosts and bioproducts or to develop new capabilities and approaches that will advance all aspects of the Design-Build-Test-Learn biomanufacturing cycle.
- **Details**: $5M to be made available to the National Labs to collaborate with industrial partners. Projects limited to two years and $500K to $2M total per project.

2017 Timeline:

June 21 - RFP live on ABF website
July 24 - Proposals due
July 31 – ABF raw scores due
Aug 4 – initial selections made
Aug 7-10 – BETO internal briefings
August 11 - BETO concurrence on final selections
August 14 - Notifications go out
August 25 - AOPs entered into EERE system
September 1 - Process initiated for $$ sent to labs

Proposal response highlights:

19 proposals submitted (18 industry | 1 academic)
$19.2M requested (4X oversubscription of resources)
Indicates industry is very interested in leveraging ABF

7 Proposals awarded and implemented as CRADAs

Agilent Technologies
Department of Microbiology, Franklin College of Arts and Sciences, University of Georgia
LanzaTech — capturing carbon fueling growth
teselagen BIOTECHNOLOGY
LYGOS
Kiverdi
VISOLIS CARBON NEGATIVE MATERIALS

FY18 DOE BEEPS FOA: Topic 2 (ABF)

- **Goal**: to accelerate innovation and adoption of new biomanufacturing approaches that will foster growth of the bioeconomy.
- **Approach**: competitive DOE FOA process to award funds for financial assistance addressing the development of technologies able to contribute to the production of price-competitive biofuels and bioproducts.
- **Details**: 0-5 awards in topic area 2 (Agile BioFoundry Industry Partnership Initiative), with a range of $1,000,000-$2,000,000 per award.

2018 Timeline:

May 3 – FOA issue date
May 30 – Letters of intent due
June 27 – Full applications due
Aug 3 – Responses to Reviewers' comments due
September 4 - BETO announces awardees
September – Award Negotiations underway

Proposal response highlights:

Number of "ABF coordination calls" with industry: 22
Number of proposals submitted without ABF call: 1
Number of letters of intent not encouraged: 2
Number of full proposals submitted: 11
Indicates industry continues to be very interested in leveraging the ABF.

3 proposals awarded

LYGOS $2M

ZymoChem $1.3M

UC San Diego $2M

FY19 DOE FOA: Topic 7b (ABF)

- **Goal**: to accelerate innovation and adoption of new biomanufacturing approaches that will foster growth of the bioeconomy.
- **Approach**: competitive DOE FOA process to award funds for financial assistance addressing the development of technologies able to contribute to the production of price-competitive biofuels and bioproducts.

2 proposals awarded

Berkeley UNIVERSITY OF CALIFORNIA — $2.5M

UNIVERSITY of WASHINGTON — $1.8M

Working with Industry: FY17 Direct-Funded Opportunities and FY18-19 FOAs

- **Why these projects and BETO investments are so important**
  - Expand the range of ABF targets and hosts
  - Stress-test ABF capabilities and identify weaknesses and opportunities
  - Bring new technologies in to the ABF and opportunities to license ABF technology out
  - Early stage investments that will be crucial to the ABF accomplishing its overall goal and its desired outcomes (many relate directly to industry impact and technology transfer)
  - Ensure that ABF development is responsive to industry
  - Increase industry exposure (beyond funded companies) to the ABF and its capabilities
  - Quantitatively demonstrate industry interest in leveraging the ABF

- **New for FY19: template ABF CRADA**
  - Publicly accessible from the ABF website: https://agilebiofoundry.org/work-with-us/
  - Non-negotiable for projects receiving DOE funding
  - Includes new “Extended Non-Exclusive Option” IP model

FY19 Milestones Completed

Milestone (synopsis)	Task	FY19 Quarter	Type
Selections of new target molecule & existing molecule in different host	Target/Host	Q1	Quarterly (Regular)
4X Build sequence validation capacity increase from FY18 to FY19	DBTL Infrastructure	Q2	Quarterly (Regular)
TEA and LCA on new FY19 target molecule	Integrated Analysis	Q2	Quarterly (Regular)
Deep Learning non-intuitive predictions	DBTL Infrastructure	Q2	Quarterly (Regular)
Titer goals in range of 1 to 10 g/L	Target/Host	Q3	Quarterly (Regular)
Transformation in new organism(s)	Host Onboarding	Q3	Quarterly (Regular)
5X Test capacity increase from FY17 to FY19	DBTL Infrastructure	Q3	Quarterly (Regular)
Promoters in new SOT organisms	Host Onboarding	Q4	Annual (Regular)
10L scale using DMR-EH hydrolysate, with 10 g/L, 100 mg/L/h, 40% yield	Process Integration & Scaling	Q4	Annual (Regular)
SWOT Analysis	Industry Engagement & Outreach	Q4	Annual (Regular)
DBTL Activity, Quarterly/Milestone, and final AOP reports sent to BETO. Updates to ABF website	Management	Q4	Annual (Regular)
Value of non-intuitive Learn predictions demonstrated	Target/Host	Q4	Go/No-Go

How we are thinking about our future work

- **We have a long term strategic vision for the ABF**
- **Our future work will focus on the technical and operational barriers to achieving the overall ABF goal and its desired outcomes**
- **Some challenges facing the ABF:**
  - Show Learn can add value through non-intuitive predictions
  - Demonstrate industry-relevant ABF competencies across targets and hosts
  - Onboard new hosts and develop tools for them
  - Increase DBTL cycle capacities and efficiencies; reduce cycle time
  - Keep current strength / weakness / opportunities / threat (SWOT) assessments
  - Demonstrate reproducible geographically distributed unit operations
  - Find sustainable ABF IP / licensing / contracting model(s)
  - Demonstrate that past work and Learnings increase the efficiency of new work
- **Next slides show our current FY19 and pending FY20-22 milestones**

Representative FY20-22 Milestones

- **FY20 Annual Smart**
  - **Reproducibility of 3 distributed Test unit operations**, including bioreactor scale-up, quantified through comparison of results post data quality assurance for on-site vs. off-site sample analysis. Identify 3 or more variables that capture unit operation variability; reproducibility will be determined by less than 10% variability in these variables at the distributed Test sites.

- **Go/No-Go Decision, Q2 FY21**
  - **5 target molecules or tools transferred between host organisms**. Successful target molecule transfers will have product titers greater than the lesser of 1 g/L or the titer established in the prior host organism at time of transfer. For 3 of 5 of these, 2X biological engineering cycle efficiency gains demonstrated over attempts made in prior host organisms

- **FY22 Annual Smart**
  - **5X efficiency** (cycle time, how many cycles, how many strains per cycle, per person/ instrument/ resource) improvement in DBTL engineering cycle compared to FY19 baseline efficiency demonstrated
  - Bring a total **20 microbial hosts** (20 species) to at least Tier 1, and provide corresponding information, resources, and tools via publicly-accessible ABF website

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 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.