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

Agile BioFoundry

Peer Review Panel

4 April 2023

Gayle Bentley

Your host for this session

BETO Conversion Technology Manager

Agenda Overview

DAY 2 – Tuesday, April 4, 2023					
8:30 AM	8:45 AM	15	Technology Area Daily Intro	BETO	Gayle Bentley
8:45 AM	9:15 AM	30	ABF - Lessons Learned and Introduction to Future Plans	ABF	Nathan Hillson
9:15 AM	9:45 AM	30	ABF Future Strategy - Strategic Plan	ABF	Nathan Hillson
9:45 AM	10:15 AM	30	ABF Future Strategy - Goals, Milestones, and Deliverables	ABF	Nathan Hillson, Gregg Beckham, John Gladden, Jon Magnuson
10:15 AM	*10:30 AM*	*15*	*Break*	*All*	
10:30 AM	11:00 AM	30	ABF Future Strategy - Implementation Plans	ABF	Nathan Hillson, Gregg Beckham, John Gladden, Jon Magnuson
11:00 AM	11:30 AM	30	ABF Future Strategy - Q&A, Open Discussion, and Feedback	ABF	All ABF members present
11:30 AM	12:00 PM	30	ABF Future Strategy - Q&A, Open Discussion, and Feedback	ABF	All ABF members present
12:00 PM	*1:00 PM*	*60*	*Lunch*	*All*	
1:00 PM	1:30 PM	30	Development of Bacillus as an industrial host for the microbial production of biopolymers	ZymoChem	Thomas Mand
1:30 PM	2:00 PM	30	Developing multi-gene CRISPRa/i programs to accelerate DBTL cycles in ABF hosts engineered for chemical production	University of Washington	James Carothers
2:00 PM	2:30 PM	30	Accelerating engineered microbe optimization through machine learning and multiomics datasets	Lygos, Inc.	Nick Ohler
2:30 PM	3:00 PM	30	Accelerating polyketide synthase engineering for high TRY production of biofuels and bioproducts	University of California Berkeley	Jay Keasling
3:00 PM	*3:20 PM*	*20*	*Break*	*All*	
3:20 PM	3:50 PM	30	A two-chamber growth and production system for robust continuous bioprocessing	Pow Genetic Solutions	Maggie Stoeva
3:50 PM	4:20 PM	30	FOA Closing Discussion		
4:20 PM	*5:00 PM*	*40*	*Closed Door Comment Review Session*	*Reviewers*	

Reviewer Introductions

Welcome back, Reviewers!

Name	Affiliation
Karen Draths	Michigan State University
Brentan Alexander	CIO, Synonym
Doug Friedman	CEO, BioMADE
Ramana Madupu	DOE Office of Science
Hanny Rivera	Ginkgo Bioworks
Gale Wichmann	Amyris
Fuzhong Zhang	Washington University in St. Louis

Ground Rules

Presenters: We will give you a 5 minute warning. When your time is up, we will verbally let you know. Please wrap up quickly.

Reviewers: Please ask questions during the Q&A period. Be considerate to allow all reviewers the opportunity to ask a question.

General public: We will field questions as time allows after the reviewers have asked questions.

FOA Projects

Funding mechanisms

	FOA	AOP
Selection Method	Competitive	Lab Call
Open to the Public	✓	☒
National Lab Participant	Only as Subrecipient	✓
Go/No-Go Decision Points	✓	✓
Verifications	✓	☒
Award Modifications Method	Contracting Officer (CO)	AOP Tool Change Control

FOA = Funding Opportunity Announcement

AOP = Annual Operating Plan

CRADA= Cooperative Research and Development Agreement

ABF Timeline and History

Visioning Meeting

Pilot Project (LBNL, NREL, PNNL, SNL)

ABF Kickoff
- Industry Advisory Board Formed
- Website Launched

Website Relaunched

Formal partnership between ABF and ABPDU established

Joint solicitation with NSF, MSRDC

Joint solicitation with NSF

We are here

FY 2015

FY 2016

FY 2017

FY 2018

FY 2019

FY 2020

FY 2021

FY 2022

FY 2023

2017 Directed Funding Opportunity Partnerships
- Lygos
- Kiverdi
- Agilent
- TeselaGen
- Neidle Lab
- LanzaTech
- Visolis

