Agile BioFoundry

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

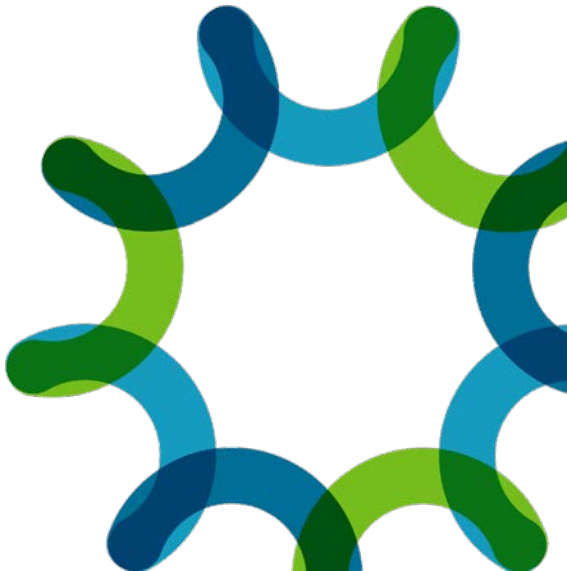
BIOENERGY TECHNOLOGIES OFFICE

Introduction to Directed Funding Opportunities

James Gardner

Program Manager, DOE Agile BioFoundry

BETO Peer Review 2021
Conversion Technologies
10:30 – 11:00
March 10, 2021



Overall ABF Goal

- **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 DFO Project overview

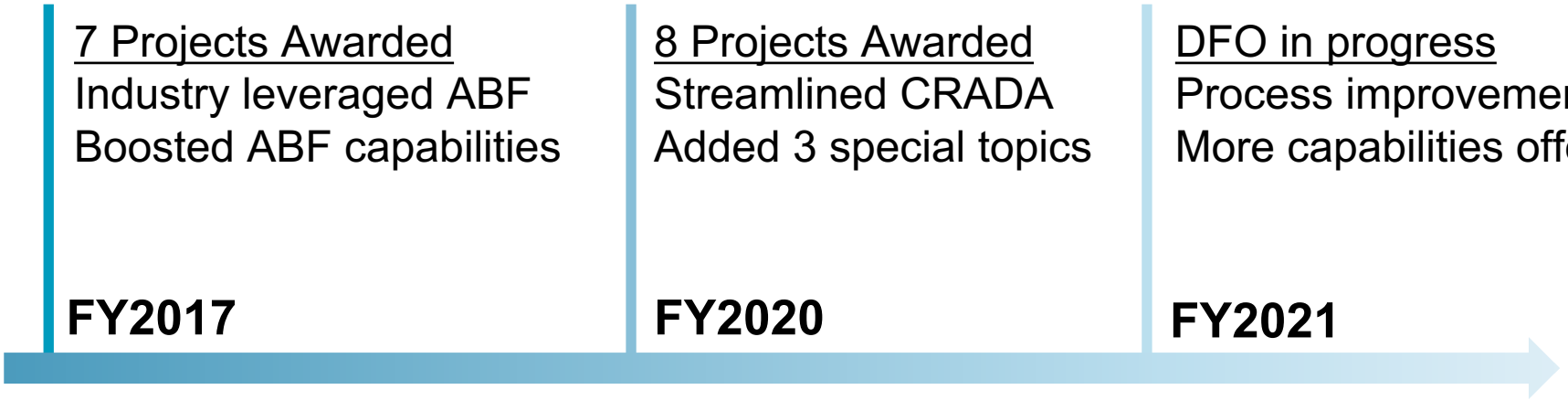
- **Goal**

  Partner with industry and universities to offer Agile BioFoundry capabilities to directly accelerate commercialization of bio-based products.

  Further develop the ABF's capabilities in an industry-responsive manner.

- **Project History**

  $15M DOE funds over 3 FY, minimum 20% partner cost share.



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

Agilent

VISOLIS
CARBON NEGATIVE MATERIALS

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

LYGOS

Kiverdi

teselagen
BIOTECHNOLOGY

LanzaTech
capturing carbon fueling growth

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

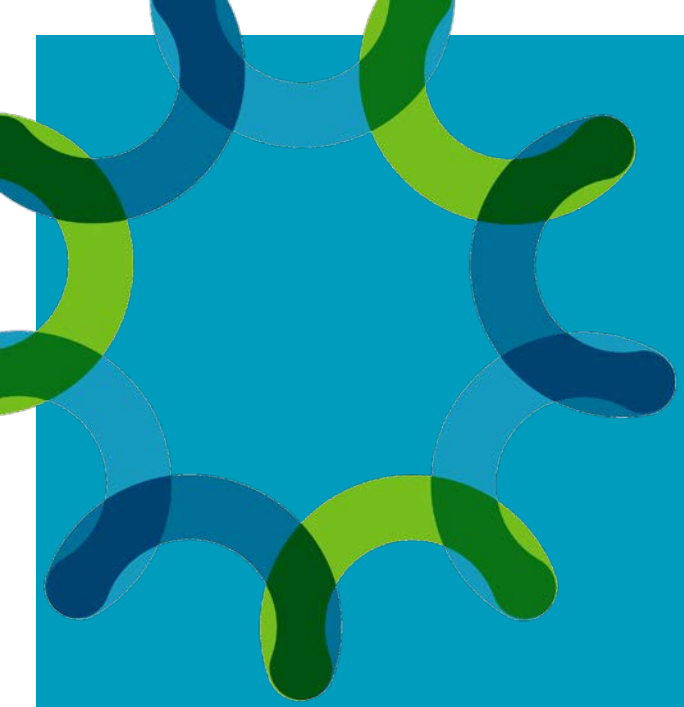
University of Delaware
Washington University in St. Louis
White Dog Labs
c16 Biosciences
Enduro Genetics
danimer scientific — A Biotechnology Company
invaio
Kalion
LanzaTech

