Agile BioFoundry

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

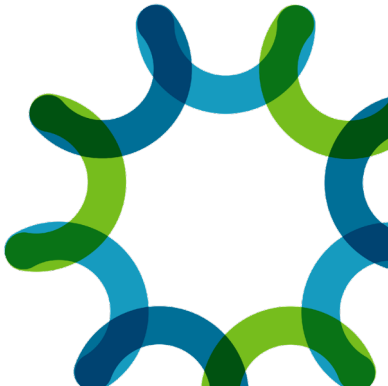
BIOENERGY TECHNOLOGIES OFFICE

DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Funding and Partnering Mechanisms

Wednesday, April 5, 2023
Partnerships

James Gardner
ABF



Overview - Partnering

Overview – Partnering Discussion

- BETO alignment: the goals and value of partnering
- Past partnering mechanisms
- Recent developments in partnering
- Risks to success
- DEI strategies
- Progress and Impact
- Future partnering goals and objectives

Overview – ABF's Partnering Goals

BETO Mission Alignment

Accelerate development of SAFs and chemicals

Support decarbonization of industry

Activities

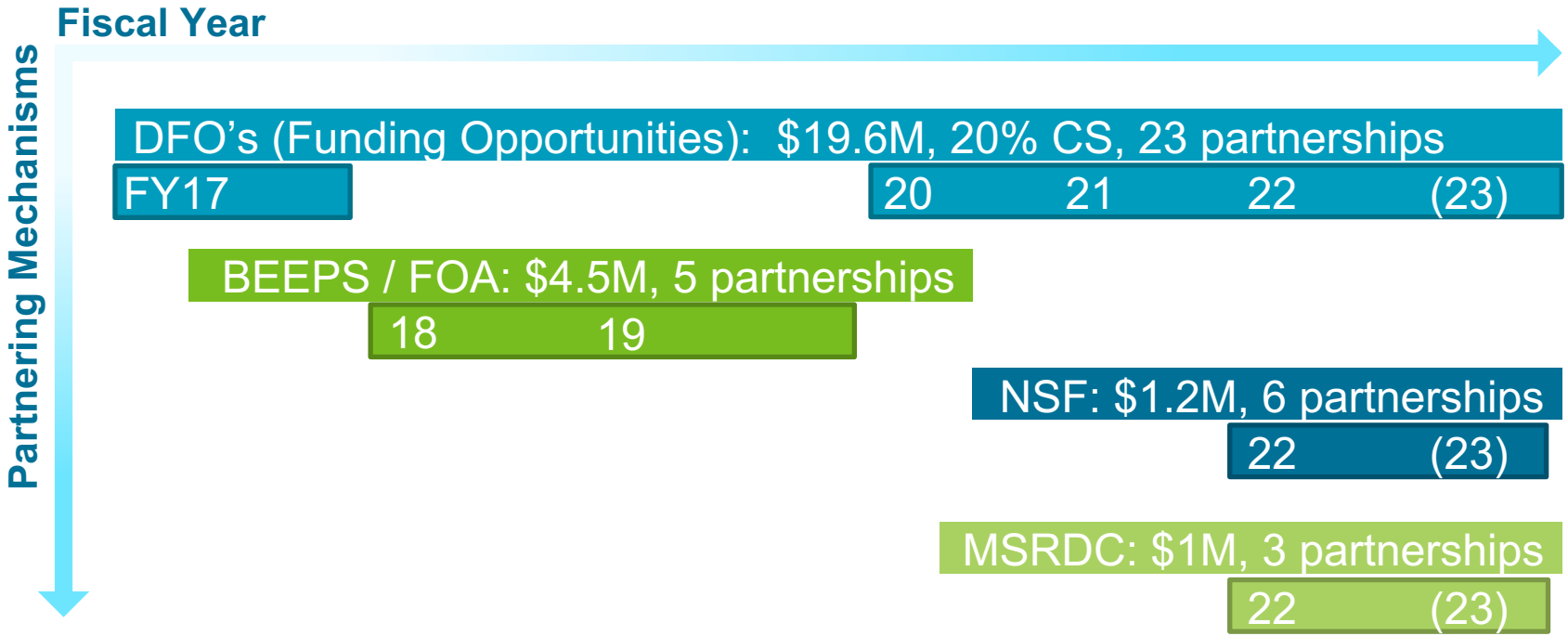
- Partner with industry & academia to offer ABF's capabilities
- Further develop industry-responsive capabilities

High level research question

- What BETO/ABF resources and research capabilities help the partner accelerate toward commercialization?

Overview – ABF's Partnering History

$26.4M DOE invested, 4 partnering mechanisms



Overview – Responses to Previous Reviewers' Comments

Excerpted Comments: …The cost-share requirements [of BETO-funded opportunities] helps secure industry commitment, but it also largely excludes academia…

…the ABF is working to establish an interagency collaboration with the NSF, to set up a funding opportunity that would allow for applicants to utilize their NSF funds as part of their cost share requirement, opening the door for greater academic engagement.

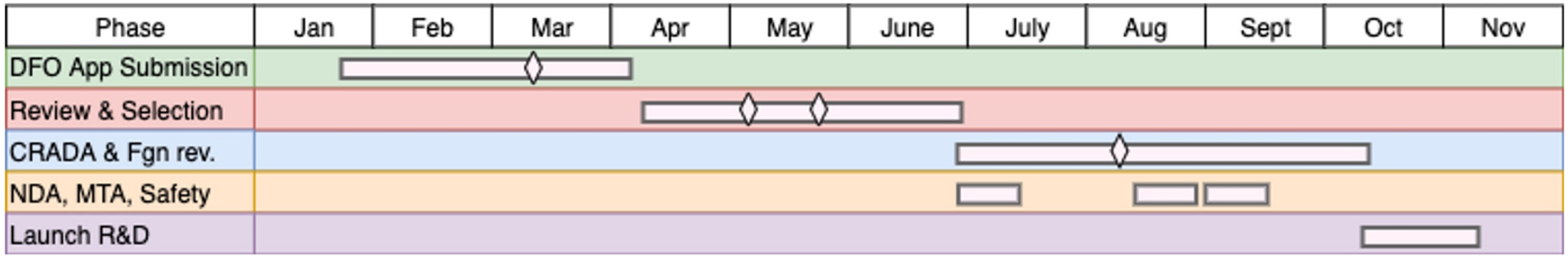
The funding strategy should balance three different priority areas: Supporting industry, increasing uptake and visibility of ABF capabilities, and developing ABF capabilities in a manner that will be of relevance to others. …For the third priority, it was not clear whether it is considered important that these interactions help the ABF build reusable infrastructure that will aid its other partnerships.

…these collaborations have indeed offered value to the ABF, beyond visibility. …the focus of the presentation was more squarely on the unique value which the ABF has immediately offered its DFO collaborators…the ABF evaluates its prospective DFO relationships for their internal strategic value too, [but] that aspect could have been better communicated…

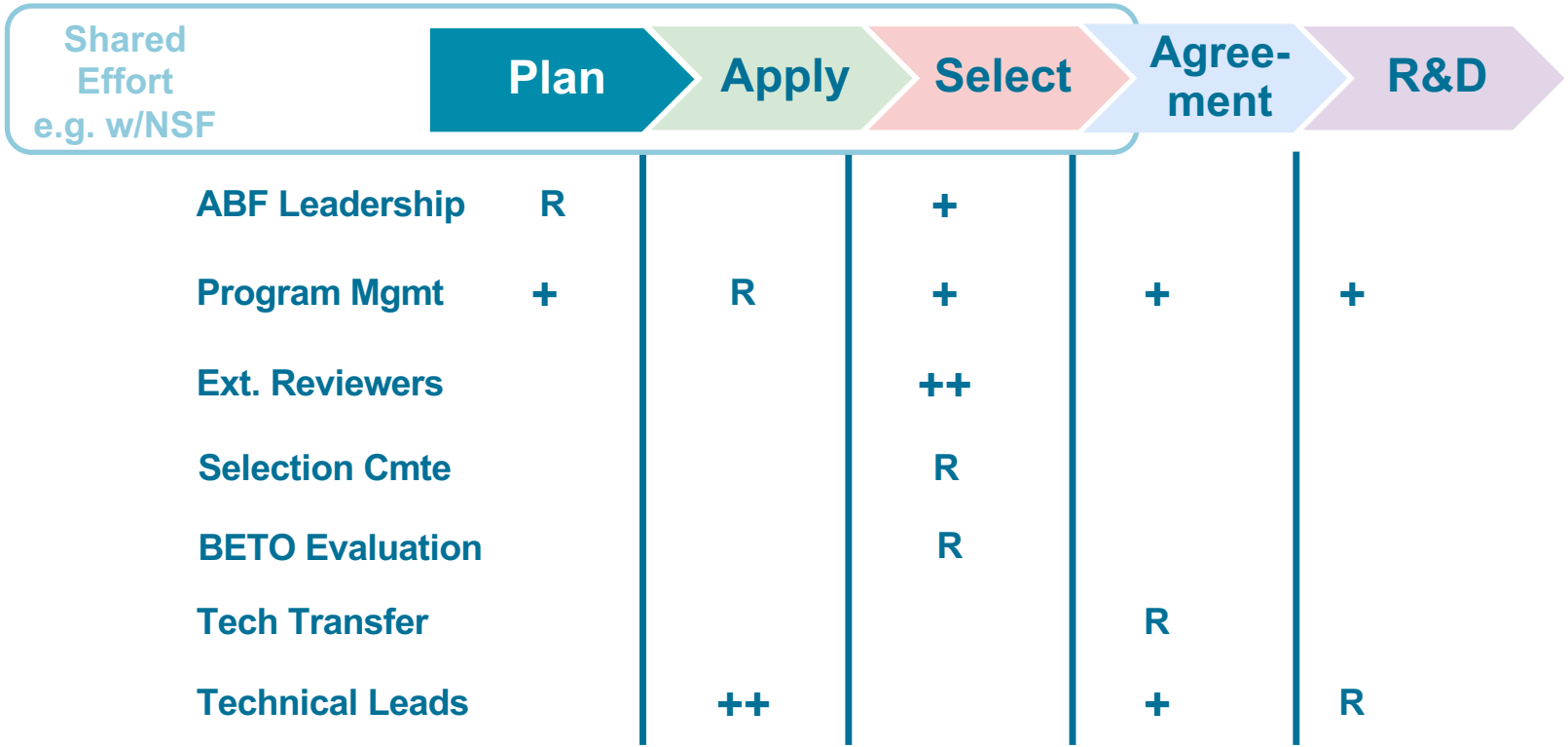
1 - Approach

1 – Approach: Funding Opp. Structure Overview

1) DFO planning; **2)** application submission; **3)** review and selection; **4)** execution of CRADA work agreements; and **5)** execution of safety & administrative reviews, MTAs, and blanket NDAs; **6)** Launch R&D.