2018 BEEPS FOA Partnerships
- Lygos
- Zymochem
- UCSD

2019 Multi Topic FOA Partnerships
- U Washington
- UC Berkeley
- POW BIO

2020 DFO C16 Biosciences
- Clemson
- LanzaTech
- White Dog Labs
- Kalion
- Danimer Scientific
- Inviao Science
- Enduro Genetics

2021 DFO Partnerships
TeselaGen
LanzaTech
Levandura
Tech Holding
iMicrobes
Huue, inc

2022 DFO Partnerships
- UC Berkeley
- Birch Biosci
- Kiverdi
- U Nebraska
- Azolla

2023 Solicitation live Now!

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2018 BioEnergy Engineering for Products Synthesis (BEEPS)

Funding for collaborative projects between an applicant and the Agile BioFoundry to address critical biomanufacturing challenges

Areas of interest:

- Development of non-model host organisms with industrially-relevant production advantages over *E. coli* and *S. cerevisiae*
- De-bottlenecking of biosynthetic pathways to take a target molecule from mg/L to tens of g/L, increase productivity and yield
- Production of datasets that enable Agile BioFoundry's Learn methodologies

Metric:

By the end of the project period, all projects will be required to demonstrate titers exceeding 20 g/L of product using cellulosic sugars or other biomass derived intermediates as a feedstock.

LYGOS

ZYMOCHEM

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2019 BETO Multi Topic FOA, Area of Interest 7b

Areas of Interest:

- Utilization of the tools developed by the Agile BioFoundry to show increases in the efficiency of the DBTL cycle;
- Development of tools which can be incorporated into the Agile BioFoundry to decrease cycle time for a broad variety of host organisms;
- Production of data sets that will enable Agile BioFoundry's Learn methodologies, which seek to use machine learning and other approaches to improve subsequent rounds of design.

Metric:

By the end of the project period applicants must demonstrate an increase in the efficiency of a DBTL cycle of 30% over the state of technology for a given organism, including a final cycle time of less than 4 months. A DBTL cycle is defined as the time that it takes to use computer-aided design software to design 100 constructs, build those constructs, transform constructs into a host strain (achieving at least 40 engineered host strains), test host strain for output (which at minimum includes cell growth and product titer), and to analyze the data in a way that is fully useable for the next design cycle. An increase in efficiency is defined as the amount of improvement in a target production metric per cycle per time.

ABF Strategic Plan

ABF presented a new plan for the 2023-2025 cycle

- What reductions in time and cost has the ABF been able to measure?
  - Substantial and meaningful work has been done, but measuring that progress remains a challenge. ABF was directed to identify a plan to measure progress toward its original mission in the FY23-25 AOP proposal. Independent, external reviewers assessed the ABF’s 3 year plan
- The feedback from this review, in addition to the 2021 Peer Review indicated that ABF’s approach may not be currently well structured to achieve their high level goals.

“It’s impossible to judge how likely the consortium is to hit this goal as there was zero data given on the current estimate of cost and time reduction through ABF…However, much of the work in the proposal (especially DBTL, HOD, and PISU) will absolutely result in a reduction in cost and time to bring new molecules to market. The only question is what level of reduction will be achieved.”

Process- update ABF's strategic plan

Two quarters were dedicated to planning, broken down by developing a new strategy and ensuring the tasks are set up to implement the strategy.

During this time, all experimental work stopped to focus on planning.

FY23Q1: strategic planning

FY23Q2: implementation of strategic plan

BETO provided a draft Vision to frame the work

Vision

ABF is a public resource to accelerate microbial strain and process development for industrial deployment, and directly builds the Nation's biotechnology capacity to provide renewable chemicals and fuels with dramatic GHG emissions reduction.

