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

Agile BioFoundry Peer Review Panel

April 3, 2023

Gayle Bentley

Your host for this session

BETO Conversion Technology Manager

Agenda Overview

Start	*End*	*Title*	*Speaker*
1:00 PM	1:30 PM	Technology Area Introduction	Gayle Bentley
1:30 PM	2:00 PM	ABF Introduction and Overview	Nathan Hillson
2:00 PM	2:30 PM	ABF Past Accomplishments - DBTL Infrastructure, Demonstration Projects, and Beachheads	Nathan Hillson, Gregg Beckham, Di Liu, Jon Magnuson
2:30 PM	3:00 PM	ABF Past Accomplishments - Industry Engagement, Outreach, Management	Christopher Johnson, Phil Laible, Emily Nelson
3:00 PM	*3:20 PM*	*Break*	
3:20 PM	3:35 PM	ABF Past Accomplishments - TEA/LCA	Bruno Klein, Thathiana Benavides
3:35 PM	3:50 PM	ABF Past Accomplishments - Host Onboarding and Development	Adam Guss, Taraka Dale
3:50 PM	4:05 PM	ABF Past Accomplishments - Process Integration, Scale Up	Deepti Tanjore, Davinia Salvachua
4:05 PM	4:35 PM	ABF Past Accomplishments - Q&A, Open Discussion, and Feedback	Nathan Hillson
4:35 PM	*5:15 PM*	*Closed Door Comment Review Session*	

Reviewer Introductions

Welcome, Reviewers!

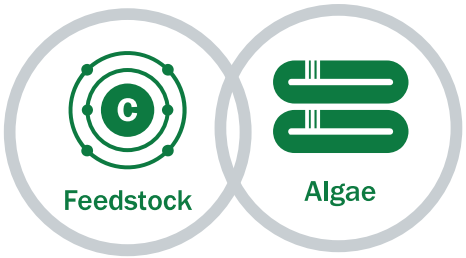
Name	Affiliation
Karen Draths	Michigan State University
Brentan Alexander	CIO, Synonym
Doug Friedman	CEO, BioMADE
Ramana Madupu	DOE Office of Science
Hanny Rivera	Ginkgo Bioworks
Gale Wichmann	Amyris
Fuzhong Zhang	Washington University in St. Louis

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.

Where Does ABF Fit within BETO

FY2023 Enacted Budget Authority = $280M

Renewable Carbon Resources



FY2023:
$77,900,000

Conversion Technologies

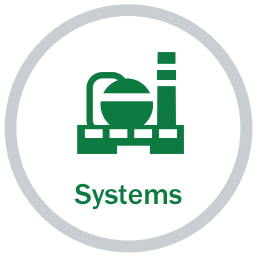
FY2023:
$100,000,000

Agile BioFoundry

FY2021 BA:
$15M National Laboratory Core

$5M Directed Funding Opportunity

Systems Development and Integration



FY2023:
$92,600,000

Data, Modeling, and Analysis

FY2023:
$9,500,000

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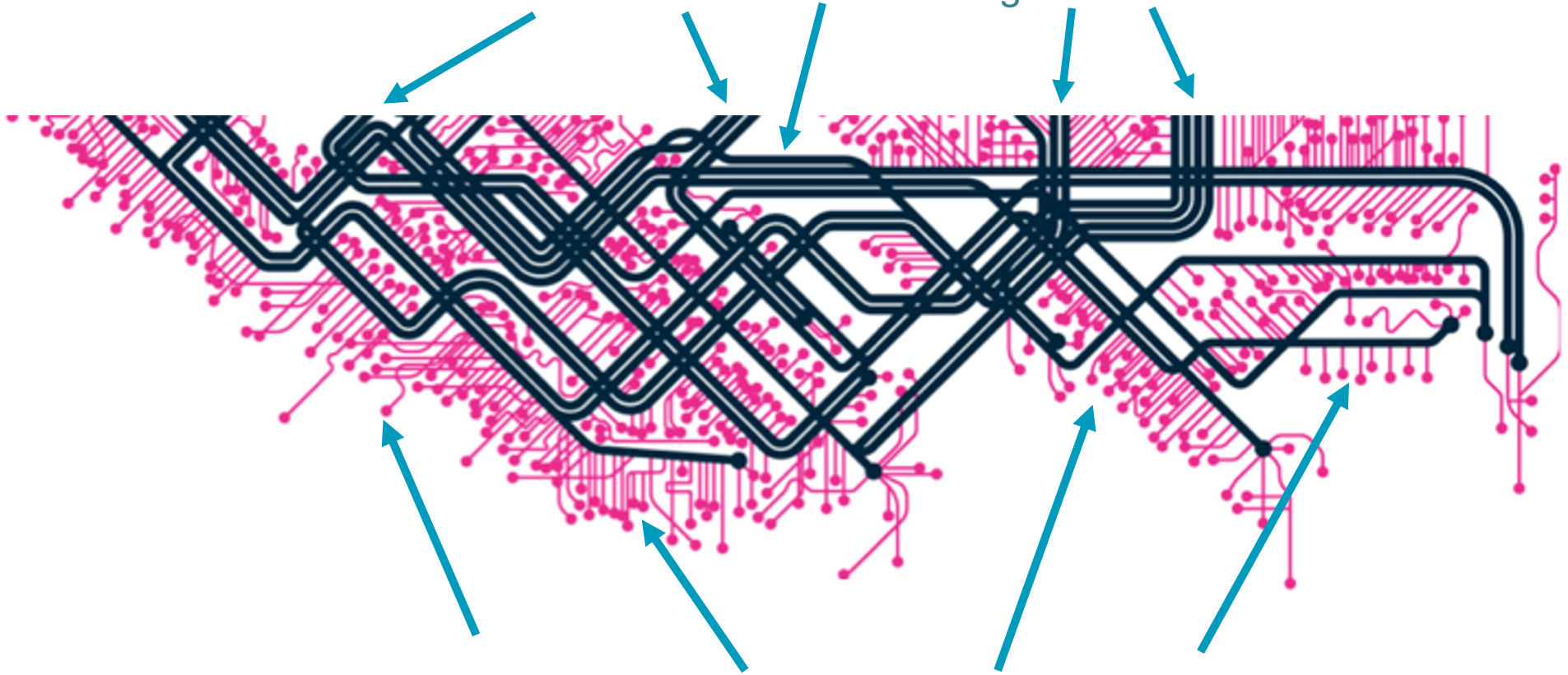
Public Infrastructure Investment Enables Private Industry

