Agile BioFoundry

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

BIOENERGY TECHNOLOGIES OFFICE

ABF CRADA Management

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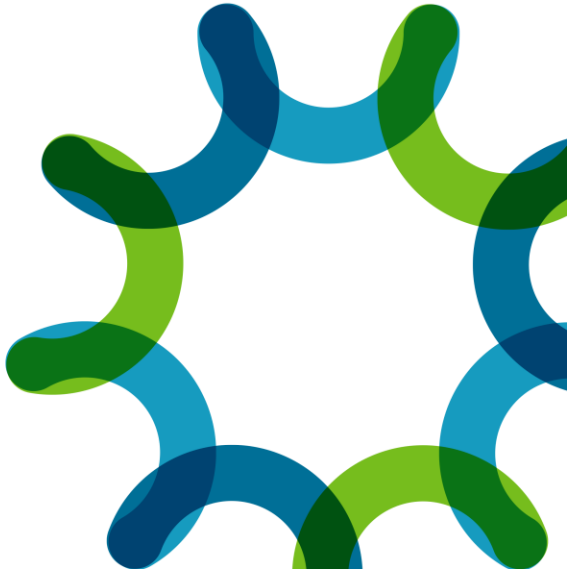
BETO Peer Review 2019
Conversion Technologies
4:00-4:30 PM
March 7, 2019
Denver, CO



ABF Goal Statement

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

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

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

Task Summary

History: Task initiated as a result of funding opportunities (ABF DFO, BEEPS FOA) that resulted in CRADAs

- coordinate and track CRADA approvals
- interface with DOE-BETO and ABF Labs

Context: Industry engagement is key to success of ABF

- ABF succeeds only as industry is engaged and adopts resulting technologies
- ABF DFO and the BEEPS FOA are strategic opportunities provided by BETO to promote ABF and industry collaborations

Task goals: Prove the value proposition of the ABF to industry through demonstrable S&T successes

- Assist and improve the CRADA development and approval process
- Assist in monitoring progress

Task Quad Chart

Timeline

- October 1, 2018
- September 30, 2019
- 55% complete

Barriers addressed

- Ct-L. Decreasing Development Time for Industrially Relevant Microorganisms
- At-D. Identifying New Market Opportunities for Bioenergy and Bioproducts
- At-G. Social Acceptance and Stakeholder Involvement
- ADO-D. Technology Uncertainty of Integration and Scaling

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	0	0	248k	2.748M

Objective

Ensure efficient and impactful industry interactions and sponsored projects (CRADAs)

End of Project Goals

- Efficient execution of CRADAs
- Streamline approval process and increase transparency
- Manage future DFOs that involve ABF review and process oversight

1 – Overview

DFO Summary

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

2017 Timeline:

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

Proposal response highlights:

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

7 Proposals awarded and implemented as CRADAs

Agilent Technologies
Department of Microbiology, Franklin College of Arts and Sciences, University of Georgia
LanzaTech — capturing carbon fueling growth
teselagen BIOTECHNOLOGY
LYGOS
Kiverdi
Visolis — Carbon Negative Materials

DOE BEEPS FOA: Topic 2 (ABF) Summary

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

2018 Timeline:
May 3 – FOA issue date
May 30 – Letters of intent due
June 27 – Full applications due
Aug 3 – Responses to Reviewers' comments due
September 4 - BETO announces awardees
September – Award Negotiations underway

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

3 proposals awarded

LYGOS $2M

ZymoChem $1.3M

UC San Diego $2M

2 – Approach

DFO - Call for Proposals

- **Focus: innovation and adoption of new biomanufacturing technologies and processes, the Agile BioFoundry consortium is overseeing a directed funding assistance for interested industry partners to use unique capabilities at national laboratories.**
- **Call for proposals posted on ABF website and broadcast emails went out on 6/21/17**
- **Proposals reviewed and scored by the Agile BioFoundry with final project approval and funds authorized to the national laboratories by DOE.**
- **Projects limited to no more than two years ranging from $500K to $2M 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 ≥ 30 percent (may be in-kind).**
- **Proposal template and FAQs posted on ABF website.**