1 – Approach: Team Responsibilities – FO Partnerships



+ contributor; ++ key contributor; Responsible

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1 – Approach: Project Tracking

Plan | Apply | Select | Agree-ment | R&D | Impact

Use of the AOP system along with project management tools help standardize projects and PM-related data management throughout the life of the project.

Tri...	Organization	Funding Year	Fund Type	$ DOE	$ Ptnr	$ Tot	% $share	Stage	Task Name	Lev...	Status	% Co...
	Kiverdi	2018	DFO	$900	$386	$1,286	30%	Project	C necator H2/CO2; Kiverdi; Steve Singer	0	In Progres	79
	LanzaTech	2018	DFO	$500	$216	$716	30%	Project	Gas ferm deep learning; LanzaTech; Phil Laible	0	In Progres	77
	Lygos	2018	DFO	$1,000	$429	$1,429	30%	Project	Isobutyric Acid; Lygos (OAs); John Gladden	0	Complete	99
	TeselaGen	2018	DFO	$800	$343	$1,143	30%	Project	ABF Informatics; Teselagen; Hector Garcia Martin	0	In Progres	88
	University of Georga	2018	DFO	$500	$214	$714	30%	Project	Agile Genetics; UGA; Ramesh Jha	0	In Progres	60
	Visolis	2018	DFO	$500	$214	$714	30%	Project	Hi Val Chems; Visolis; Carrie Eckert	0	In Progres	95
	Agilent	2018	DFO	$800	$240	$1,040	23%	Project	Omics workflows; Agilent; Kristin Burnum Johnson	0	In Progres	86
	Lygos	2019	BEEP	$1,400	$1,457	$2,857	51%	Project	Lygos (Machine Learning); BEEPS FOA; Hector Garcia Martin	0	In Progres	96
	Zymochem	2019	BEEP	$784	$573	$1,357	42%	Project	ZymoChem; BEEPS FOA; Steve Singer	0	In Progres	29

1– Approach: Challenges

- **Scalability issues of funding opportunities**
  Each application requires a round-robin coordination call with the applicant and ABF PIs, with numerous follow up discussions to set scope and budget. Process improvements have aimed to make better use of time for the ABF and its would-be partners.
- **Agreement execution lead time**
  CRADA execution timelines can easily exceed 1 year. IDing alternatives to the CRADA where applicable and speeding up CRADA execution.
- **Robust impact assessment**
  Active collection and evaluation of project impact, both during and long after the life of the project, has competed for limited resources. Expanding those resources for this effort is important.

1– Approach: Go/No-Go Decision Points

G/NG is Not Applicable to Partnering Mechanisms

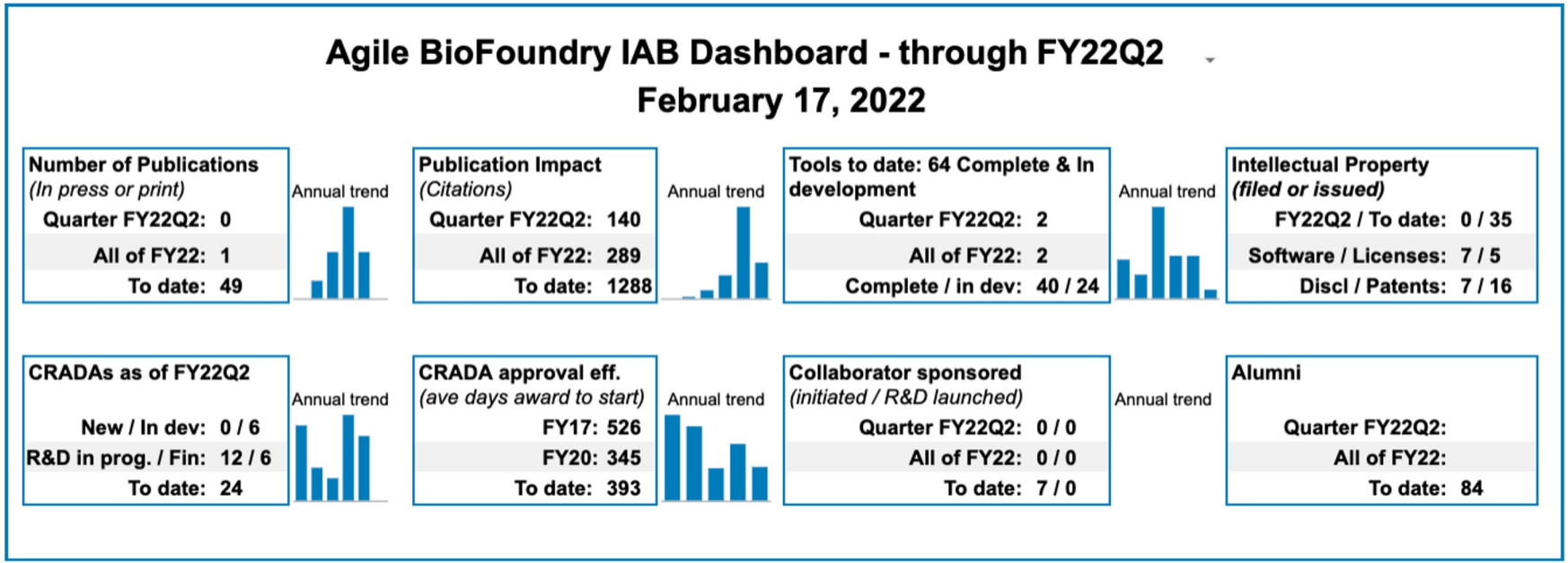
1– Approach: Metrics

- **Funding Opportunity Execution**
  - Available funds
  - Applicant numbers and oversubscription levels
  - Capabilities utilization
  - Satisfaction survey results
  - CRADA execution time

- **Partner R&D Impact**
  - Stated value for industry partner
  - Resulting intellectual property & licensing
  - Publications

1– Approach: Related Projects / IAB

Industry Advisory Board Quarterly Dashboarding as a tool for soliciting feedback on performance. *Example below*



1 – Approach: Risks of Partnership Development

Risk	Sev.	Risk	Mitigation Plan
IP licensing challenges		ABF cannot meet its strategic potential without applying past learnings to future projects.	Work with partners, Lab leads, and Lab tech transfer teams from the outset to maximize availability of IP for subsequent applications.
Untimely agreement execution		Protracted execution times may dampen community interest level for, and impact of, future collaborations.	Utilize recently expanded PM staff and management tools; apply suggestions of ABF's Partnering Working Group for more proactive process management.
Ineffective impact mgmt		Ever-growing numbers of partnerships challenge ABF's current processes for partnership onboarding & management, including impact tracking.	Reduce inefficiencies of ABF's artisanal processes. Use grant management software and greater FTE to oversee projects & measure impact.
Uncertain path to funds-in partnerships		The community appetite for funds-in work agreements with ABF is unclear.	Onboard staff dedicated to business development; work closely with teams at BETO and Nat Labs to assure success.

1– Approach: DEI in Partnering

Goal: Expand ABF's participation in research with investigators

- At minority serving institutions (MSIs)
- From underrepresented communities

MSI STEM Research and Development Consortium (MSRDC)

MSI STEM Research & Development CONSORTIUM

- $1M Funds-out, proposal-based awards
- Three awardees
- Sets stage for larger ABF engagements

ABF is evaluating additional DEI strategies in partnering

1 – Approach: Partnership Portfolio Development

Current and upcoming partnering mechanisms

- ABF funding opportunity (aka CRADA call, DFO) [1° industry]
- NSF-ABF joint solicitation [academia]
- Minority Serving Institutions funding opportunity [academia]
- BioMADE-ABF joint funding call [1° industry; MRL-focused]
- Funds-in: VC, grant subrecipients, direct