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

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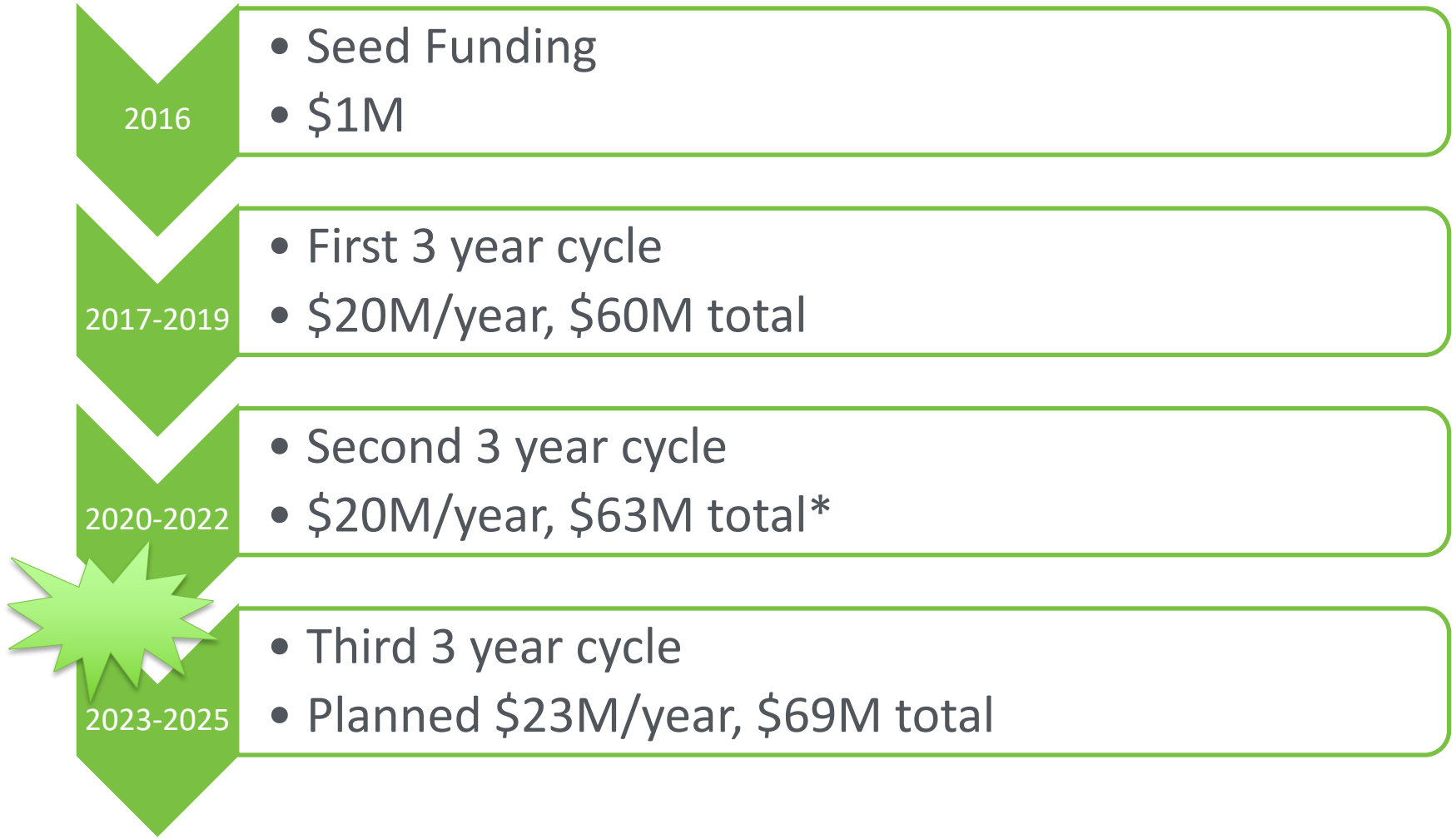
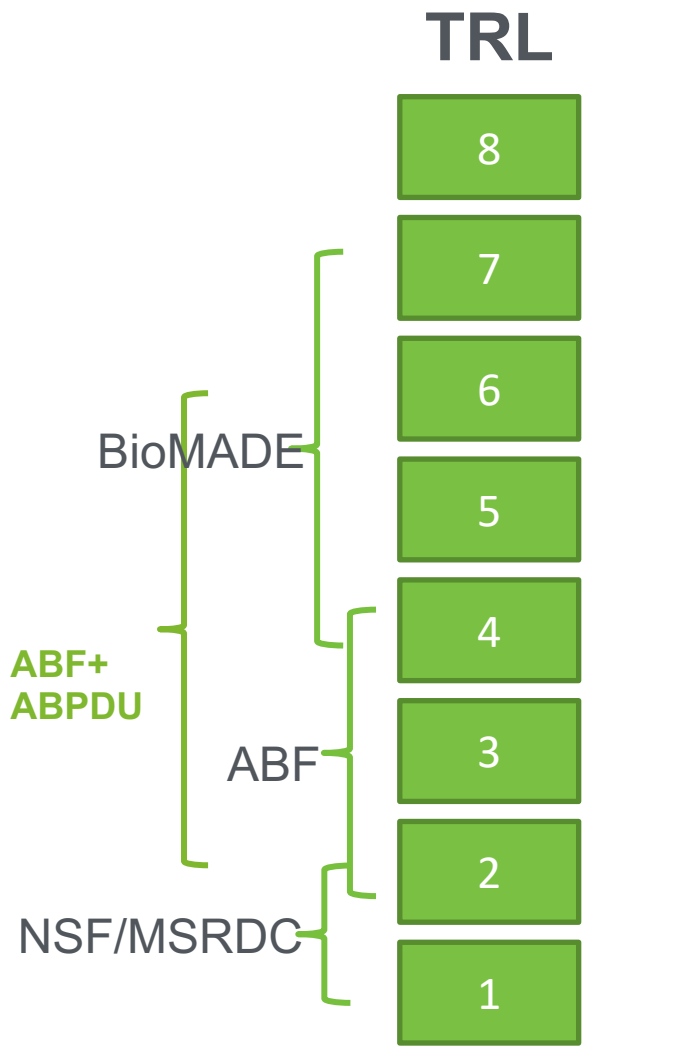
ABF Funding Cycles