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

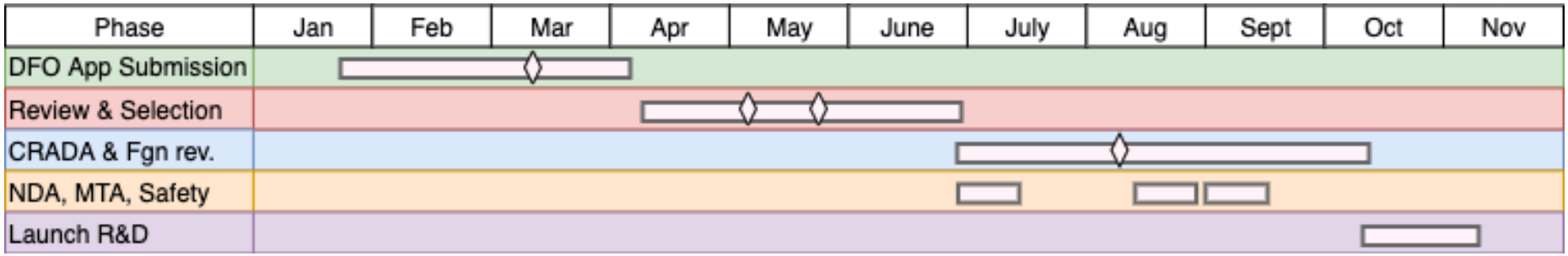
Proposal responses TBA



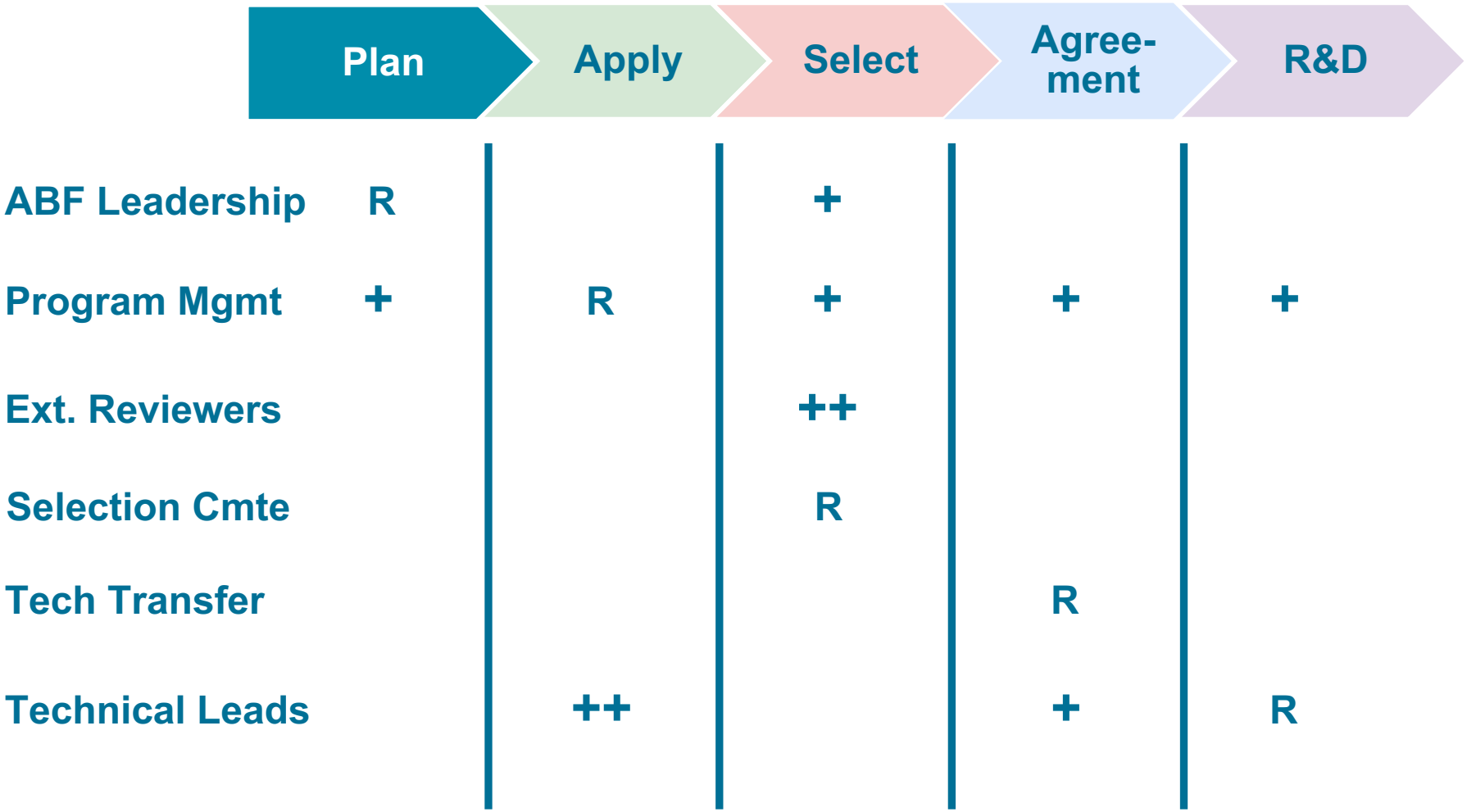
1 - Management

1 – Management: DFO 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 – Management: DFO Team Responsibilities