ABF will introduce their new vision and mission that build on this guidance

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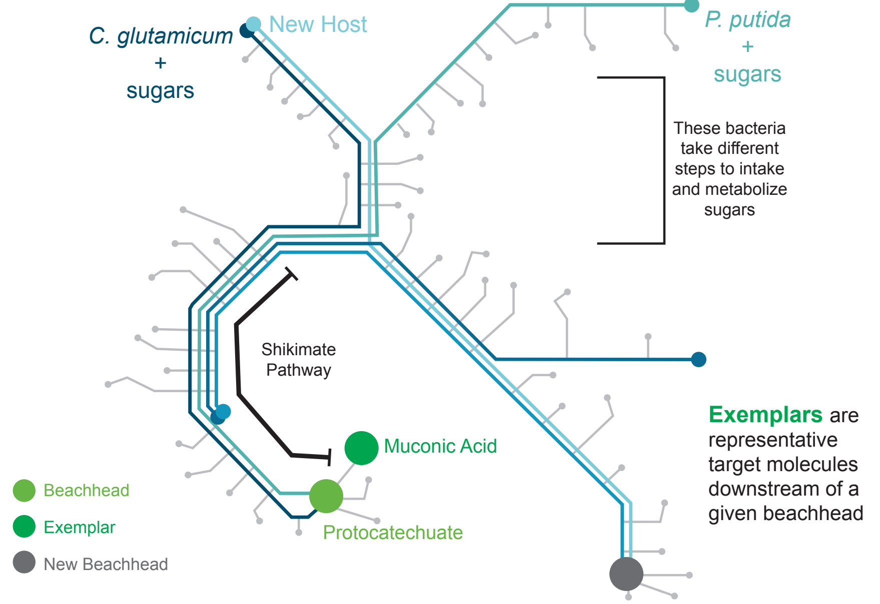
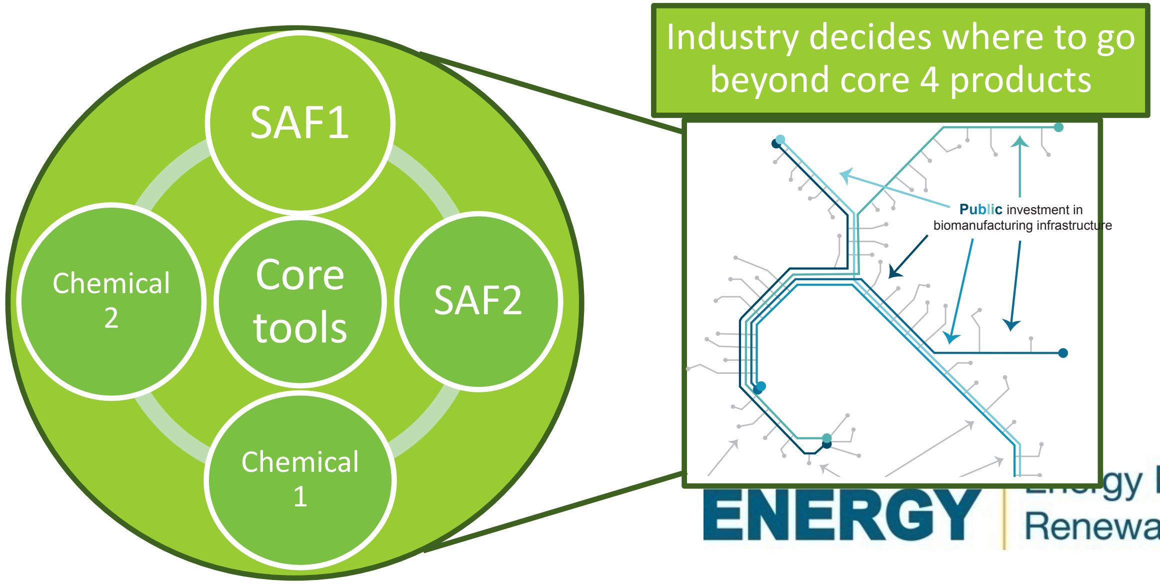
New ABF core structure

- Previously structured based on DBTL and goal to cover metabolic coverage within ABF
  - Efforts were spread thin across the ABF to cover broad metabolic space
  - Infrastructure was built in first 6 years to leverage for future partnerships

- New approach focuses all of ABF's internal activities around two core areas: SAF and chemicals.
- ONLY making 4 products total across the ABF
  - 2 SAFs
  - 2 chemicals