Proposal Review Principles and Approaches

- **The ABF Selection Committee included one person from each of the 8 National Labs participating in the ABF**
- **Proposals were scored on the following basis:**
  - **Approach (0-30 points)**
  - **Risks (0-10 points)**
  - **Impact (0-25 points)**
  - **Appropriateness (0-10 points)**
  - **Personnel (0-15 points)**
  - **Budget/Milestones (0-10 points)**
- **After all scores have been entered, the Aggregate ABF Total Raw Score served as the basis of initially rank ordering the proposals**
- **Analysis for portfolio balancing was then carried out by the Selection Committee**
- **Once final rankings were completed, results shared with BETO for concurrence**
- **Awardees notified 8/14/17, CRADA negotiations begin**
- **Projects entered into EERE FY18 AOP system for project management**

Proposal Review – Managing COI

- **The “lead” Lab is defined as the primary (majority of time and/or funding) Lab identified in an industry partner’s proposal**
- **The “lead” Lab of any ABF DFO proposal submitted was not allowed to provide any written scores to the proposal review process**
- **Any representatives of the “lead” Lab were not allowed to advocate, or participate in any discussion around that proposal during the proposal review process**
- **Any representatives of the “lead” Lab did not provide any guidance, ratings, or scores during the final decision-making process on that respective proposal during the proposal review process**
- **Any individual with a COI was not allowed to participate in reviews**

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.**

DFO Change Control cont'd

- **IF the aggregate changes are >10%:**
  - **All of the above, plus:**
  - **ABF ExComm (including Technical Manager Jay Fitzgerald) 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.**

BEEPS FOA

- **DE-FOA-0001916: BIOENERGY ENGINEERING FOR PRODUCTS SYNTHESIS (BEEPS)**
- **Topic Area 2 - Agile BioFoundry (ABF) Industry Partnership Initiative**
  - **Development of non-model host organisms with industrially-relevant production advantages (low-pH, high flux to a metabolic node, utilization of a broad substrate scope, robustness, etc.) over *E. coli* and *S. cerevisiae* for a target molecule or class of molecules;**
  - **De-bottlenecking of biosynthetic pathways to take a target molecule from mg/L to tens (10's) of g/L, increase productivity, and increase yield;**
  - **Projects which produce data sets that will enable Agile BioFoundry's Learn methodologies, which seek to use machine learning and other approaches to improve subsequent rounds of design.**

BEEPS FOA cont'd

- **BEEPS webinar held by BETO on 2/15/18**
- **ABF team held coordination calls with interested teams that led to the formation of teams that submitted proposals**
  - **Number of ABF coordination calls with industry: 22**
  - **Number of proposals submitted without ABF call: 1**
  - **Number of letters of intent not encouraged: 2**
  - **Number of full proposals submitted: 11**
  - **Number of awards: 3**
- **Proposal reviews managed by BETO**
- **Awardees currently under negotiation with BETO**
- **Industry projects will be executed by establishing a CRADA**

3 – Results

2017 ABF Directed Funding Opportunity

Lygos CRADA

- ABF PI: John Gladden
- Project Start Date: 4/1/18
- Project End Date: 3/31/20
- Percent Completed: 45%

	Year 1	Year 2	Total Funding
DOE Funded	$500k (SNL, LBNL, PNNL)	$500k (SNL, LBNL, PNNL)	$1000k
Cost Share	$167.1k	$167.1k	$334.2k

Technical Approach

- The targeted biosynthetic pathway is comprised of multiple non-native enzymes that convert pyruvate into isobutyric acid (IBA).
- At least two of the biocatalytic steps require enzyme homolog exploration and/or engineering to increase IBA production efficiency to industrially relevant levels.
- Lygos provided the ABF team a proprietary strain of engineered *P. kudriavzevii* that accumulates high levels of pyruvate while minimizing biomass production.
- ABF capabilities will be leveraged to reduce the DBTL cycle length to improve IBA production in *P. kudriavzevii*.