+ contributor; ++ key contributor; Responsible

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

Agile BioFoundry

1 – Management: DFO Project Tracking

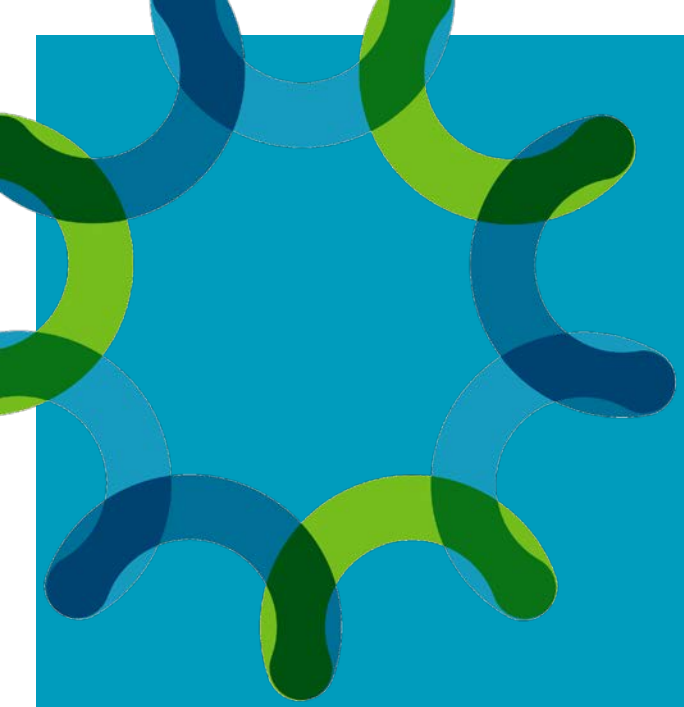
Plan | Apply | Select | Agree-ment | R&D

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 – Management: DFO risks & mitigation

Risk	Sev.	Risk	Mitigation Plan
Intellectual property usage	Med	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.
COI management challenges	Low	Collaborations or competitive relationships among reviewers, applicants and ABF Lab staff could lead to COI.	Screen for COI among reviewers, applicants, and ABF Lab staff at the outset. See COI discussion below.
Obtaining external reviewers	Low	For the FY21 DFO, all reviewers are asked to meet as a single group for score leveling, a difficult meeting to schedule.	Set the meeting time months in advance, to ensure reviewer availability.
Untimely agreement execution	Med	Protracted execution times may impact community interest level for future collaborations.	Although Lab Tech Transfers own agreement process, propose common templates for statements of work and other alignments, to accelerate teams toward project kickoff.
Relatively low visibility		DFO announcement may have lower visibility than typical FOAs.	Use all communication channels at our disposal. Share with networks and contacts. Provide ample time (75d) for application.



2 - Approach

2 – Approach: ABF DFOs

Element	FY17 DFO	FY20 DFO	FY21 DFO
Funding DOE	$5M	$5.7M	$5M*
Cost share min.	30%	20%	20%

*A portion of funds may be allocated for NSF collaborations, for strategic development of biomanufacturing technologies with ABF's university partners.

Process Improvements:

- Expand the timeline.
- Utilize external reviewers.
- Use non-negotiable CRADA.
- Standardize application steps.

Documentation Improvements:

- Provide single outlet of information.
- Clarify cost share calculations & types.
- Clarify use of milestones & tables.
- Use DOE eligible entities guidelines.

2 – Approach: FY20 DFO Announcement

- Innovation and adoption of new biomanufacturing technologies and processes, the Agile BioFoundry consortium oversee a directed funding opportunity for interested industry partners to **use unique capabilities at national laboratories**.
- Call for proposals was posted on the ABF website, broadcast via emails & social media. Proposal template, instructions, and FAQs posted on ABF website.
- External reviewers recommended proposals. The Agile BioFoundry executive committee voted to identify the final list of projects, with final project approval and funds authorized to the national laboratories by DOE.
- Projects limited to no more than two years ranging from $500K (seed) to $2M (full) total per project.
- Federal funds allocated to DOE National Laboratories to provide access to Agile BioFoundry resources only, subject to available Agile BioFoundry budget. Industry cost share ≥ 20% (may be in-kind).
- Awarded proposals were entered into the DOE system, tracked as stand alone AOPs, and tracked internally using appropriate project management tools & practices.

2 – Approach: FY20 DFO Special Topics

Culture heterogeneity and selection of desirable traits

- Culture stability and heterogeneity across scales and over time are a barrier to efficient fermentations
- Need to keep each cell producing product efficiently

AI-enhanced Biomanufacturing

- Machine Learning (ML) and Artificial Intelligence (AI) methods have become indispensable for many industries, yet their utility in synthetic biology is still being proven
- ABF has developed cutting-edge capabilities to be applied to industrial problems

ABF Host Engineering and New Host Onboarding

- Industry has rated this as one of the areas of most potential impact for ABF
- Advancing current hosts and adding new hosts will enable the biomanufacturing sector to tackle new problems more efficiently

TOPICS	Major	Minor
Culture heterogeneity and selection of desirable traits	5	5
AI-enhanced Bio manufacturing	2	5
Host Engineering and New Host Onboarding	6	8

2 – Approach: FY20 Proposal Review

- External reviewer teams comprised a mix of academic and industry perspectives, screened against COI, and offered expertise appropriate for the subset of applications in their packet.
- Each external reviewer reviewed their subset of applications, commenting on each section, overall appropriateness and responsiveness to special topics.
- External reviewer teams conferred on a group recommendation of Fund, Maybe Fund, Do Not Fund recommendations and final comments.
- ABF selection committee made final selections to balance reviewer recommendations with strategic priorities and Lab resources.
- ABF leadership shared portfolio with BETO for concurrence.
- ABF notified applicants Jul-3-2020; CRADA negotiations began thereafter.
- Projects were entered into EERE FY20 AOP system for project management.