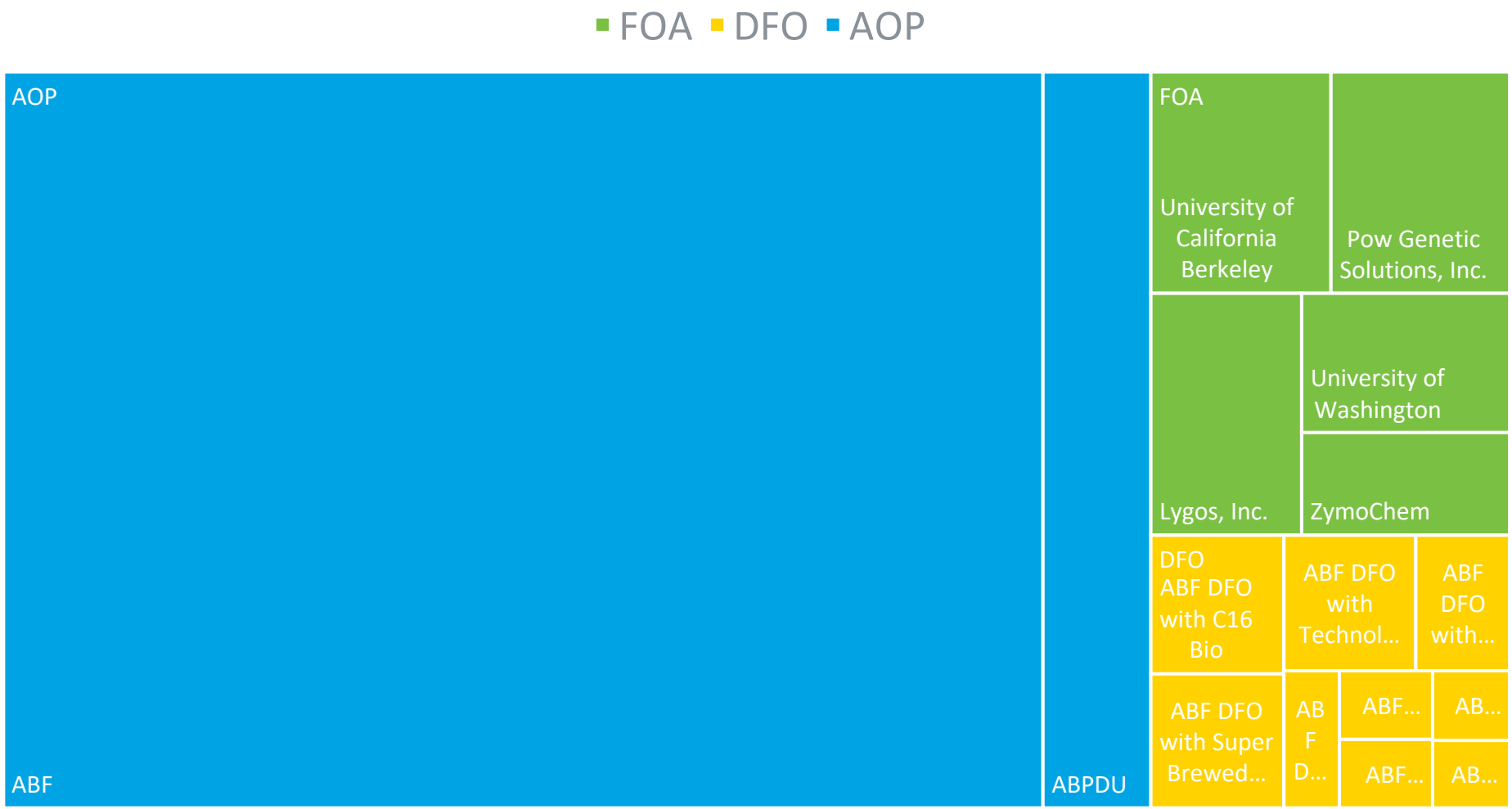
*Budget for 2022 included adding ABPDU to core

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



Portfolio Funding Overview



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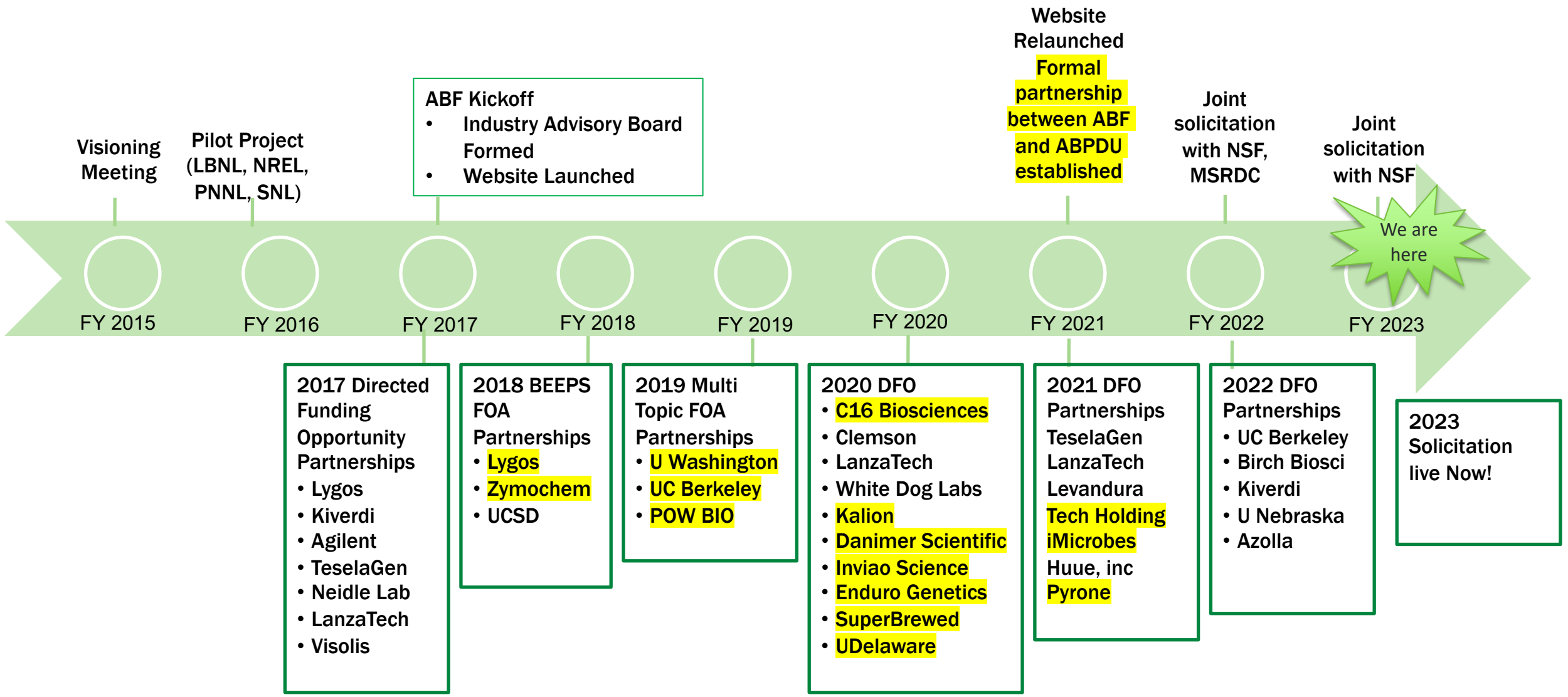
ABF Timeline and History