Metabolic engineering strategies developed for a given target can be applied to targets associated with the same beachhead





New budget structure

Proposed funding model (FY23-FY25)*

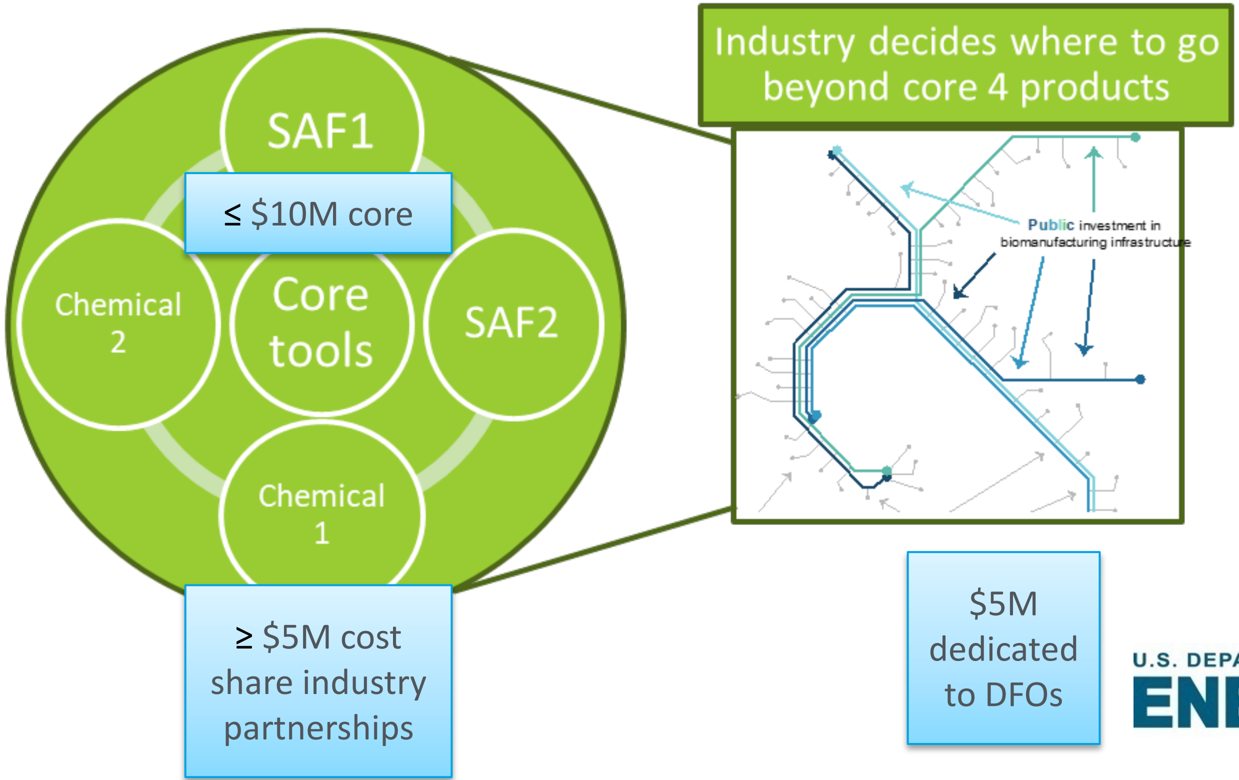
≤ $10M/year core efforts

≥ $5M/year ABF core-industry partnerships

- ABF-solicited partnerships with cost share commitments
- Overlapping interest with core ABF efforts
- Obtain in-kind cost share FY23-FY25 (with urgency)

$5M/year FO, NSF-ABF, BioMADE

*All core projects connect to a demonstration pathway, and all core-industry partnerships contain funds-in or cost share



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How will ABF measure progress?