2 – Approach: Proposal Review – Managing COI

FY20 External review

Excluded from application review process, confirmed via email

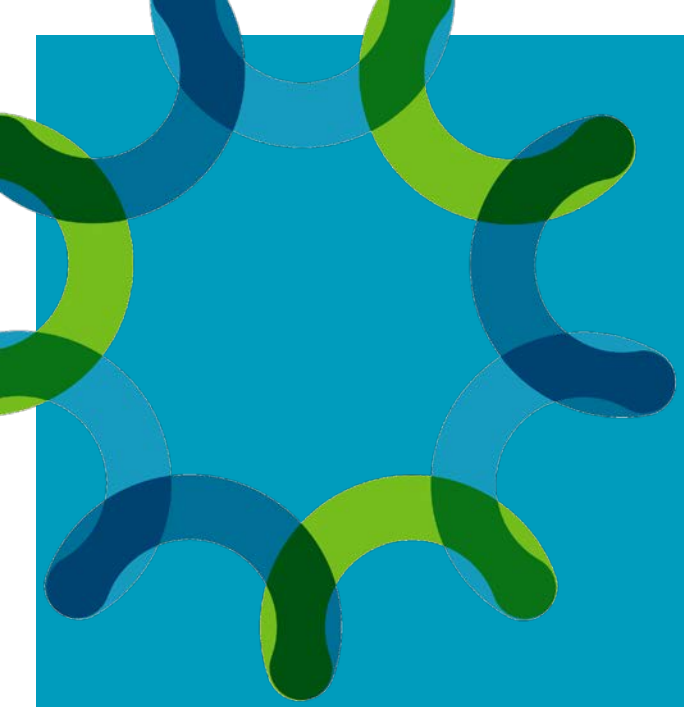
- Representatives from any of the ABF national Labs
- Representatives from, or financial interests in any proposing companies, such as: Employees, Board members, Shareholders
- Known affiliations to any of the above, including: Recent collaboration with proposer(s) (within the last 3 years), participation in previous work related to the specific proposal under consideration.
- In potential competition with proposing applicant.

Reviewers recused themselves from specific applications

- Personal connections (family / close friend)

Upon receipt of final application list.

- Confirm in writing (email) that they remain in good standing with regards to the exclusion criteria
- Propose alternate reviewers where necessary.



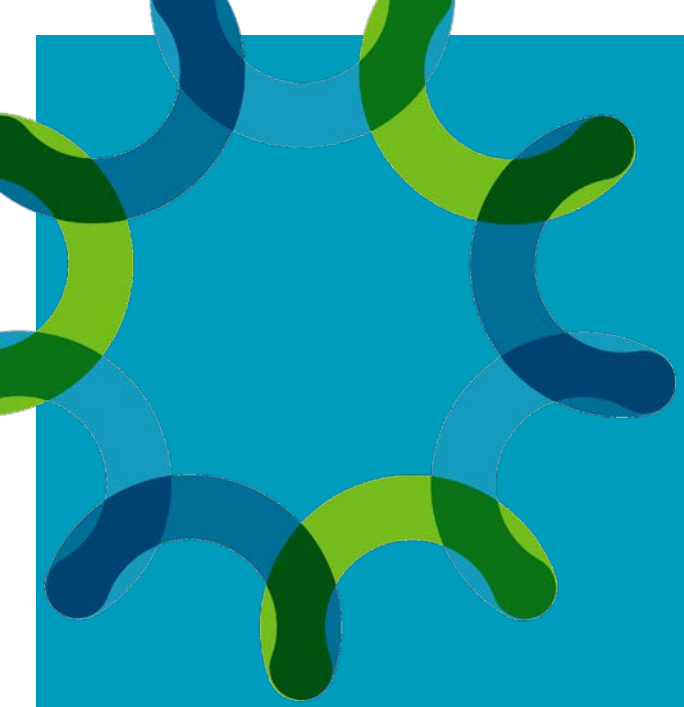
3 - Impact

3 – Impact: Directed Funding Opportunity

- **Successful mechanism for offering ABF capabilities directly to industry & universities.** Address tough challenges in biomanufacturing that otherwise would not receive the benefit of such capabilities. Accelerated bioproduct development for ABF collaborators.
  FY17 + FY20 DFOs: $10.7M DOE / $3.7M CS / 36 proposals / 15 projects.

- **Supports ABF's strategic goals.** Special topics afforded a targeted approach to the expansion of ABF capabilities into new, industry-relevant hosts, co-development of new bioinformatics, and AI capabilities, as well as growth of the ABF IP portfolio.

- **Valuable industry engagement & outreach.** The DFO application process provides a unique source of insights into the direction of the industry and its emergent challenges.



4 - Progress and Outcomes

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	20%			
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

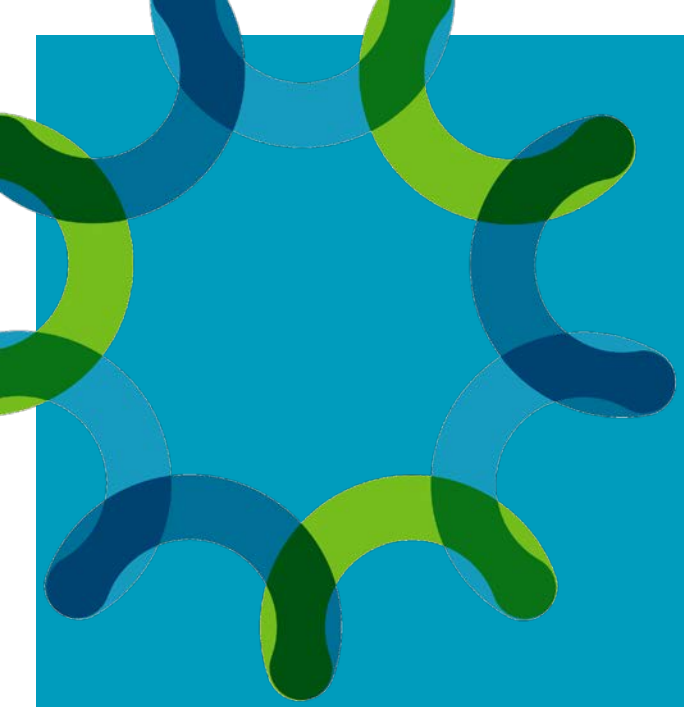
	Key Characteristics
DOE / Cost share	$500k / $125k = 20%
Special Topics	AI/ML & Onboarding
Capabilities	AI, strain development, -omics
Participating Labs	NREL, PNNL
ABF PI	Gregg Beckham (NREL)

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.