ABF R&D supports BETO's decarbonization strategies and emphasis areas

- **Decarbonizing energy-intensive industries pillar**:
  - ABF metabolic beachheads supporting and optimized routes to direct replacement chemicals, Performance-Advantaged BioProducts (through PABP mini-consortium collaborations), and CO2 utilization for chemicals (through ABF Direct-Funding Opportunity supported industry collaborations)
  - Additional TEA/LCA modeling to identify chemical markets to prioritize and **potential GHG reductions**
  - *FY22 goal: at least one target molecule achieves estimated MSP within 20% of the fossil feedstock incumbent*

- **Strategic decarbonizing transportation pillar**:
  - The ABF will be leveraging its Design-Build-Test-Learn infrastructure (and data) to the challenges and opportunities of sustainable aviation fuel

- **EERE's diversity in STEM emphasis area**:
  - The ABF is allocating $1M in Directed-Funding Opportunity resources to collaborate with the Minority Supporting Research and Development consortium
  - Multiple milestones to improve DEI within the project

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Key milestones relevant to meeting the overall ABF goal

2017: identify 15 target/host pairs. Complete >1 DBTL cycle for 5 molecules, hitting 100 mg/L for at least 2 molecules

2018: Add 3 additional targets From 10 molecules, produce at least 100 mg/L for 2018 targets and 500 g/L for 2017 targets.

2019: Add 3 additional targets From 10 molecules, produce at least 100 mg/L for 2018 targets and 500 g/L for 2017 targets.

2020: One representative host reach 20 g/L, 0.3 g/L/hr and 50% theoretical yield.

2021: 5 molecules that reach 1 g/L in first organism transferred to a new host, where titers will reach 1 g/L and 2X improvements in terms of DBTL cycle time

2022: At least 10 target/host pairs from the initial ABF meet ambitious metrics

These milestones culminate with value to industry. $15.9M in federal funds, $4.4M in cost share for DFOs.

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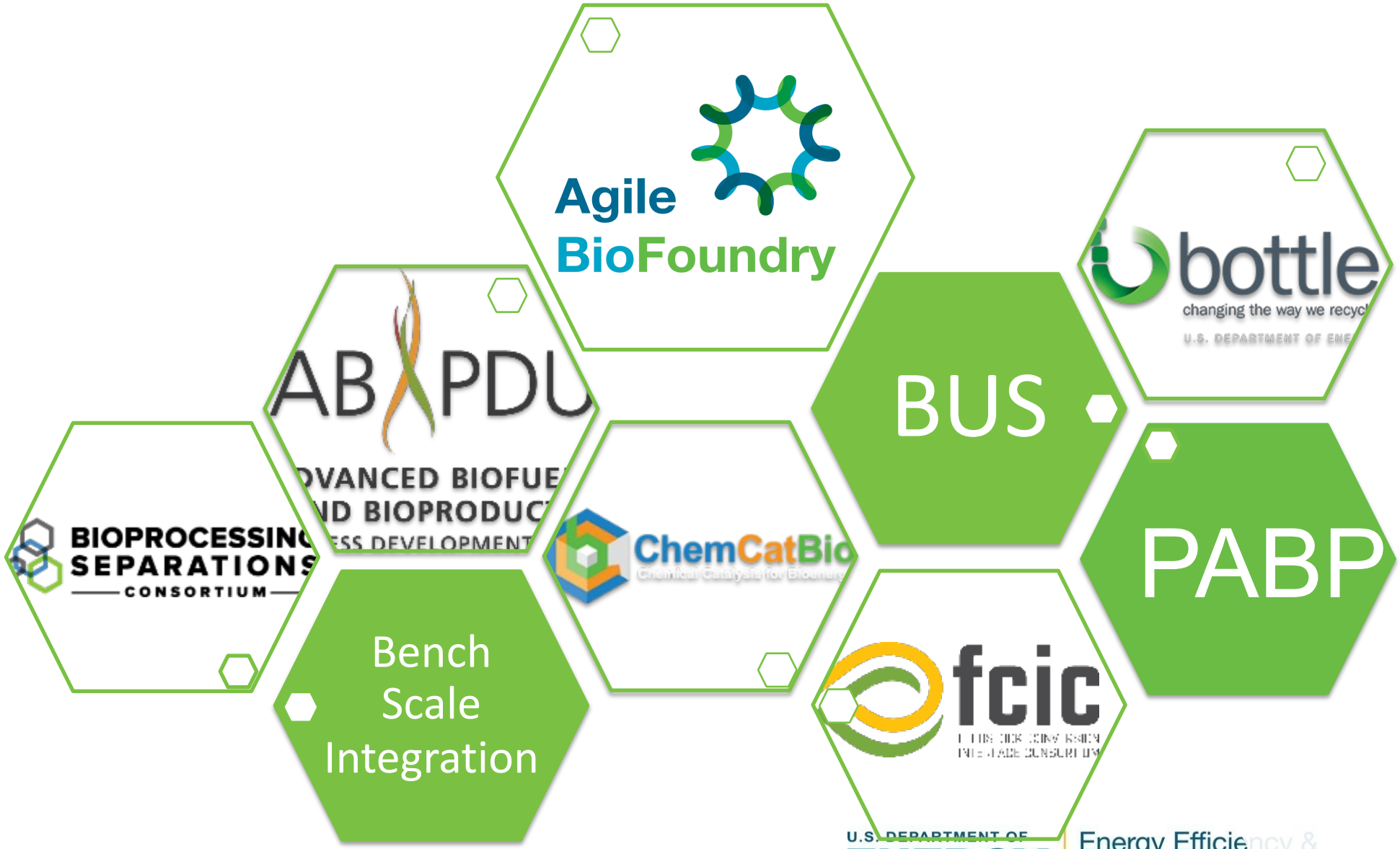
Active collaborations across the BETO portfolio

Provide three additional compounds of interest to PABP Consortium at sufficient quantities for property testing

Reduce glycerol production in BDO-producing strain by at least 30% relative to FY21 baseline.