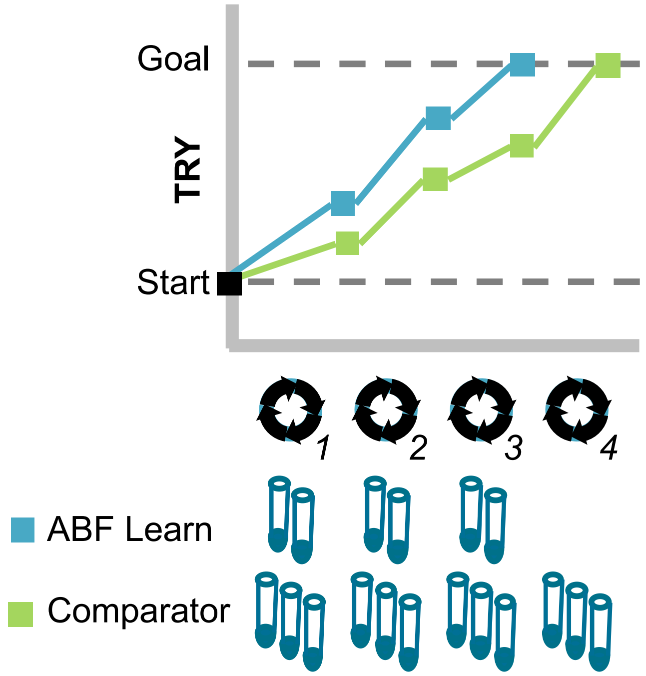
(1) Internal: Measure capability performance

- Assess technologies against best baseline
- If new approach does not outperform (e.g. by XX%) or add entirely new capability, discontinue development
- Until approach demonstrated, do not feature on the ABF website

Metrics

- Performance gains against comparators
- Heat map of SOP / unit operation / capability use in collaborator projects
- ABF unit operation capacities

All ABF capabilities will have to demonstrate quantifiable improvement compared to incumbent approach.



(2) External: Partnerships

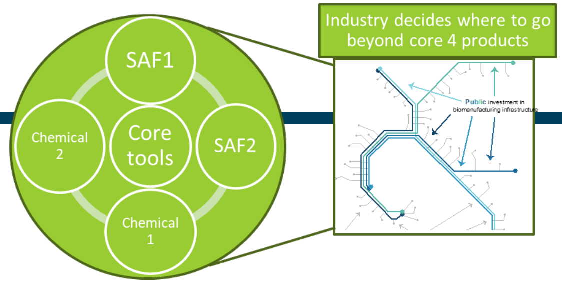
- Achieve ≥50% of ABF's budget allocated to external partnerships.
- Reach at least 500kT CO2e reduction by ABF commercial partnership projects
- Commercialization time and cost reduction savings for at least 6 partnership projects

Metrics

- BD: numbers of funds-in projects, monetary value of funds-in projects, funds-in support as a fraction of overall ABF funding.
- BETO-funded collaborations: Numbers of funding-opportunity projects, affiliates trained at ABF locations, numbers of MSI-based investigators, oversubscription of ABF funding opportunities, funds leveraged from collaborating funding agencies.

Questions?

What are the proposed core areas?



2 SAFs, selected to leverage ABF capabilities and plug directly in to existing SAF pathways

- *SAF 1: Ethanol production in anaerobic thermophiles via consolidated bioprocessing of lignocellulose for SAFs*
  - Produce ethanol from lignocellulose directly using an anaerobic thermophilic strain at industrially-relevant solids loadings, solubilization, and ethanol titer. Meet >50% GHG emissions reductions relative to fossil-based jet fuel at pilot scale
- *SAF 2: Alkane production in oleaginous microbes for SAFs*
  - Produce secreted lipid-derived products as feedstocks for ASTM certified jet fuel, e.g. HEFA, HC-HEFA, and CHJ. TEA and LCA will examine integration with current industrial SAF processes. Target lipid titers that would meet at least 50% GHG emissions reductions relative to fossil-based jet fuel at pilot scale.

2 Chemicals

- *Chemical 1: muconic acid*
- *Chemical 2: 3-hydroxypropionic acid*
  - Produce direct replacement and performance-advantaged chemicals at industrially relevant titers, rates, and yields and at 70% reduction in GHG emissions relative to fossil-based production. Exemplar biochemicals could include adipic and 3-hydroxypropionic acid.
  - Assess and develop biochemical targets with industry partners that maximize the potential to reduce GHG emissions relative to fossil-based production, reaching 1MMT CO_2e per product.