1 – Approach: Partnership Portfolio Development

Continuous Improvement

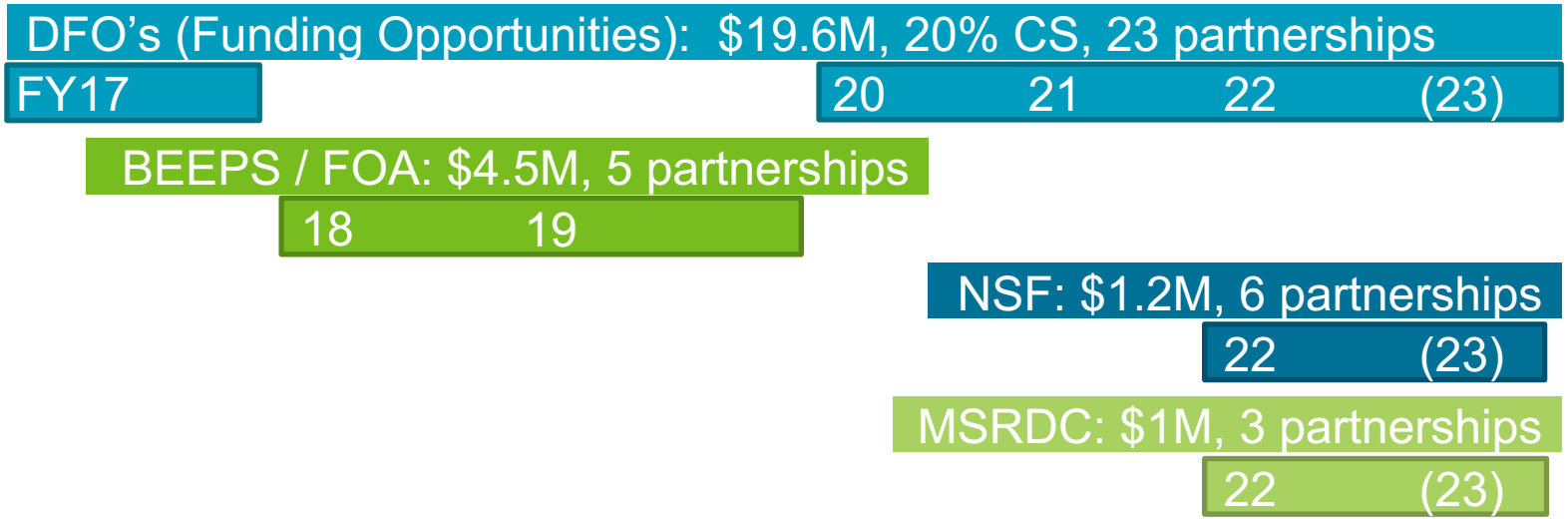
- Standardize partner dev. and speed up contracting
- Better assess impact
- Augment IP licensing

2 – Progress and Outcomes

2 – Progress and Outcomes

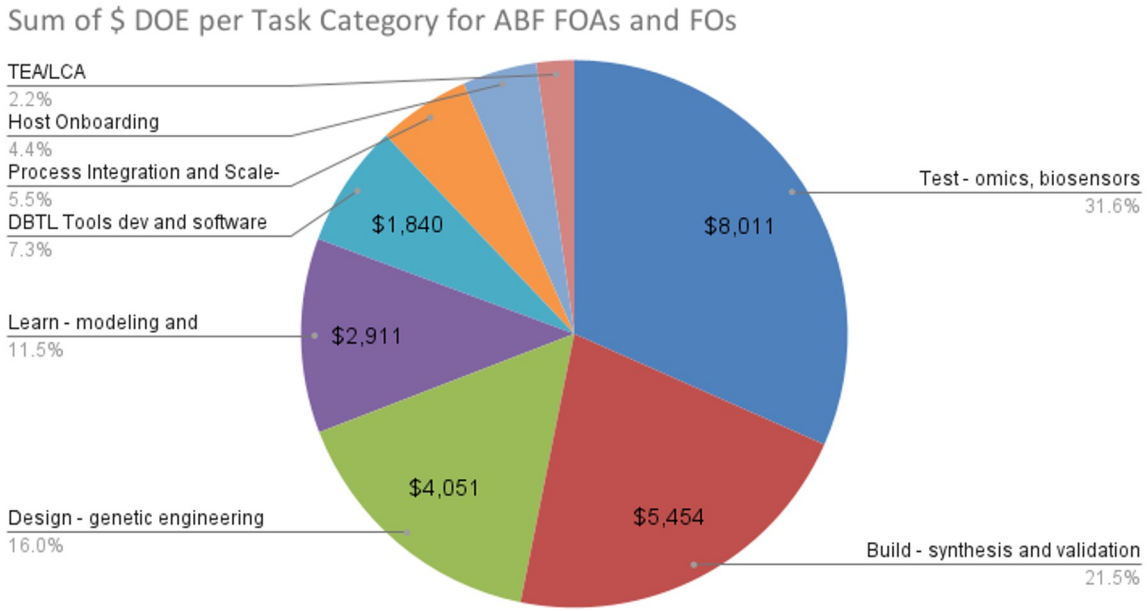
2 – Progress and Outcomes: ABF's Partnering

$26.4M DOE invested, average 3x oversubscription
4 partnering mechanisms, with others in development
22 CRADAs executed
15 CRADAs in development



2 – Progress and Outcomes: Resource Usage

Partner projects are utilizing a wide range of ABF capabilities



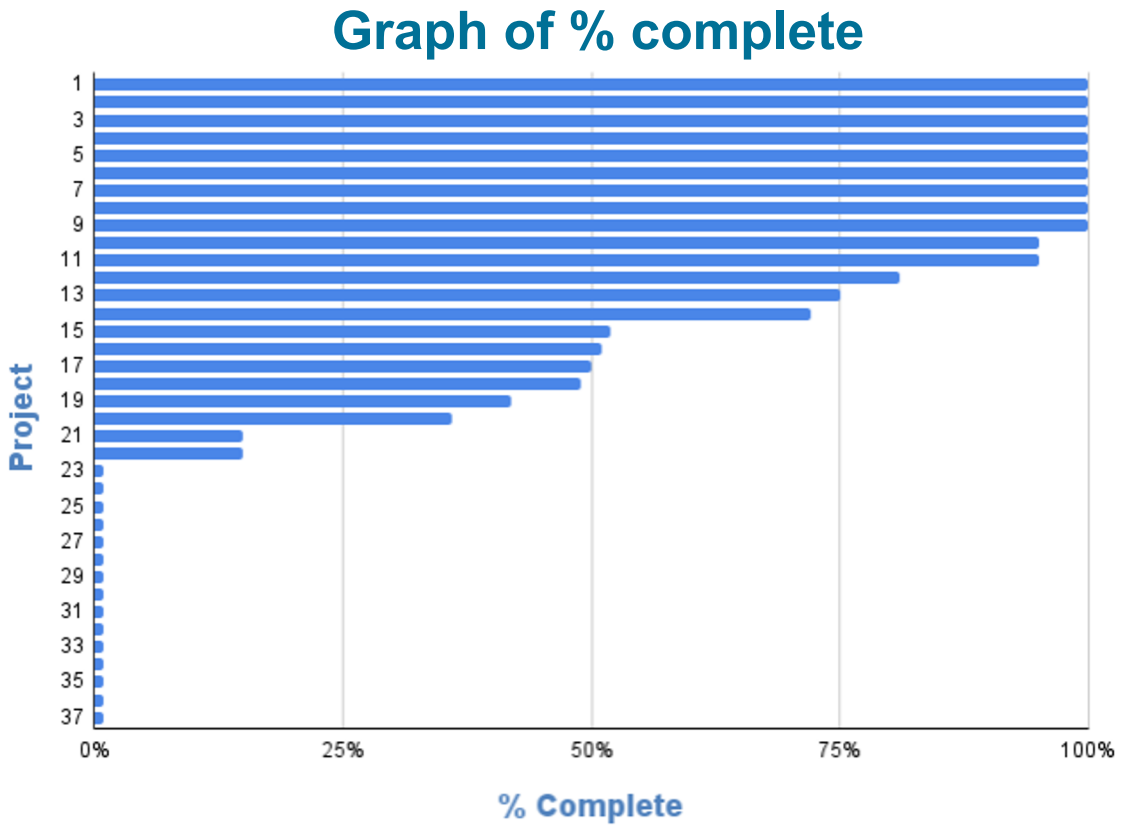
2 – Progress and Outcomes: Project status

9 Complete

13 Underway

15 In Contracting
(ave ≈ 350 days)

ABF Projects are in varying stages of completion and contracting.



2 – Progress and Outcomes: Milestones

FY21Q3 Management - Trends in the Licensing of CRADA-Based IP

- **Milestone:** Industry partner decision making processes analyzed for choice between traditional (exclusive license, shorter option period) and alternative (non-exclusive, longer option period) CRADA IP model that retains incentives for industry yet enables ABF to learn and leverage past experience.
- **Status:** Completed with time extension. CRADA partners typically exercise their option to an exclusive license. ABF is monitoring emerging trends in licensing.

FY22Q2 IE&O - Key priorities & challenges for ABF - gap analysis

- **Milestone:** Analyze ABF capabilities (as they are utilized by collaborators) to determine key priorities and challenges for industry around commercialization
- **Status:** Completed on time. Responding to 2021 Peer Review comments, surveyed respondents for ABF's ability to address 10 biomanufacturing risks, among other topics. See additional slides.

2 – Progress: Partnership Continuous Improvement

Standardize workflows of partner management
Improve impact assessment

Grant management software is in implementation. Moving to such a system will position the ABF for anticipated growth of its partnering budget as well as further diversification of the number and type of partnering mechanisms it offers.
This system will streamline, automate, centralize and standardize steps, starting with applicant eligibility, reviewer intake, application submission, reviewer evaluation, selection, project oversight, and ongoing impact assessment.

Speed up contracting

Ongoing stakeholder discussions are shedding light on bottlenecks in contracting. Strategies to accelerate problematic workflows will be employed with the goals of rapidly identifying appropriate work agreements, collapsing the time required for creating work & background IP statements, and directly shepherding the workflow with stakeholders, throughout the contracting phase.