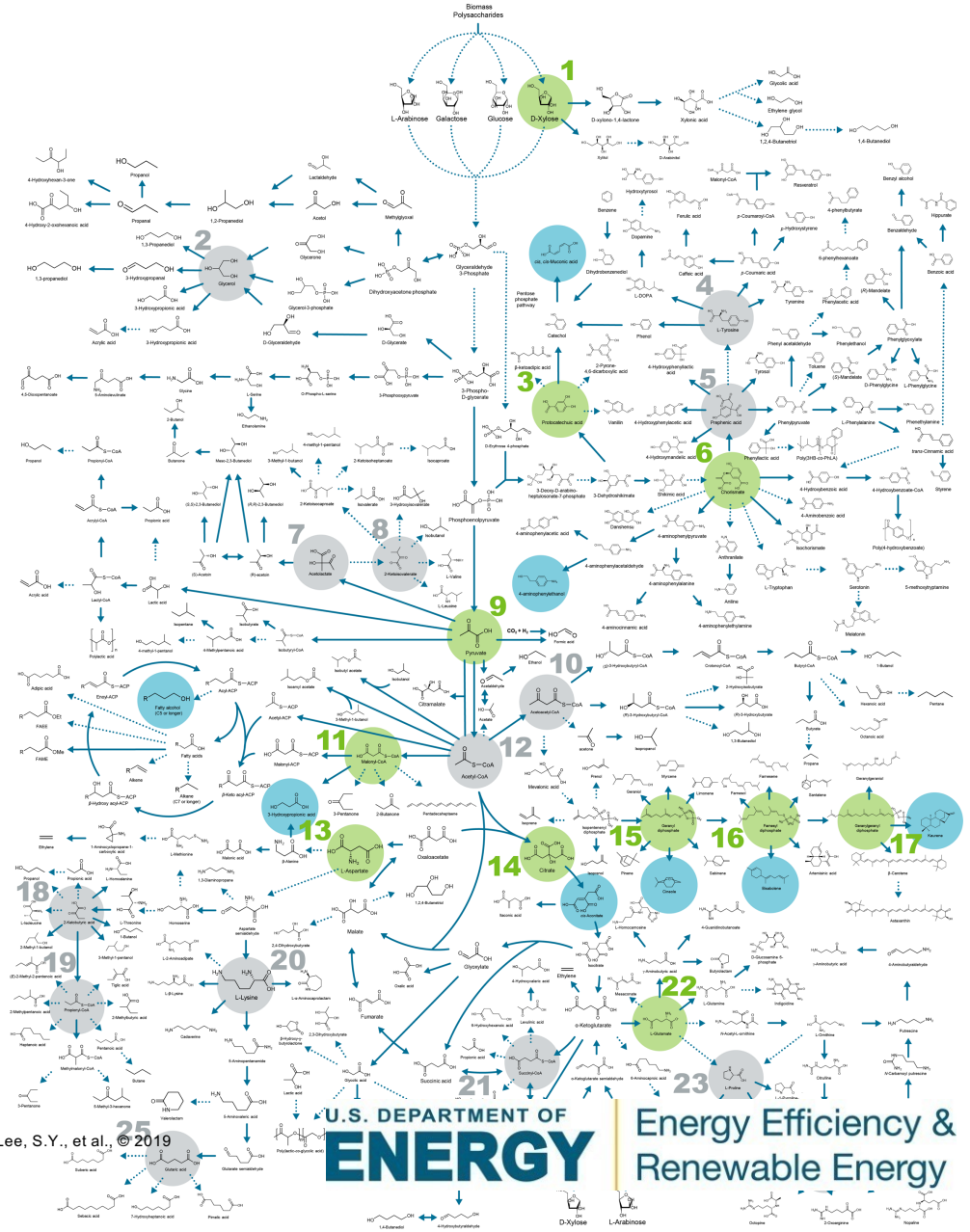
- Closely coordinating with BSI, SepCon, and CCB

Volatile products project connected with SepCon (SepCon Milestone)



ABF has enabled production of many central intermediates and products

01 Xylose (2)
02 Glycerol
03 Protocatechuic acid (3)
04 L-Tyrosine
05 Prephenic acid
06 Chorismate (2)
07 Acetolactate (1)
08 2-Ketoisovalerate
09 Pyruvate (5)
10 Acetoacetyl-CoA
11 Malonyl-CoA (2)
12 Acetyl-CoA
13 L-Aspartate (1)
14 Citrate (2)
15 Geranyl diphosphate (1)
16 Farnesyl diphosphate (1)
17 Geranylgeranyl diphosphate (1)
18 2-ketobutyric acid
19 Propionyl-CoA
20 L-Lysine
21 Succinyl-CoA
22 L-Glutamate (3)
23 L-Proline
24 L-Arginine
25 Glutaric acid



Current ABF target molecules
Current ABF beachhead molecules (# of exemplars)
Potential beachhead molecules

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Production of central intermediates was achieved in many organisms

- 12 active beachheads,
  - 21 exemplar targets
- 15 onboarded organisms
- 9 hosts scaled to >10 L