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Where Does ABF Fit within BETO

FY2023 Enacted Budget Authority = $280M

Renewable Carbon Resources	Conversion Technologies	Systems Development and Integration	Data, Modeling, and Analysis
Feedstock, Algae	Conversion	Systems	Data
FY2023: $77,900,000	FY2023: $100,000,000	FY2023: $92,600,000	FY2023: $9,500,000

Agile BioFoundry

FY2021 BA:

$15M National Laboratory Core

$5M Directed Funding Opportunity

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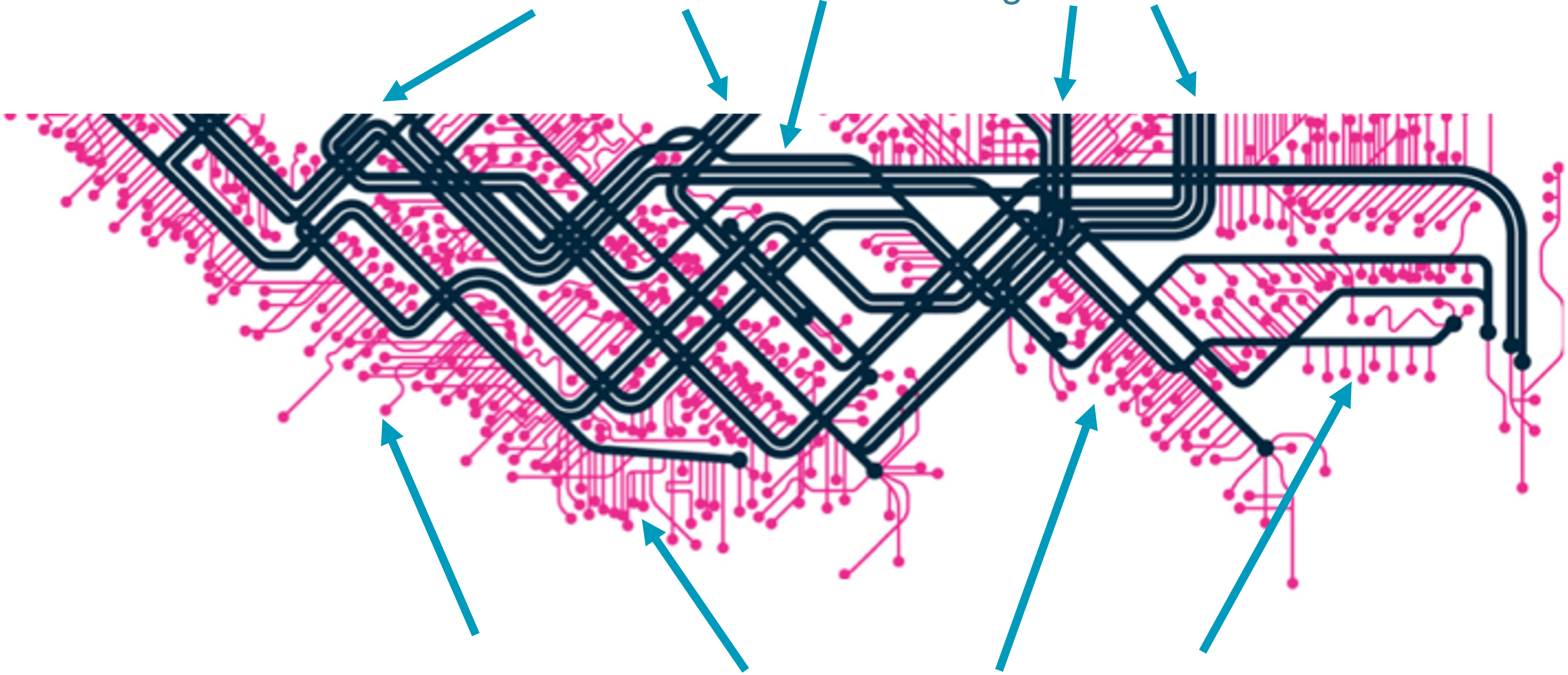
Public Infrastructure Investment Enables Private Industry