3 – Impact

3 – Impact: Example Testimonials

"The combination of the multi-omics dataset and ABF's expertise, tools, and technologies have enabled us to further accelerate the strain engineering design, build, test, and learn cycle," **Andrew Conley - Lygos**

"I wouldn't be able to develop a bioproduct on my own, but what I can do is talk to other scientists who have the skills and resources necessary to do this work that has both scientific merit and biotechnology applications." **Ellen Neidle - University of Georgia**

"We didn't have any significant capabilities in-house to engineer these kinds of microbes or fermentations. That's not something we could have done otherwise. Through this partnership, we were able to access these resources without having to build them from scratch in-house." **Deepak Dugar - Visolis**

"...However, [isoprene is] currently only made from petroleum. So it would be very interesting to show that we can produce this at scale using our microorganism and the tools we're going to develop with the Agile BioFoundry." **Michael Köpke - LanzaTech**

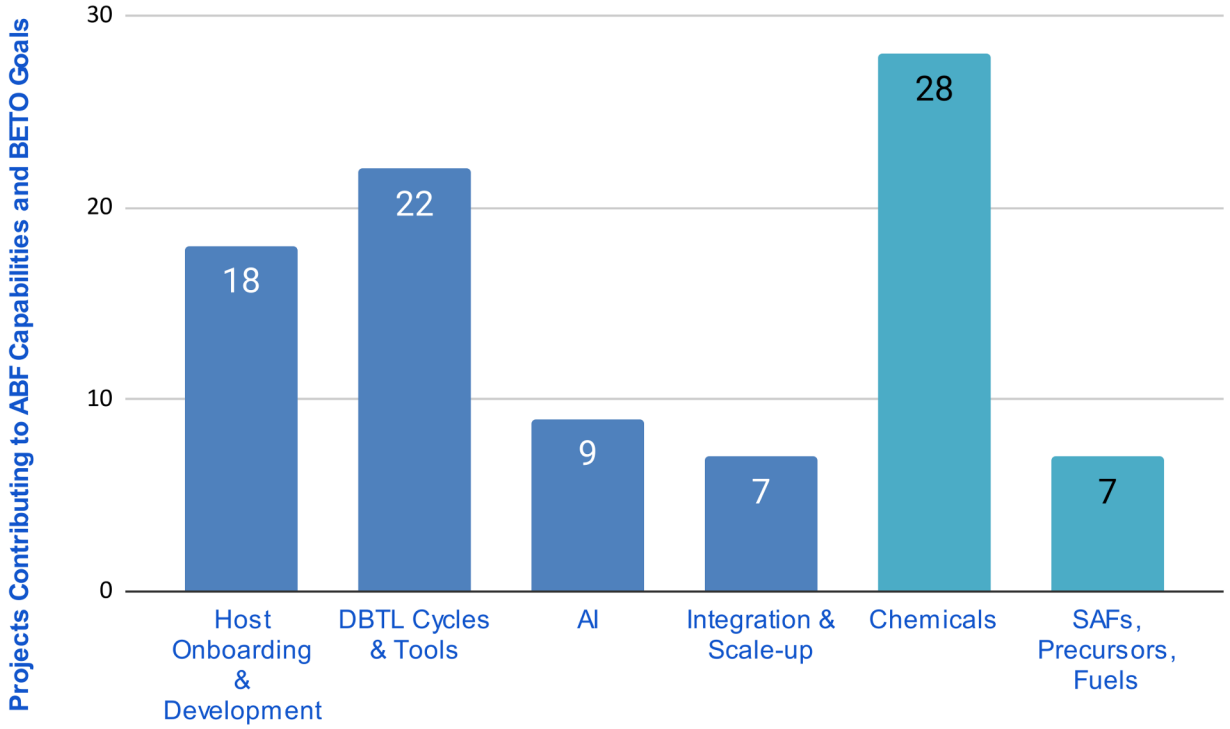
see also: agilebiofoundry.org/news/ | youtube@agilebiofoundry | BETO & ABF comms channels | IE&O

3 – Impact of Partnerships: ABF Development / Responsiveness to BETO Goals

Partner projects expanding knowledge and tools of the ABF.

Partner projects with a target chemical or fuel.

Each of ABF's 22 executed CRADAs bears an option to an IP license. Multiple partners are pursuing these.



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Summary

Summary

- **Approach:** The ABF offers its unique set of R&D capabilities to industry in order to advance BETO's decarbonization goals for bio-based SAFs and other renewable products. As part of a longer-term strategy, the team is diversifying and expanding its external partner portfolio, in part through other funding agency relationships and community partners.

- **Outcomes:** $26.4M DOE invested, 4 partnering mechanisms and counting, and 37 partnerships. ABF is pursuing new approaches to partnering and more rapid contracting strategies.

- **Impact:** Projects are advancing numerous SAFs, precursors, and chemicals as well as a cache of new ABF tools and knowledge. Continuous evaluation has been resulting in many process improvements with each funding opportunity.

Quad Chart Overview

Timeline

- *Project start date: 10/1/2017*
- *Project end date: 9/30/2022*

	FY22 Costed	Total Award
DOE Funding	*10/01/2021 – 9/30/2022*	*$4.9 M*
Project Cost Share *	20-30% in-kind	CS N/A for some partnering

Project Goal

Partner with organizations through funding opportunities and funds-in mechanisms . Use ABF's tools to advance the partners' technologies (.e.g., biologically produced SAFs and chemicals) toward commercial viability.

End of Project Milestone

Finalize communications of project awards; coordinate with Lead Lab tech transfer teams; move forward with execution of CRADA agreements.

Funding Mechanism

Funding Opportunity (aka DFO or CRADA Call)
Joint solicitations: NSF-BETO/ABF, MSRDC

Project Partners*

- ABF National Labs: ANL, LANL, LBNL, NREL, ORNL, PNNL, SNL
- Approx. 30 distinct outside entities.

*Only fill out if applicable.

Additional Slides

FY17 DFO Summary

- **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 DBTL biomanufacturing cycle.
- **Details**: $5M DFO. Projects limited to two years and $500K to $2M total per project. **Compressed timeline.**

Jun 21	RFP live on ABF website
Jul 24	Proposals due
Jul 31	ABF raw scores due
Aug 4	Make initial selections
Aug 11	BETO briefings & concurrence
Aug 14	Notify recipients
Sep 1	Initiate fund distributions

Proposal response highlights:
19 proposals: 18 industry | 1 acad.
$19.2M requested: 4X oversubscription
[Industry is very interested in ABF]

7 Proposals awarded as CRADAs & entered in DOE system as stand alone AOPs

Department of Microbiology
Franklin College of Arts and Sciences
UNIVERSITY OF GEORGIA

VISOLIS
CARBON NEGATIVE MATERIALS

Agilent

LYGOS

Kiverdi

teselagen
BIOTECHNOLOGY

LanzaTech
capturing carbon fueling growth

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FY20 DFO Summary

- **Approach:** Build on successes of FY17 DFO. Expand the timeline. **Involve external reviewers.** Publish special topics to indicate areas of strategic value.
- **Details:** $5.7M DFO. Projects limited to two years and $500K to $2M total per project. Use of **non-negotiable CRADA agreement** to streamline time to project kickoff. External reviewers commented and recommended *Fund*, *Maybe Fund*, or *Do not fund* for each application.

Feb 10	Begin soliciting reviewers
Feb 19	Announce DFO
Feb 20	Applicant discussions begin
Apr 10	Application deadline
May 31	**Complete external review**
Jun 17	Lab vote & final selection
Jul 08	Announce recipients

Proposal response highlights:
17 proposals: 14 industry | 3 acad.
$13.9M requested: 2.4X over

8 Proposals awarded as CRADAs & entered in DOE system as stand alone AOPs

KALION

danimer scientific
A Biotechnology Company

UNIVERSITY OF DELAWARE

WHITE DOG LABS

c16 BIOSCIENCES

Enduro Genetics

invaio

LanzaTech

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4 – Progress and Outcomes: FY20 DFO

Eight successful applications responded well to special topics and demonstrated a breadth of scope, both full and seed.

- Topic 1 Culture heterogeneity and selection of desirable traits
- Topic 2 AI-enhanced Biomanufacturing
- Topic 3 Host Engineering and New Host Onboarding

Organization	Scope	Total $	% Match	Hetero-geneity	AI/ML	Onboard-ing
C16 Bio	Full	$2,047	32%			
U. Delaware	Seed	$625				
Enduro Genetics	Seed	$672	28%			
LanzaTech	Seed	$625	20%			
White Dog Labs	Full	$1,770	20%			
Kalion, Inc	Seed	$532	20%			
Invaio	Seed	$535	20%			
Danimer	Seed	$625	20%			

4 – Progress and Outcomes: FY20 DFO

	Key Characteristics
DOE / Cost share	$1.54M / $598k = 32%
Special Topics	Host Engineering and Onboarding
Capabilities	Strain engineering and -omics
Participating Labs	Sandia; PNNL
ABF PI	John Gladden (Sandia)

Goal: Leverage ABF's onboarded host *Rhodosporidium toruloides* to accelerate biomanufacturing of mid-chain fatty acids, palm oil alternatives.

Approach: Modify endogenous & heterologous fatty acid synthases & reductases, use –omics tools to understand metabolic impacts. Generate commercially relevant titer, rate, and yield.

Impact: Develop a more sustainable alternative for the $60B palm oil market. Palm oil is used in myriad applications but its production is very environmentally destructive.

Risks: Challenges of targeting mid-chain fatty acyl-CoA substrates within a complex fatty acid biosynthesis pathway to ultimately yield a production strain.