		Organisms	Criteria: Metabolic Diversity: Beachead: Aromatics	Terpenes	Fatty Acids/ Alcohols (Malonyl-CoA)	Citrate	Pyruvate	Glutamate	Acetoacetyl CoA	Processing Conditions: Low pH: Multi-Celled	Single-Celled	Aerobic/Anaerobic: Aerobe	Anaerobe	Facultative Anaerobe	Feedstock Utilization: Default: Hydrolysate	Alternates: Syngas	Methane/ MeOH	Formate/ CO2/ H2	Photosynthetic (CO2, sunlight)	Unhydrolyzed Biomass
Existing ABF Organisms	1	***Pseudomonas putida***	■				■		■			■			■					
	2	*Acinetobacter baylyi ADP1*	■			■						■			■					
	3	***Corynebacterium glutamicum***	■			■	■	■						■	■					
	4	***Aspergillus pseudoterreus***				■				■		■			■					
	5	***Aspergillus niger***				■				■		■			■					
	6	***Rhodosporidium toruloides***		■	■							■			■					
	7	***Pichia kudriavzevii***									■			■	partial					
	8	***Zymomonas mobilis***					■		■					■	■					
	9	***Bacillus coagulans***					■							■	■					
	10	***Cupriavidus necator***					■					■						■		
	11	***Clostridium tyrobutyricum***							■				■		■					
	12	***Clostridium ljungdahlii***											■			■				
	13	*Clostridium carboxidivorans*											■			■				
Potential Organisms	14	*Rhodococcus jostii*	■		■		■							■	■					
	15	***Lipomyces starkeyi***			■						■			■	■					
	16	*Yarrowia lipolytica*			■	■			■			■			■					
	17	*Rhizopus oryzae*					■					■			■					
	18	***Zygosaccharomyces bailii***					■					■			partial					
	19	*Aspergillus oryzae*				■				■					■					
	20	*Candida boidinii*					■								■		MeOH			
	21	***Methylomicrobium buryatense***					■		■								■			
	22	*Bacillus methanolicus*					■	■									■			
	23	white rot fungus such as *Ganoderma lucidum*																		■
	24	*Myceliophthera thermophila*																		■
	25	*Parageobacillus thermoglucosidasius*					■								■					■
	26	*Synechocystis sp. PCC 6803*		■	■										partial				■	
	27	***Rhodobacter sphaeroides***		■	■									■	partial				■	

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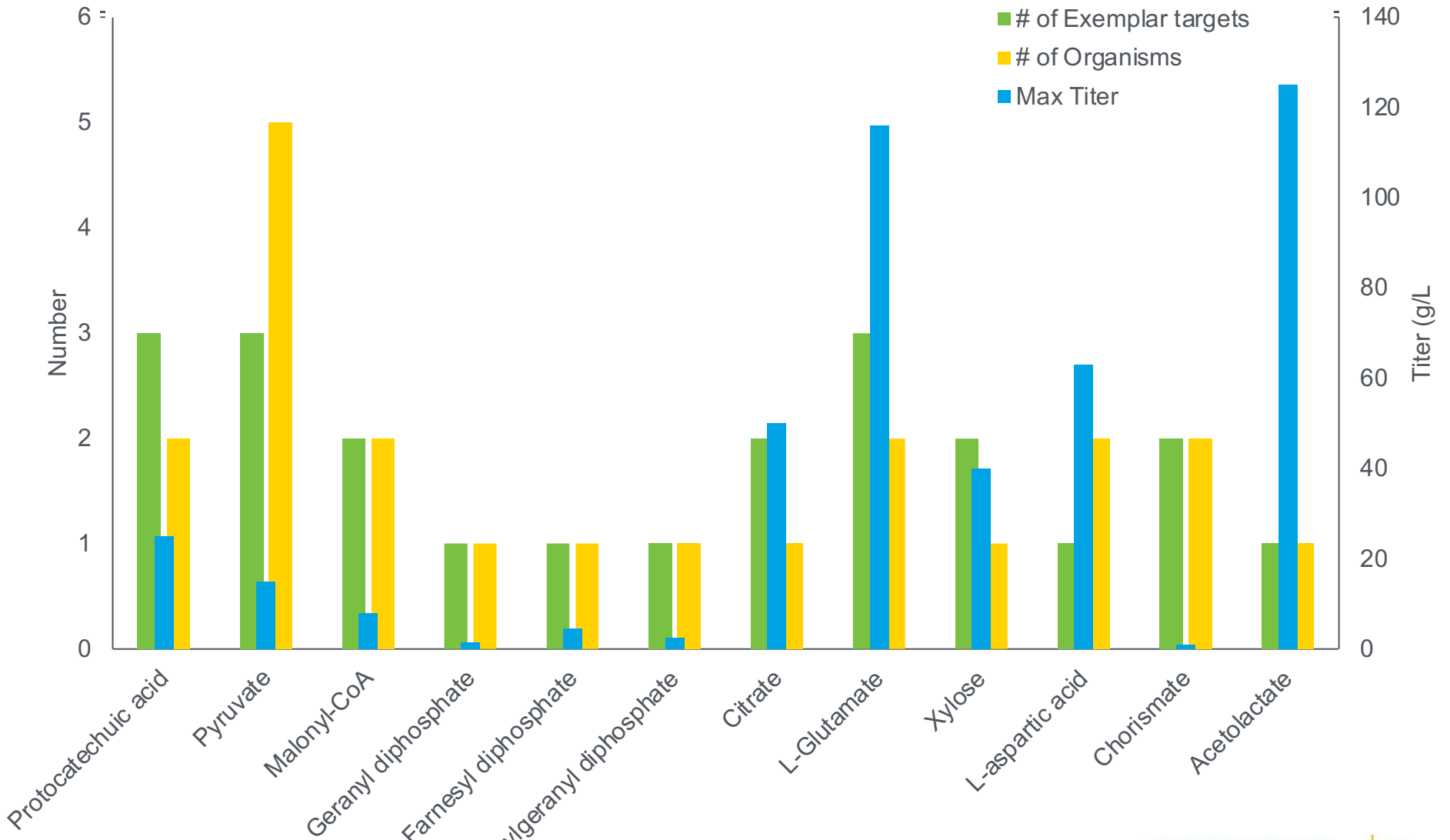
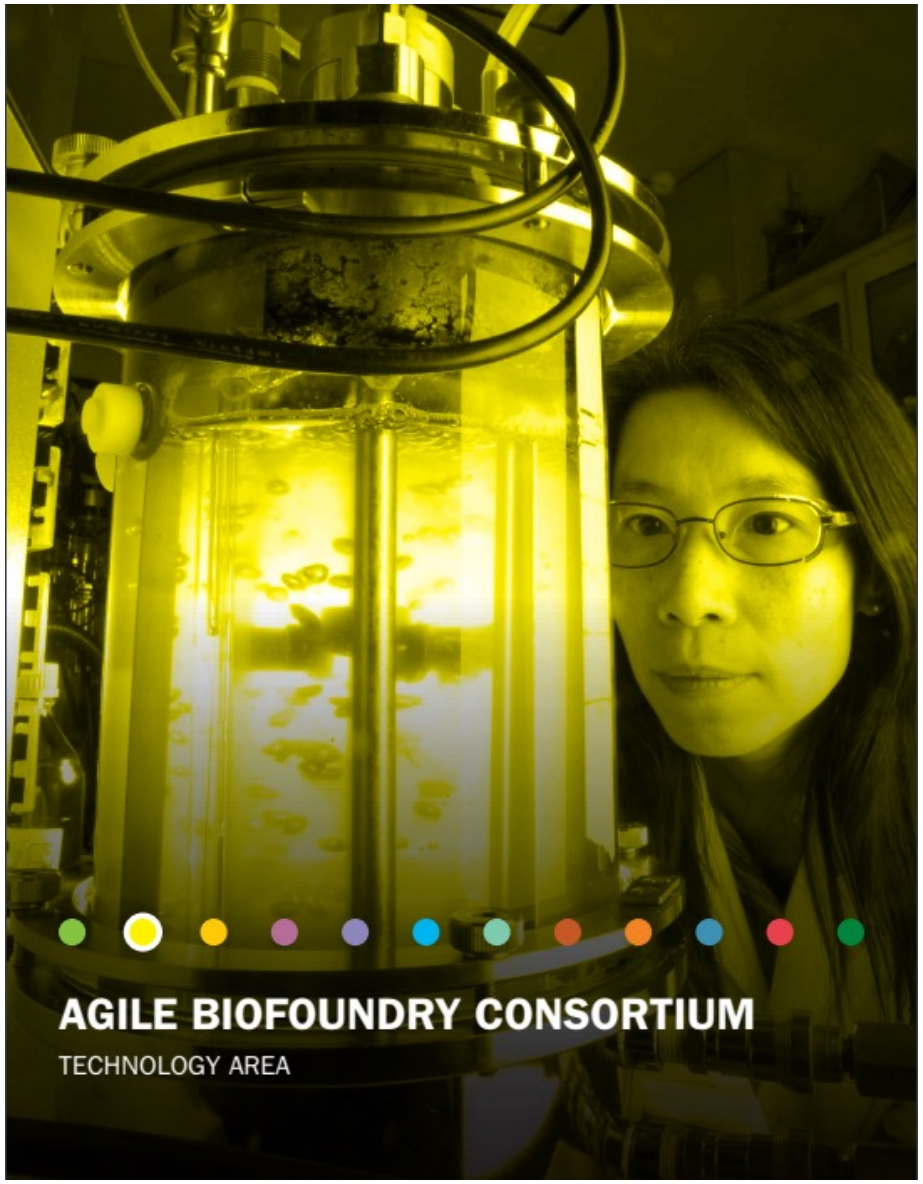
High levels of production have been achieved in many hosts