Public investment in biomanufacturing infrastructure



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

Adapted from Lyft

ABF Goals, Outcomes, Relevance, Risks

- **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

ABF Funding Cycles

2016	• Seed Funding • $1M
2017-2019	• First 3 year cycle • $20M/year, $60M total
2020-2022	• Second 3 year cycle • $20M/year, $63M total*
2023-2025	• Third 3 year cycle • Planned $23M/year, $69M total

We are here

*Budget for 2022 included adding ABPDU to core

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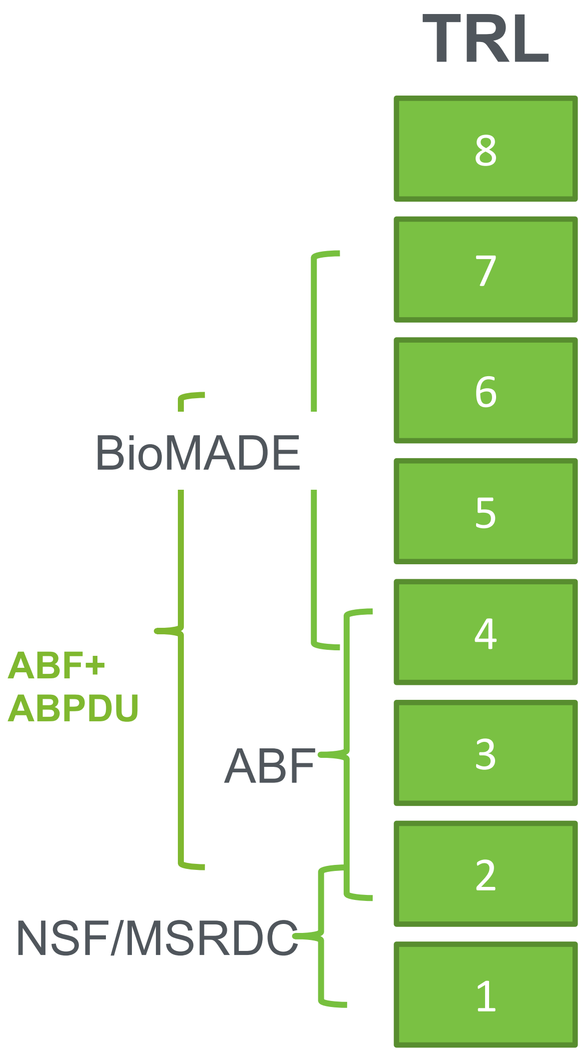
Strategy for collaborative work

- **ABF + NSF**: $1M ABF DFO, plus $4-5M from NSF. Support for collaborations between NSF-supported academic PIs and DFO-supported ABF teams.
- **ABF + MSRDC**: $1M DFO. Expand ABF partners to PIs at minority serving institutions
- **ABF + BioMADE***: $2M DFO. Provide a route to mature ABF technologies and provide ABF support to BioMADE
- **Core ABF DFO**: $1-5M. Depends on budget. Will focus on advancing core BETO decarbonization goals



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ABF developed a new Vision and Mission

Old

- **Vision:** The Agile BioFoundry will unite and expand the capabilities of the national laboratories to develop a robust, agile biomanufacturing platform accessible to researchers across the private and public sectors.