4 – Progress and Outcomes: FY20 DFO

	Key Characteristics
DOE / Cost share	$500k / $125k = 20%
Special Topics	Heterogeneity; AI; Host onboarding
Capabilities	Strain engineering, omics, data modeling
Participating Labs	LBNL; PNNL
ABF PI	Deepti Tanjore (LBNL)

Goal: Solve common expression heterogeneity problems in non-conventional yeast *Yarrowia lipolytica*, using ß-carotene as a model system.

Approach: Follow culture productivity, alongside omics & genetic sequencing and advanced modeling to discern alterations as a function of growth and stress.

Impact: Yarrowia is a promising host for high titers of complex lipids. The molecular level understanding of diverse terpenoids will enable an industrially relevant platform.

Risks: It's possible that there are many underlying genetic changes associated with titer instability.

Representative milestone: Task 3: Determine the mechanism by which metabolic burden and culture conditions (e.g., oxygen) alter the overall and local mutagenesis rate, by way of RNAseq (CU/WUSTL), targeted metabolomics & proteomics (PNNL)

4 – Progress and Outcomes: FY20 DFO

	Key Characteristics
DOE / Cost share	$500k / $172k = 28%
Special Topics	Heterogeneity and Onboarding
Capabilities	Strain engineering and -omics
Participating Labs	LBNL, NREL, LANL
ABF PI	Deepti Tanjore (LBNL)

Goal: Demonstrate Enduro Genetics' product addiction technology in non-model organisms to test its portability and scalability.

Approach: Reduce genetic heterogeneity in a 300-liter commercial *Bacillus subtilis* protein production process and develop/replicate this in ABF's *Corynebacterium glutamicum* muconate production strain.

Impact: This will show its portability and help ABF achieve commercially-relevant titers rates and yields of this product.

Risks: Reviewer comment: **"**Although the proposers state that the approach is product and organism agnostic, that clearly is not [proven] based on the proposed scope…"

Representative milestone: Generate a library of stabilized muconate addicted *C. glutamicum* strains.

4 – Progress and Outcomes: FY20 DFO

	Key Characteristics
DOE / Cost share	$500k / $125k = 20%
Special Topics	Onboarding & Heterogeneity
Capabilities	Strain engineering, biosensors
Participating Labs	LANL, ORNL
ABF PI	Taraka Dale (LANL)

Goal: Accelerate strain selection & engineering. Enable parallel development of multiplexed genome editing tools and high-throughput screening methods for volatile or gaseous products via anaerobic fermentation.

Approach: Develop transcription-factor based anaerobic selection and multiplexed genome editing tools for the industrial acetogen *Clostridium autoethanogenum.*

Impact: Demonstrate ABF's genetic manipulation tools in a very significant non-model host, streamlines LanzaTech's synthetic biology efforts, demonstrate the tools' effectiveness to the broader community. CRADA is pending.

Risks: Reviewers: The proposed tasks are doable but ambitious for the proposed timeline. Not clear if the sensor will work in *C. autoethanogenum*.

Representative milestone: Establish an anaerobic fluorescence marker for *C. autoethanogenum* (YFAST).

4 – Progress and Outcomes: FY20 DFO

	Key Characteristics
DOE / Cost share	$1.4M / $370k = 20%
Special Topics	Heterogeneity & Onboarding
Capabilities	Strain engineering, biosensors
Participating Labs	ORNL, LANL, PNNL
ABF PI	Adam Guss (ORNL)

Goal: Develop and demonstrate a stable isopropanol-producing strain of *Clostridium ljungdahlii* to overcome culture instability.

Approach: Use –omics and sequencing to identify changes the microbes undergo as the culture shifts from productive to non-productive. Engineer sensor circuitry to suppress loss of productivity.

Impact: White Dog Labs is near commercial TRY for its mixoferm process to isopropanol, but culture instability limits fermentation times and yields. Applying ABF omics capabilities to solve this problem will pioneer development of a sensor circuit for a *Clostridium* (and be applicable to other anaerobes).

Risks: Reviewers: lacks detailed plan to “mine” the -Omics data from Task 1. As well as identifying & implementing a novel IPA biosensor. The extent of genomic and proteomic changes could complicate which heterogeneity to focus on.

Representative milestone: Task 3: Develop an isopropanol-specific biosensor.

4 – Progress and Outcomes: FY20 DFO

KALION, INC.

	Key Characteristics
DOE / Cost share	$425k / $107k = 20%
Special Topics	AI/ML & Onboarding
Capabilities	Machine learning, -omics
Participating Labs	NREL, PNNL, LBNL, ANL
ABF PI	Violeta Sanchez i Noge (NREL)

Goal: Lower media production cost, increase productivity, and increase the overall commercial feasibility of glucaric acid production.

Approach: Use machine learning to simplify media, use –omics tools to identify correlations between specific metabolites and glucaric acid production rates, to understand production losses over time.

Impact: Glucaric acid substitutes for phosphate in water treatment and other applications (DOE 2004 top 10 molecule). Economically viable production is limited by complex, expensive media, though current titers can reach >90 g/L.

Risks: Little experience at this scale with *E. coli* to know how well the results will translate to bioreactors. “If machine learning fails for the data collected in Task 1.2, we will use a rational approach.” Reviewers: ML/AI experiments should be done after the applicant picks apart [yeast extract] issue to ensure that targets identified are relevant.

Representative milestone: 1.3. Evaluation of diverse cultivation conditions and/or strains derived from machine learning.

4 – Progress and Outcomes: FY20 DFO

	Key Characteristics
DOE / Cost share	$410k / $103k = 20%
Special Topics	Onboarding
Capabilities	fungal strain engineering
Participating Labs	PNNL, NREL
ABF PI	Jon Magnuson (PNNL)

Goal: Develop an efficient bioprocess for the production of an antimicrobial peptide (AMP) in a current ABF host or readily onboarded host for commodity agricultural use.

Approach: Develop GRAS/USDA approved, fungal host expression systems for AMP, identify AMP insensitive hosts, build & test multiple expression strains, and optimize for production.

Impact: Producing a key biological peptide at a scale that has not previously been achieved, could prove revolutionary for agriculture, and it will add to ABF's core capabilities, likely extending to other peptides, proteins, and potentially small molecules. Agricultural pesticides are used at a massive scale in the US (1 billion lbs/year) accounting for 5-15% of the energy input to crop production.

Representative milestone: Task 4: Scale up the process in stirred tank reactors and produce at least 100 grams of the target AMP.

4 – Progress and Outcomes: FY20 DFO

danimer scientific
A Biotechnology Company

	Key Characteristics
DOE / Cost share	$500k / $125k = 20%
Special Topics	AI/ML & Onboarding
Capabilities	AI, strain development, -omics
Participating Labs	NREL, PNNL
ABF PI	Gregg Beckham (NREL)

Goal: Use ABF's Learn tools and systems biology capabilities to produce tunable combinations of PHAs, with different chain lengths, enabling new biodegradable plastics with novel properties.

Approach: Use an integrated DBTL cycle workflow to tune PHA polymer composition. Integrate exogenous gene cassettes enabling production of C8, C10, and C12 monomers into Danimer's NODAX PHA strain and iteratively engineer targets derived from multi-omic modeling.

Impact: Enable ABF to contribute to the development of non-model strains currently in use in a true industrial biomanufacturing process. Danimer is a large company and the only current US producer of PHAs. Products resulting from this collaboration could be commercialized in <5 years.

Representative milestone: Task 3: Evaluate PHA compositions produced by at least 4 strains with different expression levels of phaG, alkK, and phaC.

FY21 DFO Summary

- **Approach:** Apply lessons learned for over a dozen process improvements. Continue use of non-negotiable CRADA. Use **scoring and ranking of applications** to augment the role of external reviewers. Pool top ranked applications; cutoff = 1.5x available funds. ABF selection committee votes per strategic program-building.
- **Details:** $5M DFO. Projects limited to two years, minimum $400K scope and maximum $2M DOE contribution per project.

Jan 21	RFP live on ABF website
Mar 15	**Abstracts due**
Apr 9	**Applications due**
May 10	Reviewers normalize **scores**
Jun 4	ABF Selection committee
Jun 29	DOE complete review
Jul 3	Announce recipients
Aug 17	Complete SOWs
Oct 1	Execute agreements
Nov 1	Commence R&D

BETO Mission: *Decarbonize the industrial sector*

7/7 selection committee votes; Score = 7.463, Excellent

Goal: Leverage current best practices to develop and validate an interoperability standard for fermentation process data, near real-time intelligence gathering, and fermentation system tuning.

Approach: In partnership with Phycus, use production optimization of polyglycolic acid in *E. coli* and 3-hydroxypropionic acid in *R. toruloides* as model systems for fermentation management, data acquisition of large datasets including HT FACS screening assays, online & offline fermentation data, and analytics via Teselagen and ABF modeling platforms. Evaluate recommendations to reduce DBTL cycles.

Impact: Establishing universal data formats holds promise for advancing in silico bioreactor experiments by laying the foundation for deploying AI on community-generated data, saving resources and reducing the commercialization timeline. These technologies are essential to the robust bioeconomy required to decarbonize the industrial sector.

Representative milestone: Task 4, MS1: Development of a parser generation tool that can accept arbitrary fermentation data and generate a savable, shareable parser for reading the data into an internal data structure.

Proposed Budget: 2 years, 480 LBNL, 240 SNL, 80 PNNL, 200 CS

LanzaTech

Expanding the Non-Model Organisms Clostridium spp. *Genetic Engineering Toolbox to Accelerate the Discovery Process of Next-Generation Biofuels and Chemicals*

BETO Mission: 1. *Decarbonize the transportation sector, 2. Route to SAF*

6/7 selection committee votes; Score = 7.667, Excellent/Outstanding

Goal: Accelerate strain engineering in *Clostridium spp*.