2021 Peer Review



Recommendations Overview

- Establish clear metrics around ABF goals and outputs.
- Partnership projects should be strategic, with a look at helping companies mature, advancing ABF capabilities, and the entire bioindustry.
- Increase dissemination of ABF capabilities and outreach to stakeholders

RECOMMENDATIONS:

The panel unanimously concurred that the ABF should systematically analyze its medium- and long-terms goals with an eye towards identifying gaps in knowledge and process to ensure a high impact on progressing the U.S. bioeconomy. Currently funded projects fall within the ABF's mission; however, the contribution of each project towards addressing a specific technical gap was not always clear. Further, performing such an evaluation in a continuous fashion will allow the ABF to identify new barriers, ensuring that it will always be working at the leading edge of the field.

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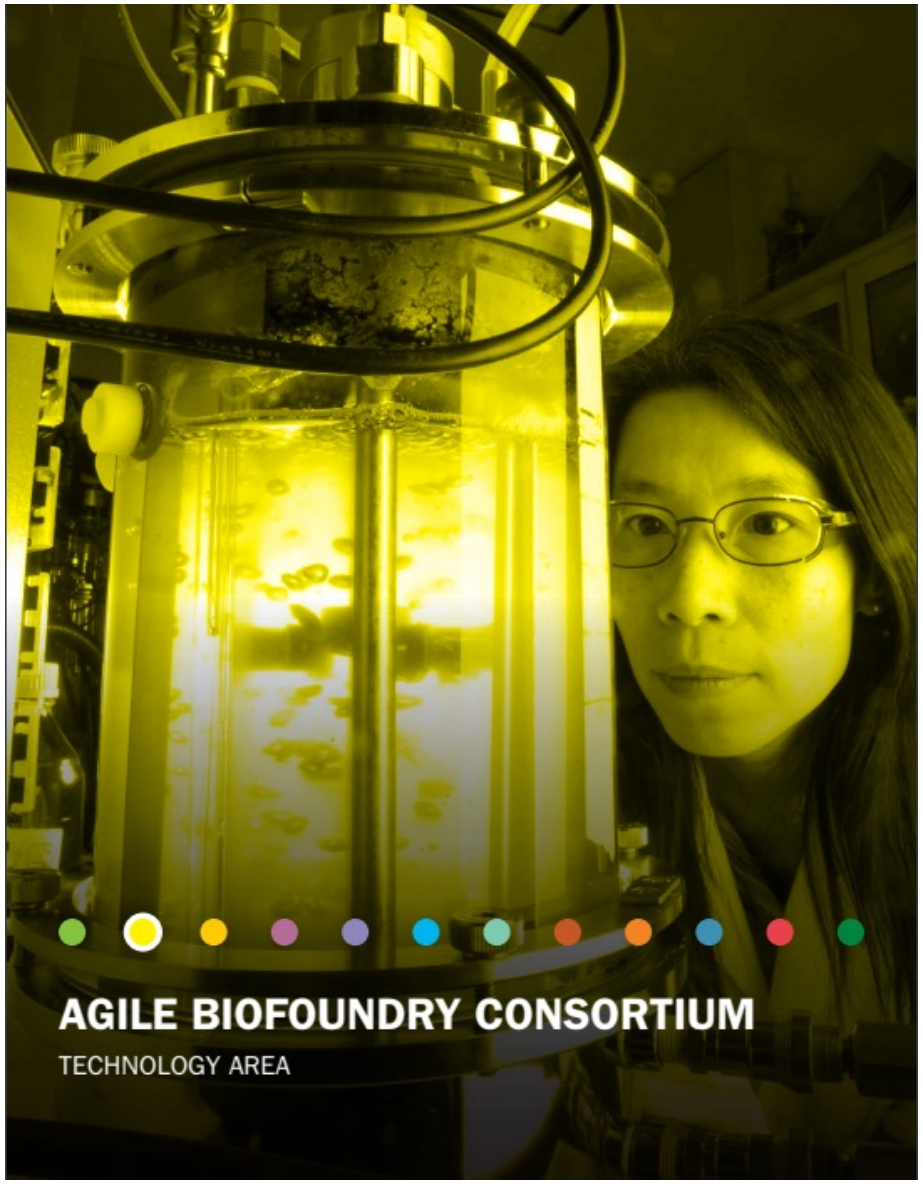
2021 Peer Review