Summary

Summary – ABF Directed Funding Opportunity

- **Industry interest is high.** Continued oversubscription to the DFO demonstrates a demand-driven allocation of funds. $10.7M DOE funds / $3.8M cost share. Multiple groups exceeded the cost share minimum.
- **Robust management and review protocols.** Standardized intake and messaging. Diverse, knowledgeable, and COI-screened reviewers. Strategy-responsive selection. Dedicated process monitoring. Semi-annual presentations, quarterly reports, cost share report forms, and AOP tracking.
- **Continuous process improvement.** Identify improvements to the processes of planning, application, review, selection, & reporting, informing the execution of current and future funding opportunities.
- **Fifteen DFO projects complete, underway, or in development.** Derived from competitively & strategically selected applications – reviewer recommendations, to use capabilities & expertise not readily available, solve tough challenges, and respond to broader strategies of the ABF and BETO.

Quad Chart Overview

Timeline

- Start: October 1, 2019
- End: September 30, 2022

	FY20	FY21	FY22	Total Active
DOE Funding	$5M	$5M	$5M	$15M

Project Partners

- ANL, LANL, LBNL, NREL, ORNL, PNNL, SNL
- FY20: C16 Bio, White Dog Labs, U. of Delaware, Washington U, Invaio, Enduro Genetics, Danimer, LanzaTech, Kalion

Barriers addressed

- Ct-L. Decreasing development Time for Industrially Relevant Microorganisms
- Ct-D. Advanced Bioprocess Development

Project Goal

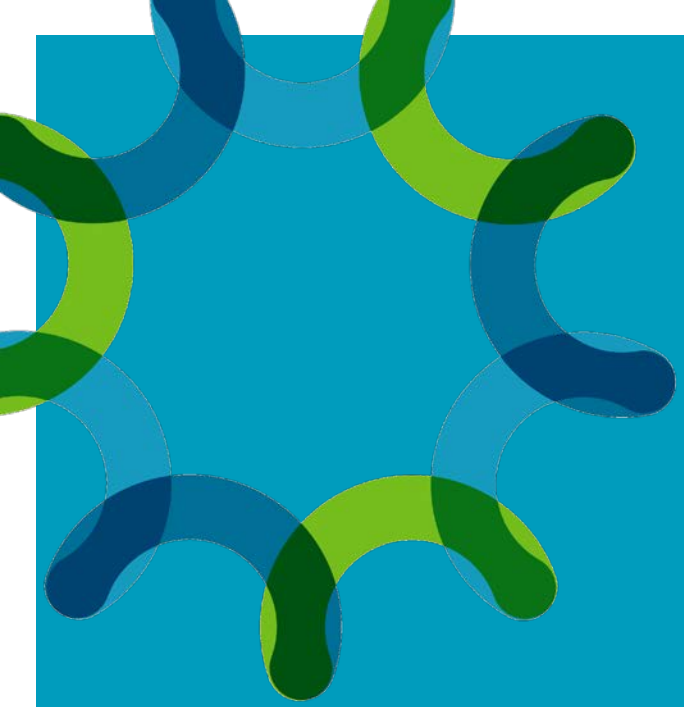
Provide a competitive application process and funding pool to facilitate collaborations with industry and academia, in order to directly accelerate its partners' development and commercialization objectives. Strategically expand the ABF's capabilities, through these partnerships, across the DBTL cycle.

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

Directed Funding Opportunity



Additional Slides

Additional Slides – DFO Change Control

- No changes that require funds transfer between National Labs are allowed.
- IF the aggregate (staffing + materials, summed across National Lab participants) proposed resource reallocation from the established / current scope of work is strictly less than 10% of the overall total (all years) National Lab budget for the project, and there will be no milestone changes:
    - Revise the written scope of work, red-lining all changes. Detail how these changes will be managed within the existing budget and timeline, including what tasks / activities will be downscoped or eliminated if other tasks are up-scoped or instantiated.
    - Calculate the aggregate proposed resource reallocation. IF the reallocation is greater than or equal to 10% of the overall total (all years) National Lab budget for the project, or if there will be any milestone changes, ExtensiveProcess (below) is required.
    - ABF PI, ABF CRADA PI (of lead lab), and supervisors/work leads of all affected staff must sign off on the revised scope of work.
    - Ensure that project management, progress / financial reporting documents and plans, etc. are updated to reflect the changes to the scope of work.

Additional Slides – DFO Change Control

- IF the aggregate changes are >10%:
    - All of the above, plus:
    - ABF executive committee (including Technical Manager) sign off on revised scope of work.
    - Proceed through standard BETO AOP change control process (including process for changing milestones, if applicable).

- IF there are substantial / substantive changes to the scope of work, the formal CRADA contractual documents may also require revisions - consult with legal / contracting representatives.

Additional Slides – Responses to Previous Reviewers' Comments

- **C**: This subproject focuses on external engagement through partnerships and external funding agreements. The major success was creating a template for the CRADA process that works with all of the ABF partnerss. This resulted in a streamlined process and increased transparency. Overall, these activities are critical to the success of the Agile Biofoundry. In addition, opening up the Agile Biofoundry to external partners though different funding mechanisms is a critical step in broadening the impact of the foundry. Developing a robust management and review/evaluation protocols is clearly important.
- **R**: We thank the reviewers for these comments and agree with all of the issues raised.