Approach: Develop tools to overexpress bottleneck enzymes (e.g., isoprene synthase) and pathways and develop microfluidic anaerobic HTS techniques. Combine these tools into LT's modular vector system.

Impact: Waste carbon oxides enables biomanufacturing with GHG emissions, saving 60% to 90% compared to current production routes from fossil feedstock. Gas-to-acetone *Clostridium autoethanogenum* outperforms other bioprocesses and is competitive with current petrochemical production routes. But production of longer chain molecules remains a challenge. Arduous genomic engineering workflows and lesser screening methods cannot keep pace with strain throughput, when compared to model organisms. Improving isoprene production will enable SAF production. GHG metrics directly contribute to decarbonizing the transportation sector.

Representative milestone: Subtask 3.3 - Apply CRISPR to optimize flux to isoprene (Q6-Q8, ORNL): Use CRISPR tool to increase flux towards isoprene. Optimize pathway balance with promoter systems developed in Task 1.

Proposed Budget: 2 years, 400 ORNL, 275 PNNL, 75 ANL, 187.5 CS

Levadura Biotechnology

Production of Triacetic acid Lactone (TAL)

BETO Mission: *Decarbonize the industrial sector*

6/7 selection committee votes; Score = 6.433, Very good

Goal: Efficient production of the ABF beachhead molecule malonyl-CoA for biosynthetic purposes, primarily as applied to polyketide production.

Approach: Microbial production of TAL via unconventional yeasts can be cost competitive. Fatty acids will serve as direct source of the polyketide metabolic precursor, acetyl-CoA, and are an underutilized waste stream from a range of industries. Starting strains efficiently consume fatty acids already. The novel pathway will: 1) use beta-oxidation derived acetyl-CoA to produce malonyl-CoA, 2) reside in the peroxisome, 3) facilitate adaptive lab evolution (ALE), and 4) be optimized by efficient carbon dioxide fixation.

Impact: TAL production from fatty acid waste streams (e.g, vegetable oil production, water treatment, and paper production) itself leads to many otherwise fossil-derived products. Raising flux through malonyl-CoA improves the viability of a wide range of downstream targets. TAL is an attractive platform molecule that can replace several commodity chemicals derived from petroleum. Biological production will contribute to reduced environmental impacts.

Representative milestone: Task 2 - Adaptive Lab Evolution Production of strains that can produce TAL from fatty acids by a fermentation process. Target titer of 5 g/L in shake flasks, ≥10 g/L by stirred tank.

Proposed Budget: 2 years, 400 SNL, 200 PNNL, 150 CS

TECHNOLOGY HOLDING
SUSTAINABLE SOLUTIONS

Engineering b-ketoadipic acid production in* Pseudomonas putida *KT2440

BETO Mission: *Decarbonize the industrial sector*

6/7 selection committee votes; Score = 6.333, Very good

Goal: Develop performance-advantaged bioproduct, ß-ketoadipic acid (ßKA), production in *Pseudomonas putida* at 40 g/L titer, 0.5 g/L/h productivity, and a 40% molar yield from hydrolysate sugars, at the 1 kg scale.

Approach: Generate non-intuitive targets through transposon library construction, with an existing ßKA biosensor as a screening tool; use ALE in parallel. ID bottlenecks and competing pathways. Test newly engineered strains in 10 L bioreactors for growth, production, and purified material properties.

Impact: This project capitalizes on BETO investments in the production of an adipic acid replacement and aims to generate enough material for testing with earnest offtake partners in the fashion industry. This work also introduces new approaches to developing non-intuitive genetic targets, and it further matures the host *P. putida* by offering rich datasets for subsequent Learn efforts. Replacing adipic acid with bio-derived ßKA holds the potential to eliminate the single largest contributor to nitrous oxide emissions, adipic acid, therefore providing a route to decarbonize the industrial sector.

Representative milestone: Task 2, global metabolomics milestone: ID of bottlenecks, off-target carbon sinks

Proposed Budget: 2 years, 740 NREL, 360 LANL, 300 PNNL, 350 CS

Deep Learning for Process Improvement and Predictive Scale-Up of Gas Fermentation

BETO Mission: *Decarbonize the industrial sector*

5/7 selection committee votes; Score = 7.175, Excellent

Goal: Lack of commercial gas fermentation scale-up facilities and publicly available knowledge are a barrier to use of gaseous feedstocks. This collaboration will leverage multi-scale experimentation to create a predictive model that identifies productivity improvements, forecasts performance, and enhances process robustness for gas fermentation using IM's synthetic methanotroph *E. coli* strain and 3HP production.

Approach: Identify key drivers of protein expression and impacts on 3HP TRY. Bioprocess and proteomic data will undergird predictive models of robustness and performance. Validation experiments will identify strain & process improvements followed by iterative validation of improvements at bench-scale and 300 L. This will reduce gas fermentation scale up risk prior to significant capital investment.

Impact: This collaboration will allow national laboratories to gain experience with gas fermentation technology across scale and provide the data required to raise capital for a large-scale system. Gas fermentation enables conversion of potential GHGs into a sequestered product, providing an opportunity to avoid emissions from the feedstock and also reduce emissions associated with 3HP production from fossil sources.

Representative milestone: Task 5 Build and test 10 model predictions at Ambr scale MS: 3x improvement of 3HP productivities over baseline.

Proposed Budget: 2 years, 750 LBNL, 250 ANL, 500 CS

huue.

Omics-driven iterative development of indigo strain

BETO Mission: *Decarbonize the industrial sector*

5/7 selection committee votes; Score = 6.733, Very good/Excellent

Goal: Build on existing biotechnology for bioproduction of indigo dye, a drop-in replacement for fossil derived indigo. Use high throughput engineering and other advanced strategies to characterize the cellular metabolism and develop new strains.

Approach: Determine abundances of proteins and metabolites. Develop a fluorescent biosensor for key bottleneck enzymes, rational mutation identification, library generation, screening, and analysis. Develop a colorimetric colony-picking assay. Multiple rounds of semi-rational engineering. Combine top enzyme hits with optimized pathway. Determine protein and metabolite levels of top strains.

Impact: New biosensors, new insights into highly exploitable, aromatic amino acid pathways, and raising supply for robust industry demand of sustainable alternatives to fossil-derived dyes. A new route to a fossil-derived product introduces an opportunity to reduce the climate impacts of indigo production.

Representative milestone: Task 6: Combine top enzyme hits with optimized pathway Outcome: Consolidation of top hits from task 3 and 5 into 24-96 attempted designs

Proposed Budget: 2 years, 230 PNNL, 225 LANL, 225 LBNL, 170 CS

FY21 DFO Lessons Learned

Changes ABF made to the application & review process were a significant improvement relative to the FY20 DFO process:

- The applicant requirements response form ensured all parties stated their understanding of the process requirements at the outset.
- Starting outreach about a month earlier added much needed breathing room.
- COI reviewer screening forms explicitly included ABF lab leads, along with applicants.
- Scoring applications added rigor to the review and ranking process.
- The selection committee only focused on top-ranked applications.
- Applicants used milestones & milestone tables more effectively and consistently.
- Documentation was much clearer and more consistent for eligible entity and cost share information, along with addition to FAQs from individual questions, where appropriate.

New improvement opportunities:

- Consider asynchronous meetings and recorded mock coordination calls to simplify the laborious and error-prone process of coordinating team discussions among ABF leads and prospective applicants.
- Consider a restructuring of the introduction section so that it serves to succinctly summarize the entire application, to better inform all reviewers during the final ranking.

FY22 Funding Opportunity Summary

- **Approach:** Apply lessons learned from previous cycle such as coordination call scheduling and reviewer panel development.
- Continue using non-negotiable CRADA and continue practice of scoring & ranking of applications by external reviewers.
- Pool top ranked applications; cutoff = 1.5x available funds. ABF selection committee votes, per strategic program-building.
- **Details:** $3.75M Funding Opportunity. Projects limited to two years, minimum $400K scope and maximum $2M DOE contribution per project. Max

Mar 18	RFP live on ABF website
May 20	Coordination calls complete
July 8	Applications due
Aug 22	Reviewers ranked proposals
Aug 26	ABF Selection committee

Targets

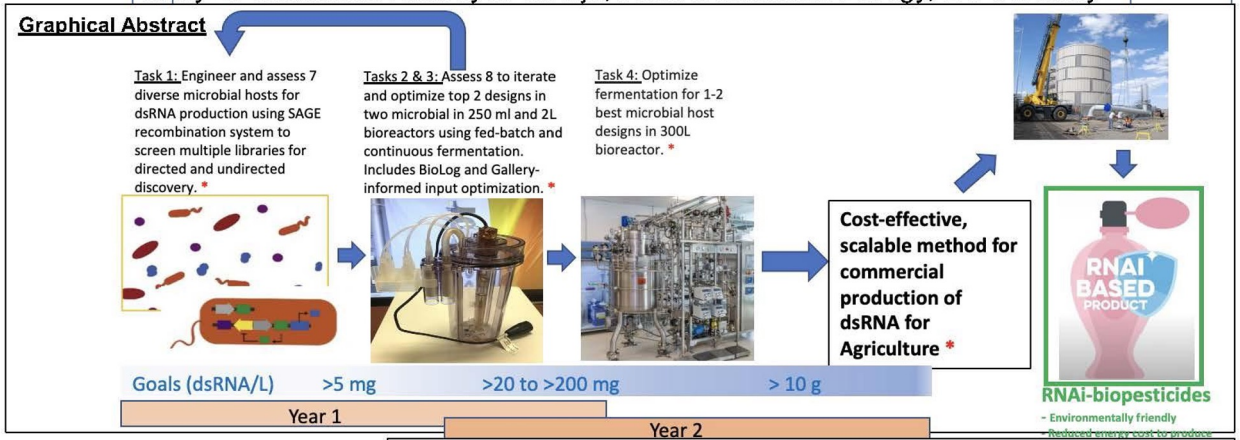
Aug 30	DOE completes review
Aug 31	DOE obligates funds
Sep 06	Inform applicants
Oct 26	Announce recipients
Nov 31	Complete SOWs & BIPs
June 30	Execute agreements
July 31	Commence R&D