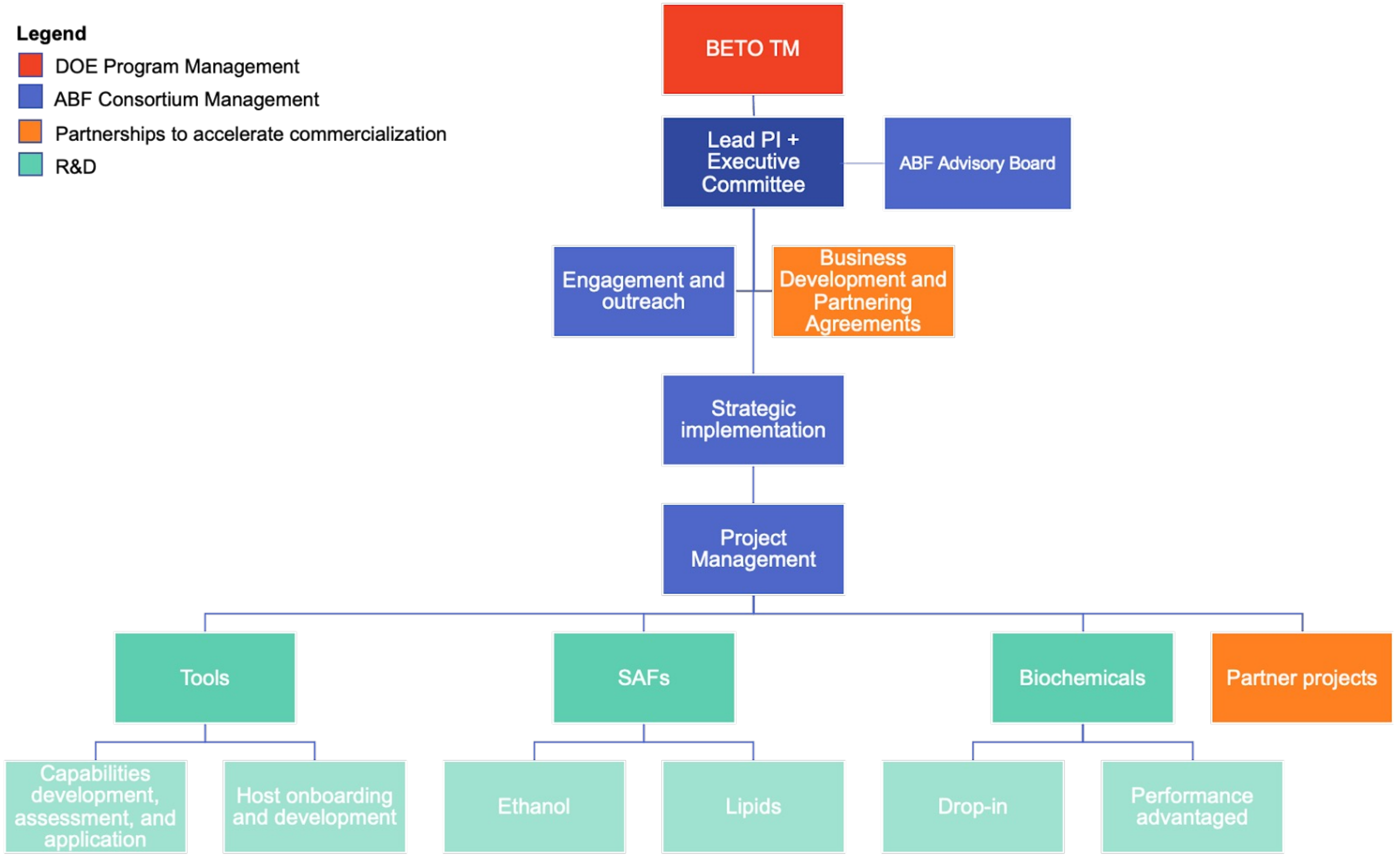
- **Mission:** The Agile BioFoundry will integrate industrially-relevant production microbes, advanced tools for biological engineering and data analysis, and robust, scaled up processes for integrated biomanufacturing.

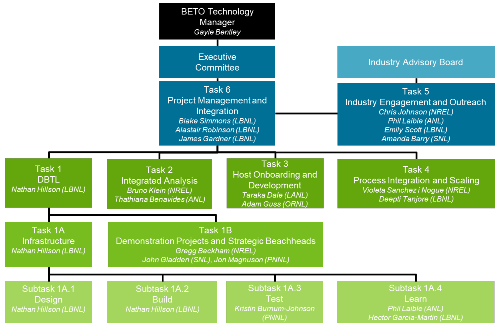
New

- **Vision:** A world running on the sustainable biomanufacturing of affordable fuels and chemicals

- **Mission:** Develop biomanufacturing tools, processes, and partnerships that enable industrial production of sustainable fuels and chemicals for the nation

New org chart





✓ Roles and responsibilities developed for each role on org chart