Additional Slides – Responses to Previous Reviewers' Comments

- **C**: ABF/BETO has established FOAs/DFOs in order to establish formal engagements that will be critical for for dissemination of ABF approaches, ensuring they maintain relevance, and for providing feedback to ABF regarding its technical strategy and operations. The funding mechanism is effective in that it incentivises industry, while mainly providing funds within the NL system. This, along with cost-share, ensures that that industrial partners are vested in the research and in its success. Throughout the process, the ABF team has noted ways to improve the engagement. In particular, the administrative burden for establishing these CRADAs has been substantial, which has led the team to innovate on the process for future engagments.
- **R**: We thank the reviewers for these comments, and hope that the lessons learned from this DFO process will assist in future efforts. In particular, the development of a template CRADA should significantly improve overall efficiency of getting projects underway in a timely fashion.

Additional Slides – Responses to Previous Reviewers' Comments

- **C**: Development of funding opportunities to drive the industry oriented mission of the ABF is critical to its success. The description of DFO and FOA successes shows progress in fulfilling this mission, as well as establishing a scoreboard. Difficulty in bringing some CRADAs to closure highlight the challenges of working with customers with different business needs. A non-negotiable CRADA template was developed and may streamline this process in future. What level of traction this provides remains to be established, as well as whether it changes the customer landscape going forward. This should be tracked to determine impact/benefit, and fed back into business development to refine messaging, funding and collaborative opportunity development.
- **R**: We thank the reviewers for these comments and agree that tracking projects and monitoring the rate of placing contracts and initiating projects will be a key metric, and we will be sure to collect and share that data.

Additional Slides – Responses to Previous Reviewers' Comments

- **C**: Weakness: It is not clear yet whether effective project governance is yet in place once projects are awarded. If not already in place or covered by BETO, it may be wise to consider a project management infrastructure/office to establish clear practices/tools to manage ongoing programs and governance thereof. This is likely to be more cost and effort effective than ad hoc management of individual programs by potentially inexperienced project managers.

- **R**: We agree with these concerns, but note that these projects will be entered into the EERE AOP system that woll be the primary performance tracking tool, and each project will provide all regularly scheduled progress and milestone completion reports. These should be sufficient in terms of project management and governance, but the ABF Project Management team will follow this closely to ensure the desired outcome is achieved.

Additional Slides – Responses to Previous Reviewers' Comments

- **C**: Weakness: Certain of the CRADAs had significant difficulty in being brought to closure, highlighting that different partners may have different business needs. The effectiveness of the non-negotiable CRADA template will need to be established in future, and may need to be refined if there is not a "one size fits all" CRADA solution.
- **R**: We agree with these concerns and we will be tracking the progress and utility of the CRADA template to ensure it is meeting our needs.

- **C**: Weakness: The CRADA includes options for exclusive licensing. Further, in order to facilitate dissemination, if not already present, there should also be options for non-exclusive licensing. (This might already be in the new template.)
- **R**: We agree that both options are important and, indeed, the template CRADA will provide a means for covering both exclusive and non-exclusive options.

Additional Slides – Responses to Previous Reviewers' Comments

- **C**: Weakness: Some potentially strong academic partners may be excluded by cost-share requirement, but it should continue to be a "must" for industrial applicants. This may miss some innovative technology development opportunities, particularly in areas to early-stage for industry.
- **R**: We agree that cost-share may be problematic for academic institutions, but note that one of the DFO awardees is from a university. Moving forward, we will monitor the participation of academic institutions and if there are apparent lost opportunities we will look for alternatives with BETO leadership for these interested parties.

- **C**: Weakness: Additional thought around project governance and expertise should be included so that it is clear how projects will be managed and reported on to ensure communication and successful programs. This seems within the scope of managing funding opportunities.
- **R**: All of the CRADA projects will be entered into the EERE AOP system that will be used to track and monitor progress. As per all other AOP entries, these projects will provide monthly and quarterly reports and submit them to BETO, and these will also be shared with the ABF leadership team.

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization

- **50 publications, 126 presentations to date**
  - 16 publications and 20 presentations since FY20
    - The following slides provide explicit lists thereof

- **2020 R&D 100 Award**
  - Awarded to Smart Microbial Cell Technology for rapid optimization of biocatalysts
  - Special Recognition (Silver Medal) for Market Disruptor in the Services category

- **36 patents, records of invention, software disclosures, & licenses**
  - The following slides list these intellectual property assets

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Publication)** Peabody GL, Elmore JR, Martinez-Baird J, and Guss AM. “Engineered Pseudomonas putida KT2440 co-utilizes galactose and glucose.” Biotechnol Biofuels 12, 295 (2019).
- **(Publication)** Christopher B. Eiben, Tristan de Rond, Clayton Bloszies, Jennifer Gin, Jennifer Chiniquy, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Oliver Fiehn, Jay D. Keasling. "Mevalonate Pathway Promiscuity Enables Noncanonical Terpene Production", ACS Synth. Biol. (2019).
- **(Publication)** Yan Chen, Deepwanita Banerjee, Aindrila Mukhopadhyay, Christopher J. Petzold. “Systems and synthetic biology tools for advanced bioproduction hosts”, Curr. Op. Biotechnol. (2020).
- **(Publication)** Jacquelyn M. Blake-Hedges,Jose Henrique Pereira, Pablo Cruz-Morales, Mitchell G. Thompson, Jesus F. Barajas, Jeffrey Chen, Rohith N. Krishna, Leanne Jade G. Chan, Danika Nimlos, Catalina Alonso-Martinez, Edward E. K. Baidoo, Yan Chen, Jennifer W. Gin, Leonard Katz, Christopher J. Petzold, Paul D. Adams, Jay D. Keasling. “Structural Mechanism of Regioselectivity in an Unusual Bacterial Acyl-CoA Dehydrogenase”, J. Am. Chem. Soc. (2019).