BETO Mission: *Decarbonize the industrial sector*

dsRNA PRODUCTION TO SCALE FOR AGRICULTURE

Mary C. Wildermuth and Jyoti Taneja, Plant & Microbial Biology, UC Berkeley

Graphical Abstract



Outside groups & national labs

Adam Guss Lab, Biosciences, ORNL
Carrie Eckert Lab, Biosciences, ORNL
Deepti Tanjore, Biosciences, ABPDU, LBL

Two Year Project: Total $625K

(in thousands)	Total =$625
Cost share	$125
DOE budget ($)	$500

Technical Approach

Using the unique expertise and resources of our ORNL and LBL ABPDU team with its deep expertise in high throughput strain engineering in phylogenetically diverse microbes and bioconversion scale up capabilities, this project aims to address the pressing need for a scalable method for dsRNA production for Agriculture allowing for high throughput, iterative, and integrated optimization of dsRNA production to scale. For example, for just one product (to powdery mildew control of grapevine) we estimate 75000 kg/yr are needed. Our approach integrates examination of seven diverse microbial hosts, optimization of the dsRNA design, microbial construct for dsRNA production via iterative engineering, media and nutrient/micronutrient inputs, and fermentation mode and conditions. Specific national lab resources to facilitate this work include use of the SAGE recombination system, CRISPRi and Rb TnSeq libraries, BioLog Phenotype MicroArrays for Microbiology, expertise with RNA-aptamer hybrids, and expertise to efficiency optimize fermentation of diverse bioproducts at scale.

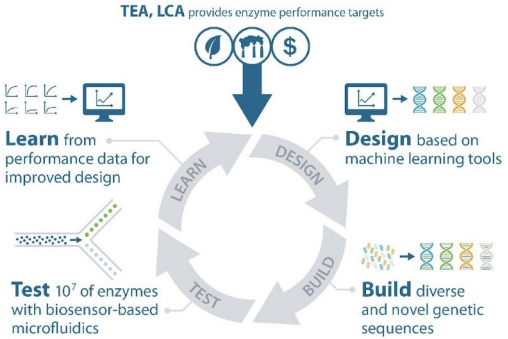
*mark key innovations

BETO Mission: *Decarbonize the industrial sector*

New enzymes for plastics recycling from biosensor-guided evolution and machine learning

Johan Kers, PhD, Co-founder and CEO, Birch Biosciences

Graphical Abstract



Techno-economic analysis and life cycle assessment targets will inform PETase DBTL cycles over a 24 month period.

Key expected innovations:

- Deep Learning models that enable prediction of optimal enzyme activity as a function of pH and temperature
- Novel quantitative droplet screening assays
- High performance **acid tolerant** PETase enzymes for **crystalline PET** depolymerization

Key expected outcomes

- Cost-competitive enzyme-mediated PET recycling
- > 50% reduction in plastic recycling CO_2 emissions
- Technical progress that emboldens investor support for commercial scale enzyme-mediated plastic recycling

Outside groups & national labs

Phil Laible, ANL
Gregg Beckham, NREL
Johan Kers, Birch Biosciences

(in thousands)	Total = $
Cost share	$200,000
DOE budget ($)	$800,000

Technical Approach

The overall goal of this proposal is to develop process-advantaged enzymes that would be superior to state-of-the-art biocatalysts for PET plastic recycling by leveraging the Design-Build-Test-Learn (DBTL) capabilities of the ABF. TEA and LCA analyses will establish enzyme performance specifications for subsequent protein engineering efforts. Deep Learning will be used to predict protein engineering designs of candidate PETase enzymes that enable depolymerization of >75% of fiber-grade PET across a pH range of 3.5-8. Microfluidic screening technologies will be developed to ensure quantitative, high-throughput screening of 10^7 member protein variant libraries secreted from *E. coli*. Optimization of microfluidic screens will include evaluation of pH sensitive dyes and biosensor reporter systems to quantify terephalate (the principal monomer of PET). Importantly, this work will be performed in an iterative cycle that will generate multidimensional training data for subsequent refinement of deep learning models and enable robust data-driven improvements in protein features.

1

BETO Mission: *Decarbonize the industrial sector*

A Platform for Expression and Secretion of Recombinant Proteins

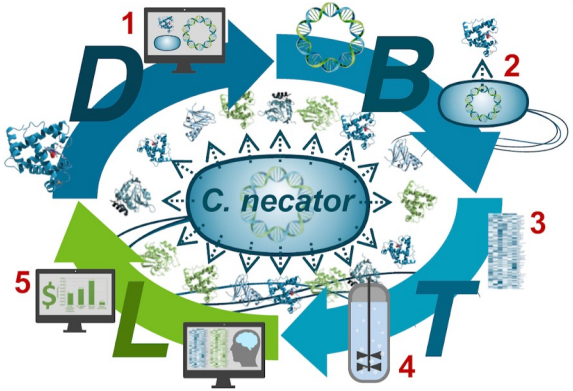
Dan Robertson, Kiverdi

Graphical Abstract

We propose a two-year project leveraging the facilities and expertise of the ABF in metabolic modeling, multiomics, metabolic engineering, and TEA to develop a ***Cupriavidus necator*-based recombinant protein expression and secretion platform** to enable scaled industrial production.

Key innovations and outcomes:

1. A versatile model for determining reaction stoichiometries and theoretical protein and energy yields sufficient for TEA
2. Identification of signal peptides that enable secretion of heterologous proteins, a *C. necator* strain with improved protein secretion, and strains engineered to overcome bottlenecks in protein production
3. Discovery proteomics to describe secretome and identify secretion signals, targeted proteomics to evaluate heterologous protein secretion, and multiomics to identify bottlenecks in protein production
4. Protein expression and secretion under fermentation conditions; protein recovery method
5. TEA for scaled production of the protein targets



Outside groups & national labs

National Renewable Energy Laboratory
Pacific Northwest National Laboratory

(in thousands)	Total = $
Cost share	$239K
DOE budget ($)	$950K

Technical Approach

Task 1: Metabolic modeling for prediction of protein mass and energy yield (NREL). We will extend the previously published Flux Balance Analysis model to estimate the maximum yield of proteins on CO_2 and H_2 as a function of their amino acid composition.

Task 2: Multiomics analysis of *Cupriavidus* protein production host (PNNL). LC-MS/MS global proteomics analysis of the whole cell proteome and secretome will be used to identify secretion signals as well as bottlenecks in protein biosynthesis.

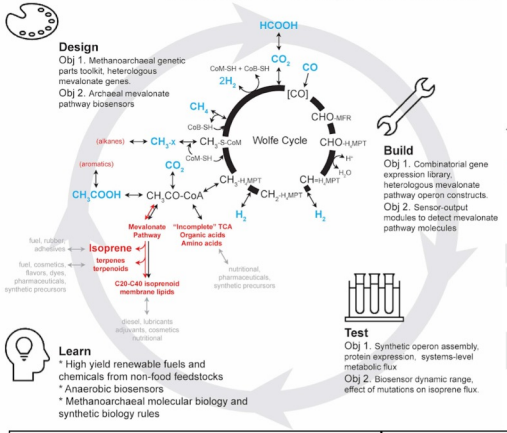
Task 3: Construction and optimization of protein expression and secretion system (NREL). Putative signal peptides will be screened by appending them to amylases. Selection based on amylase secretion will used to evolve *C. necator* for improved protein secretion.

Task 4: Lab scale oxyhydrogen fermentation (Kiverdi). Kiverdi will use 2L and 500 mL bioreactors to optimize the fermentation process.

Task 5: Techno-economic analysis (NREL). Dedicated steady-state process simulations will be developed for biorefineries centered around oxyhydrogen fermentation.

Expanding the synthetic biology biosensor toolkit for *Methanosarcina*

Nicole Buan, Associate Professor, University of Nebraska-Lincoln



Outside groups & national labs

Nicole Buan - University of Nebraska-Lincoln
Taraka Dale - Los Alamos National Laboratory
Adam Guss - Oak Ridge National Laboratory
Ramesh Jha - Los Alamos National Laboratory

(in thousands)	Total = $687.5
Cost share	$137.5
DOE budget ($)	$550

Technical Approach

Methanosarcina is a promising archaeal chassis for synthesis of sustainable fuels and chemicals from non-food feedstocks. The Buan lab has generated a strain that converts methanol to isoprene (4%) and methane (73%). We will expand the genetic toolkit for this non-model organism by developing combinatorial parts for gene expression and metabolite sensors which we will use to increase isoprene synthesis up to 20x. ***Obj. 1a:*** Design and measure fluorescence reporter signals. ***Obj 1b:*** Design reporter constructs and measure expression from constitutive and inducible methanoarchaeal promoters. ***Obj 2:*** Design and develop a methanoarchaeal biosensor for a mevalonate pathway metabolite. By Q8 of this 2-year project we will use the new genetic parts to increase isoprene yield and titer in *Methanosarcina* by stably expressing parallel ortho/heterologous mevalonate pathways of up to 10 genes from the chromosome.