RECOMMENDATIONS:

The panel also agreed on the fact that ABF should develop clear metrics around overall ABF success and the success of specific ABF areas. If the ABF's goal is to reduce the time in bioprocess scale up by 50%, then the metrics at the forefront should be those that are relevant toward this outcome, rather than peripheral measures (e.g. number of onboarded organisms, new equipment acquisitions, academic publications) that do not allow the evaluation of the effectiveness of the ABF to its stakeholders and the government. In particular, standardized metrics across different projects to assess the improvement of the DBTL cycles over time should be implemented. For example, how many strains can be assembled in one day, how many omics experiments can be run in a month, how often are ABF hosts and tools used outside ABF. These metrics, and others like them, should be used to not only assess future projects but current projects to determine their continuation. Overall, the ABF should see an improvement in the metrics, in particular speed and cost reduction, on improving the bioproduction of new molecules over time."

Milestones in response to peer review feedback

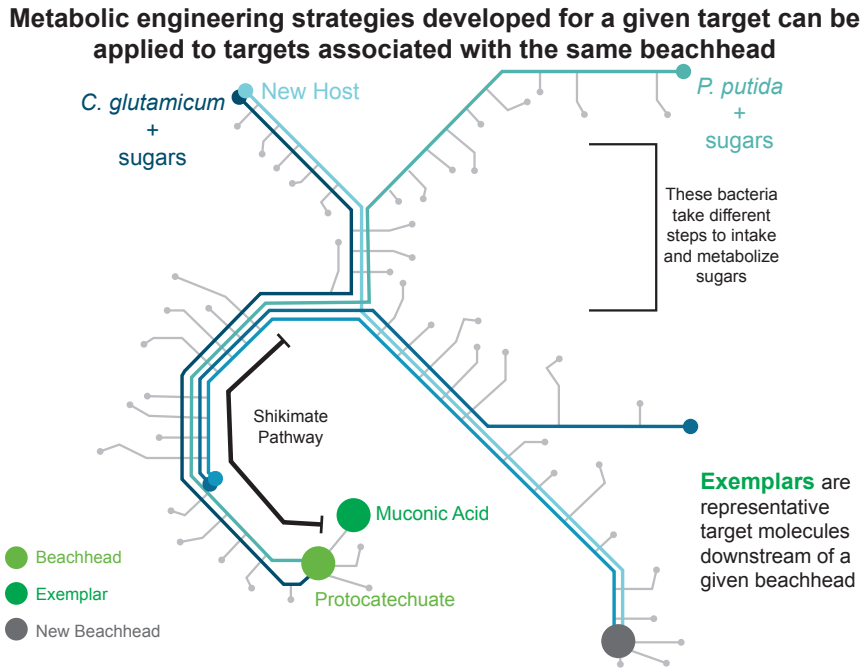
Internal assessment and improvement

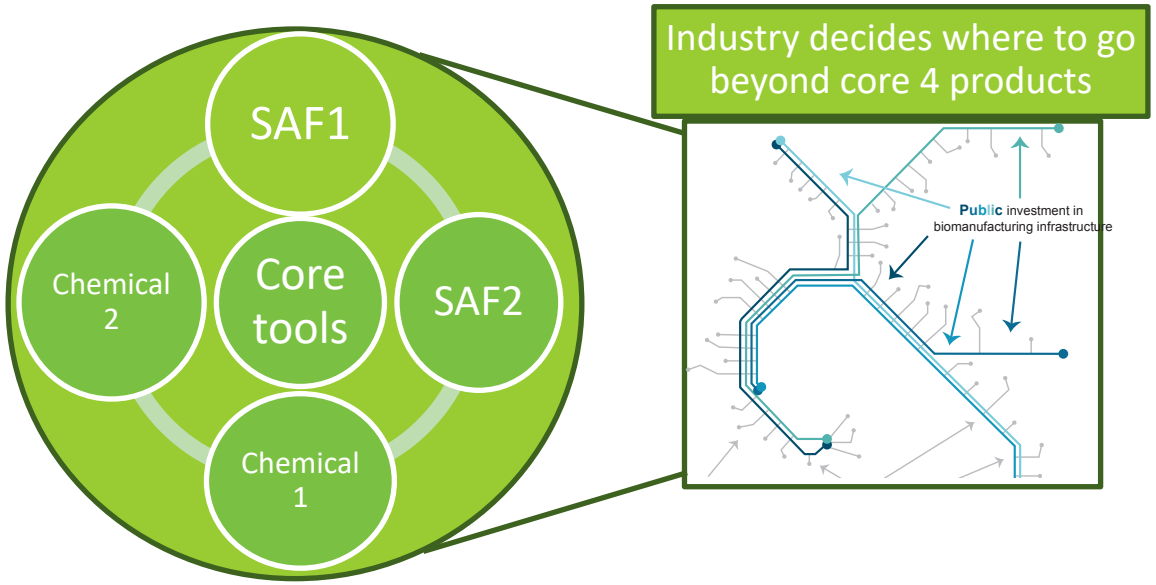
- Develop appraisal framework to determine “breadth vs depth” for host onboarding. Will use input from ABF, industry, BETO, and other stakeholders.
- Develop SOPs, informatic and physical workflows demonstrated across multiple ABF facilities for the design, construction, and sequence analysis of cloned DNA oligo pool libraries; and sequence analysis

External communication and partnerships

- Gap analysis to determine key priorities and challenges for industry around commercialization. Synthesize findings in a report to guide directions and foci of future partnerships.
- Public report released documenting technical advances of the consortium, with emphasis on process economics. The report will also summarize feedback received on the consortium’s progress and impact

New strategic plan to be introduced 4/4/23





New ABF core structure developed to leverage infrastructure built over 6 years, and enable better assessment of progress

Questions?