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Publication)** Thompson, Mitchell G., Allison N. Pearson, Jesus F. Barajas, Pablo Cruz-Morales, Nima Sedaghatian, Zak Costello, Megan E. Garber et al. "Identification, characterization, and application of a highly sensitive lactam biosensor from Pseudomonas putida." ACS Synthetic Biology (2019).
- **(Publication)** Geiselman GM, Zhuang X, Kirby J, Tran-Gyamfi MB, Prahl JP, Sundstrom ER, Gao Y, Munoz Munoz N, Nicora CD, Clay DM, Papa G, Burnum-Johnson KE, Magnuson JK, Tanjore D, Skerker JM, Gladden JM. “Production of ent-kaurene from lignocellulosic hydrolysate in Rhodosporidium toruloides.” Microb Cell Fact. 19(1):24. (2020).
- **(Publication)** Gayle J. Bentley, Niju Narayanan, Ramesh K. Jha, Davinia Salvachúa, Joshua R. Elmore, George L. Peabody, Brenna A. Black, Kelsey Ramirez, Annette De Capite, William E. Michener, Allison Z. Werner, Dawn M. Klingeman, Heidi S. Schindel, Robert Nelson Lindsey Foust, Adam M. Guss, Taraka Dale, Christopher W. Johnson*, Gregg T. Beckham*, “Engineering glucose metabolism for enhanced muconic acid production in Pseudomonas putida KT2440,” Metabolic Eng. (2020).

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Publication)** Chen, Y; Guenther, J.; Gin, Jennifer; Chan, Leanne J.; Costello, Z.; Ogorzalek, T.; Tran, Huu; Blake-Hedges, J.; Keasling, J. D; Adams, P.; Garcia Martin, H.; Hillson, N.; Petzold, C. "An automated 'cells-to-peptides' sample preparation workflow for high-throughput, quantitative proteomic assays of microbes" Journal of Proteome Research (2019)
- **(Publication)** Isabel Pardo, Ramesh K. Jha, Ryan E. Bermel, Felicia Bratti, Molly Gaddis, Emily McIntyre, William Michener, Ellen L. Neidle, Taraka Dale, Gregg T. Beckham, Christopher W. Johnson. "Gene amplification, laboratory evolution, and biosensor screening reveal MucK as a terephthalic acid transporter in Acinetobacter baylyi ADP1." Metabolic Engineering, (2020), Vol 62, 260-274
- **(Publication)** Radivojević, T., Costello, Z., Workman, K., & Martin, H. G. "A machine learning Automated Recommendation Tool for synthetic biology." Nature Communications, 11(1), 1-14.(2020).
- **(Publication)** Zhang, J., S. D. Petersen, T. Radivojevic, A. Ramirez, Andrés Pérez-Manríquez, E.Abeliuk, B. J. Sánchez et al. "Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism." Nature Communications 11, no. 1 (2020): 1-13.

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Publication)** Ernst Oberortner, Robert Evans, Xianwei Meng, Sangeeta Nath, Hector Plahar, Lisa Simirenko, Angela Tarver, Samuel Deutsch, Nathan J. Hillson, and Jan-Fang Cheng. "An Integrated Computer-Aided Design and Manufacturing Workflow for Synthetic Biology". In: Chandran S., George K. (eds) DNA Cloning and Assembly. Methods in Molecular Biology, vol 2205. (2020).
- **(Publication)** Gledon Doçi, Lukas Fuchs, Yash Kharbanda, Paul Schickling, Valentin Zulkower, Nathan Hillson, Ernst Oberortner, Neil Swainston, Johannes Kabisch. "DNA Scanner: a web application for comparing DNA synthesis feasibility, price, and turnaround time across vendors". OUP Synthetic Biology, ysaa011 (2020).
- **(Publication)** Somtirtha Roy, Tijana Radivojevic, Mark Forrer, Jose Manuel Marti, Vamshi Jonnalagadda, Tyler Backman, William Morrell, Hector Plahar, Joonhoon Kim, Nathan Hillson, and Hector Garcia Martin. "Multiomics Data Collection, Visualization, and Utilization for Guiding Metabolic Engineering". Frontiers in Bioengineering and Biotechnology 9, 45 (2021).

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Publication)** Chris Lawson, Jose Manuel Martí, Tijana Radivojevic, Sai Vamshi R. Jonnalagadda, Reinhard Gentz, Nathan J. Hillson, Sean Peisert, Joonhoon Kim, Blake A. Simmons, Christopher J. Petzold, Steven W. Singer, Aindrila Mukhopadhyay, Deepti Tanjore, Josh Dunn, and Hector Garcia Martin. "Machine learning for metabolic engineering: A review" Metabolic Engineering (2020)
- **(Publication)** Riley LA and Guss AM*. “Approaches to genetic tool development for rapid domestication of non-model microorganisms”. Biotechnol 14:30 (2021)

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Presentation)** Nathan J. Hillson "U.S. DOE Agile BioFoundry: Organization and Capabilities", Invited Talk, ABF Industry Day 2019, Emeryville, CA October 4, 2019
- **(Presentation)** Garcia Martin, H. "Machine Learning, Synthetic Biology and Automation: Engineering Life for the Benefit of Society". NERSC data seminar, Berkeley CA, November 1st, 2019
- **(Presentation)** Benavides PT, Davis R, Klein, B. "Economic and environmental assessment of biological conversions of Agile BioFoundry (ABF) bio-derived chemicals". 2nd Bioenergy Sustainability Conference 2020, Virtual meeting, October 15th, 2020
- **(Poster)** Tijana Radivojevic, Zak Costello, Kenneth Workman, Soren Petersen, Jie Zhang, Andres Ramirez, Andres Perez, Eduardo Abeliuk, Benjamin Sanchez, Yu Chen, Mike Fero, Jens Nielsen, Jay Keasling, Michael K. Jensen, Hector Garcia Martin, "ART: A machine learning Automated Recommendation Tool for synthetic biology", BRC Workshop on AI and ML for Biosystems Design, Washington, DC, February 27, 2020