BETO Mission: *Decarbonize the industrial sector*

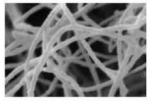
Strain engineering of a novel phototrophic bacterium for cellulosic fiber production utilizing CO_2

Milan Hanacek, CTO, Azolla, Inc.

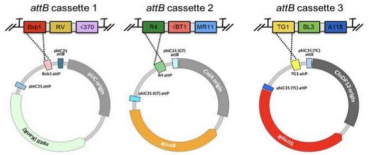
Graphical Abstract

Synechococcus sp. 7002*

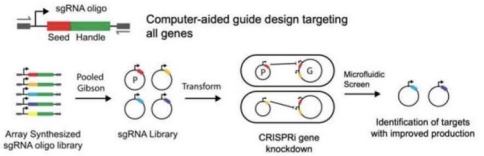
PBR



Bacterial Nanocellulose (BNC)

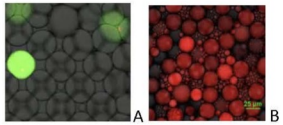


Multiplexed pathway construction



Genome-wide CRISPRi knockdown libraries

- **Leverage ABF**'s HODT's tools and approaches - develop a site-specific DNA integration tool for multiplexing insertion of DNA into safe chromosomal sites
- **2 years**: Adaptive laboratory evolution of engineered strains
- **Target innovation**: 2g/l/d* baseline production rate



Analytics and ALE in microfluidic droplets

Outside groups & national labs

Adam Guss - ORNL
Carrie Eckert - ORNL
Phillip Laible - ANL
Gyorgy Babnigg - ANL
Rosemarie Wilton – ANL
Christopher Fry – ANL (CNM)

(in thousands)	Total = $
Cost share	$212.5K
DOE budget ($)	$850K

Technical Approach

- Unique synthetic organism, a phototrophic host, engineered with cellulose synthase machinery to overproduce and secrete BNC with a high degree of crystallinity and polymerization.
- Leverage ABF's genetic-toolkit capabilities, library-based engineering, microfluidics-based screening and laboratory evolution for metabolic engineering to optimize BNC production.
- Genome-scale library to knockdown targets that increase nanocellulose production and identify additional engineering strategies for optimized production strains in subsequent DBTL cycles.
- Nanocellulose production levels measured using fluorescence-based assays at a variety of scales. The evaluation of promoter-ortholog parings (input) with BNC production (response) will be used to further iterate on strain and pathway design.
- Structural analysis of *Synechococcus* BNCs to optimize product quality characteristics through genetic modifications and preserve scaled performance in 180L and 10KL PBRs (available through Azolla).

*mark key innovations

FY22 Funding Opportunity Lessons Learned

Changes ABF made to the application & review process were a significant improvement relative to the FY21 Funding Opportunity process:

- Each application received reviews from 5 external reviewers, up from 3-4 in past years.
- Each reviewer evaluated a slightly larger number of applications, giving better overlapping reviewer coverage across the applicant pool.
- Each application review panel was custom tailored, based on applicant-stated focus areas and reviewer expertise level for ABF's 16 capability areas.
- A late-stage lengthening of the application acceptance period proved important for growing the applicant pool and may point to a need for earlier Funding Opportunity communication in future cycles.
- Use of executive summaries and 1-slide summaries (above) provided a means of more rapidly communicating the gist of each project.

Improvement opportunities:

- The group is evaluating grant management software for better process centralization and standardization across the multiple collaboration vehicles which are currently in development. This offers a more scalable approach where stakeholders use a specifically structured portal to execute the many tasks of application, review, and award impact-tracking.
- Develop better coordination within and among the multiple funding opportunities which ABF will have available in future funding cycles.
- Reconsider reviewer score weighting for team evaluation (less weight) and strategic program building (more weight).
- Strategize and manage to drive more licensing of IP from CRADAs.
- Consider paths for well-scored, albeit unfunded projects to other funding vehicles (e.g., BioMADE or Strategic AOP).

Excerpt from Gaps Analysis milestone report

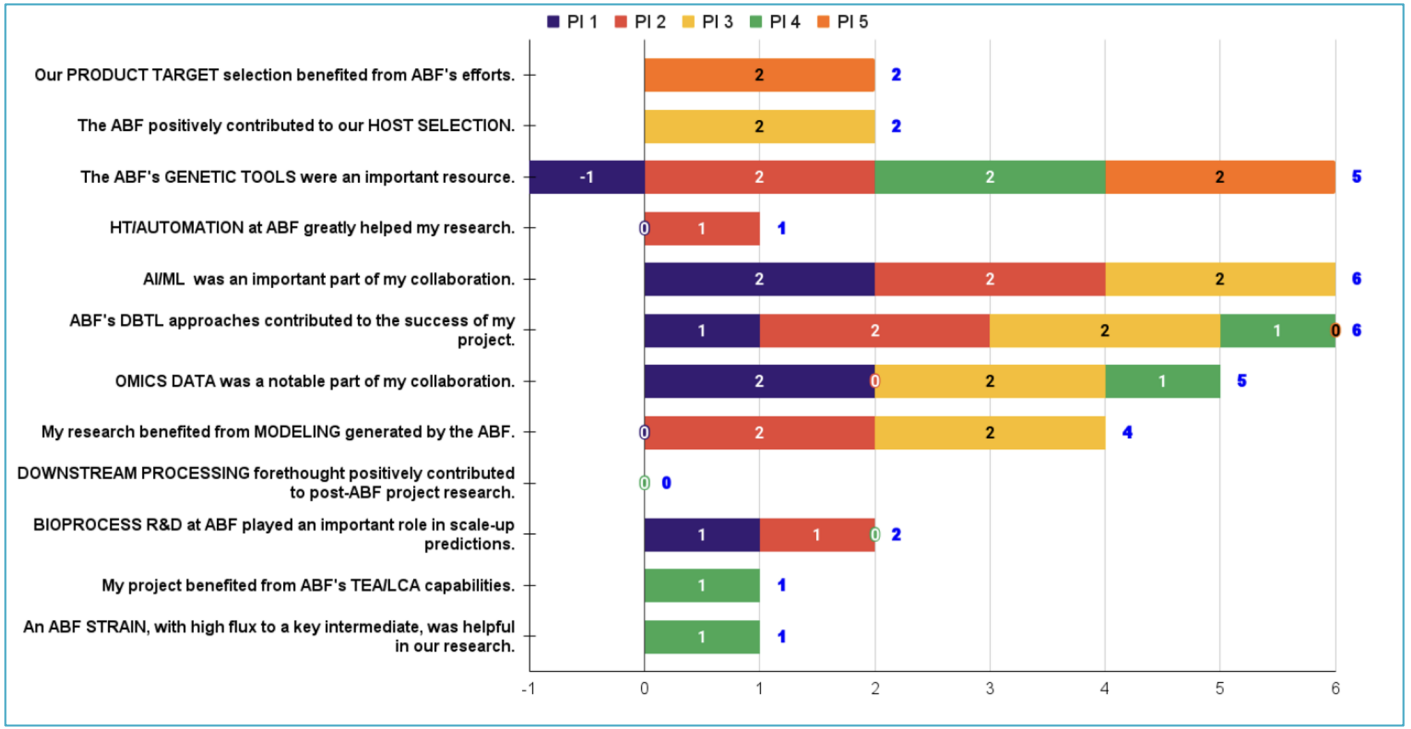


Figure 6. Five external collaborating PIs see ABF's level of capability deployment as very strong in some areas and nearly nil in others. The perceived level of satisfaction was high, with approximately 2/3 of all responses being positive or very positive. This survey evaluates the level of usage and benefit the community receives from ABF capabilities, as reported by 5 collaborating groups. Respondents indicated 1 of 5 Likert levels for each item (very negative, negative, neutral, positive, and very positive on a scale of -2 to 2, respectively).

Respondents indicated N/A for capabilities not relevant for their ABF collaboration. Colors correspond to the individual responding (see legend). Within each bar, the width of a colored section corresponds to the magnitude of the applicant's response for the Likert item (provided in full) on the Y-Axis; neutral responses score as zero and thus have no width, appearing as a legend-colored (0) label instead. Dark blue numbers to the right of each bar indicate total scores.

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

Suggestions: ABF Partnering Mechanisms Working Group

- We probably don't need CRADAs for academic/non-profit partnerships
- CRADA language is in the NSF FOA because they say the ABF requires it
- We are currently asking BETO if we can drop the CRADA requirement for the NSF call
- This will have implications for MSRDC collaborations, and possibly (some) BIOMADE

Suggestions (part II): ABF Partnering Mechanisms Working Group

- Have a menu of (usually more narrow) projects that would be funded by AOP money, probably concurrent with the DFO proposals and review
- Company (or academic?) still provides non-federal cost share
- Expectation that results would be published in a timely fashion
- Each lab needs to think of things they could offer that companies might support without exclusive access to IP/materials
  - Genetic tool development (host onboarding; application of existing tools to new organisms)
  - Biosensors?
  - Software/models/TEA/LCA?
  - Fermentations (ABPDU/NREL)?
  - Protocol development?
  - Equipment usage (automation, omics)?

Suggestions (part III): ABF Partnering Mechanisms Working Group

- Each lab is required to have a person (not the lab PI) covered at least 5% to shepherd CRADAs through the system quickly
- Determine how we can add value to large/established companies (BizDev person?)
- Keep non-negotiable CRADA, but might we offer the option of negotiation if they are ok with an extended approval timeline?
- Put out an RFI to see what pre-competitive things industry might want
- Reach out to venture capital about funding TEA/LCA for due diligence?
- We can create an IP Management Plan if we feel we need it for the non-CRADAs

See also

Additional Slides section within

BETO Peer Review 2023 ABF Introduction and Overview

for presentations, publications, patents, and other related information