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Presentation)** Garcia Martin, H. “ART: a machine learning Automated Recommendation Tool for guiding synthetic biology”. AI4Synbio Symposium, Arlington VA, November 8th, 2019.
- **(Presentation)** Garcia Martin, H. “Opportunities in the intersection of:Artificial Intelligence & Synthetic Biology & Automation”. Army Science Planning and Strategy Meeting, Burlington MA, November 13th, 2019.
- **(Presentation)** “ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- **(Presentation)** Garcia Martin, H. “Opportunities in the intersection of machine learning, synthetic biology, and automation”. ABLC 2020, Virtual meeting, July 10th, 2020.
- **(Presentation)** Garcia Martin, H. “Leveraging machine learning and automation to make synthetic biology predictable”. SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- **(Panel)** Garcia Martin, H. “Sustainable Living Systems”. LA Life Summit, Virtual meeting, October 15th, 2020.

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Presentation)** T. Radivojevic, “Automatic Recommendation Tool”, Invited Talk, Agile BioFoundry Learn Summit 2020, Argonne/Lemont, IL, March 4, 2020
- **(Presentation)** T. Radivojevic, “Using ART to improve tryptophan production”, Invited Talk, Agile BioFoundry Learn Summit 2020, Argonne/Lemont, IL, March 4, 2020
- **(Presentation)** T. Radivojevic, “Guiding synthetic biology via machine learning”, Invited Talk, Biofuels & Bioproducts Division Meeting, JBEI, Emeryville, CA, March 11, 2020
- **(Presentation)** T. Radivojevic, “ART: A machine learning Automatic Recommendation Tool for guiding synthetic biology”, Invited Talk, Computational Bio-Science Meeting, Berkeley, CA, April 23, 2020
- **(Presentation)** Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **(Presentation)** Nathan J. Hillson, "Perspectives from the U.S. DOE Agile BioFoundry", OECD BNCT Virtual Workshop, Session 1: Biofoundries and COVID-19, via Zoom, July 29, 2020
- **(Presentation)** Garcia Martin, H. "Opportunities in the intersection of machine learning, synthetic biology, and automation". ABLC 2020, Virtual meeting, July 10th, 2020.
- **(Presentation)** Garcia Martin, H. "Leveraging machine learning and automation to make synthetic biology predictable". SPIE Optics + Photonics 2020, Virtual meeting, August 24th, 2020.
- **(Presentation)** Nathan J. Hillson, "FY20 ABF CRADA Call: Process, Applications, and Selections", Conversion R&D Standing Lab Update Call, via WebEx, July 27, 2020
- **(Presentation)** Nathan J. Hillson, "Perspectives from the U.S. DOE Agile BioFoundry", OECD BNCT Virtual Workshop, Session 1: Biofoundries and COVID-19, via Zoom, July 29, 2020

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **License partners**
  - University of Georgia
  - Kiverdi, Inc.
  - LanzaTech, Inc.
  - Visolis, Inc.
  - Danimer Scientific
- **Patent Applications**
  - Terephthalate biosensor and applications thereof
  - Mutant transporters for bacterial uptake of terephthalic acid
  - Alleviating the bottleneck in enzyme evolution and pathway optimization using novel biosensors (Disclosure Title) Modified Biosensors and Biocatalysts and Methods of Use (Application Title)
  - Mutant transporters for bacterial uptake of terephthalic acid
  - ART: A machine learning Automated Recommendation Tool for guiding synthetic biology

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **Patent Applications (cont.)**
  - A Generative Model for Protein Sequences for the Purpose of Protein Design or Phenotypic Inference
  - Predicting Metabolic Pathway Dynamics from Time Series Multiomics Data Using Machine Learning Techniques
  - Use of Statistical Learn Approaches to Predict Next Generation Sequencing Subsequence Depth of Coverage
  - Mutant transporters for bacterial update of terepthalic acid
  - Method and strain for sugar conversion
  - Engineered Microorganisms for the Production of Intermediates and Final Products (1st)
  - Engineered Microorganisms for the Production of Intermediates and Final Products (2nd)
  - Production of organic acids from *Aspergillus pseduoterreus* cadA deletion strain (1st)
  - Production of organic acids from *Aspergillus pseduoterreus* cadA deletion strain (2nd)

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **Patent Applications (cont.)**
  - Genetically engineering an industrial filamentous fungus *Aspergillus niger* for 3-hydroxypropionic acid production
  - A specific exporter responsible for aconitic acid high production in *Aspergillus pseduoterreus*
- **Records of Invention**
  - Bioproduction of limonene from syngas
  - Mutant transporters for bacterial update of terepthalic acid
  - Method to produce branched chain polyhydroxyalkanoates and branched chain 3-hydroxyacids
  - A genetic circuit to reduce cell-to-cell production heterogeneity
  - High yield conversion of D-xylose to D-arabitol in *R. toruloides*
  - Manipulation of tRNA thiolation gene ncs2 for enhanced production of fatty-acyl-CoA derived chemicals in *R. toruloides*

Additional Slides – Publications, Patents, Presentations, Awards, and Commercialization (cont.)

- **Software Disclosures**
  - Automated Recommendation Tool (ART) v2.0
  - Kinetic Learning v0.1
  - Automated Recommendation Tool (ART): v1.0
  - PIACE: Parallel Integration and Chromosomal Expansion of Metabolic Pathways
  - OMG, Omics Mock Generator Library: v0.1.1
  - Fermentation Data Processing
  - Fermentation Data Manipulation and Analysis Once imported