Visolis CRADA

- ABF PI: Carrie Eckert
- Project Start Date: 3/21/18
- Project End Date: 6/30/20
- Percent Completed: 30%

	Year 1	Year 2	Total Funding
DOE Funded	$250k (NREL $170k, ORNL $80k)	$250k (NREL $170k, SNL $80k)	$500k
Cost Share	$107.15k	$107.15k	$214.3k

Technical Approach

- Develop a hybrid biomass-conversion technology integrating thermochemical gasification with syngas fermentation ($CO/H_2/CO_2$) to improve biorefinery economics.
- Use low pH yeast (*Pichia kudriavzevii*) for the conversion of sugars to PM1 to bypass the need for pH buffering during fermentation.
- Use *Clostridium ljungdahlii* for anaerobic fermentation of Syngas ($CO/H_2/CO_2$) to improve biomass utilization and increase carbon conversion efficiency (compared to carbon loss from glycolysis).
- ABF will develop genetic tools to enable the Design Build Test Learn cycle for pathway engineering to improve product yields.

Lanzatech CRADA

- ABF PI: Philip Laible
- Project Start Date: 5/1/18
- Project End Date: 4/30/20
- Percent Completed: 25%

	Year 1	Year 2	Total Funding
DOE Funded	$250k (ANL $130k, NREL $120k)	$250k (ANL $130k, NREL $120k)	$500k
Cost Share	$108k	$108k	$216k

Technical Approach

- Develop an 'in-line' AI-guided process that will monitor and adjust industrial, waste gas-fed fermentation conditions in real time to optimize fermentation output of biofuels
- Integrate extensive LT database of fermentation data across multiple scales (bench top to industrial)
- Leverage detailed metabolic model of fermentation organism
- Construct AI module that provides operational feedback to maximize fermentation output and minimize bioreactor crashes
- This approach aims to be generalizable with the plan to design in-line AI control modules that are applicable to a wide variety of industrial fermentation processes

Teselagen CRADA

- ABF PI: Hector-Garcia Martin
- Project Start Date: 6/29/18
- Project End Date: 6/29/20
- Percent Completed: 25%

	Year 1	Year 2	Total Funding
DOE Funded	$400k (LBNL $200k, SNL $180k, PNNL $20k)	$400k (LBNL $200k, SNL $180k, PNNL $20K)	$800k
Cost Share	$171,429	$171,429	$342,858

Technical Approach

- The goal is to enhance tools that facilitate biomanufacturing design and enable efficient data collection:
  - BOOST(Build Optimization Software Tools)
  - BLiSS (Black List Sequence Screening)
  - EDD (Experiment Data Depot)
- Top challenges:
  - Divergent needs in Teselagen's and ABF's users
  - Integration of different codebases
- Critical success factors:
  - Continuous feedback from industrial/academic users

UGA CRADA

- ABF PI: Taraka Dale
- Project Start Date: 10/1/2018
- Project End Date: 09/30/2020
- Percent Completed: 12.5%

	Year 1	Year 2	Total Funding
DOE Funded	$250k (LANL $140k, NREL $110k)	$250k (LANL $140k, NREL $110k)	$500k
Cost Share	$107,143	$107,143	$214,286

Technical Approach

- Establish a sensor-reporter system for the target molecule (Terephthalic acid, TPA)
- Introduce and evolve enzyme that can convert renewable substrates to TPA
- Link the evolution of strain and efficiency of enzyme to fluorescence signal: High fluorescence and High enzyme efficiency
- Development of a biosensor needs TPA to be transported into the cells or produced intracellularly :
- Transporters need to be established or an enzyme needs to be minimally functional to produce TPA

Kiverdi CRADA

- ABF PI: Steve Singer
- Project Start Date: 1/1/19
- Project End Date: 12/31/21
- Percent Completed: 5%

	Year 1	Year 2	Total Funding
DOE Funded	$450k (LBNL $175.5k, NREL $153k, ORNL $121.5k)	$450k (LBNL $175.5k, NREL $153k, ORNL $121.5k)	$900k
Cost Share	$192,850k	$192,850k	$385,700k

Technical Approach

- Combine improved transformation efficiency, reliable gene editing and an expanded set of RBSs to demonstrate bioreactor production of 1-dodecanol from H_2/CO_2 by *Cupriavidus necator*
- Challenges addressed
  - *C. necator* transformation efficiency is low
  - It is difficult to inactivate multiple genes in *C. necator*
  - A restricted set up genetic parts exists for heterologous expression in *C. necator*
  - There are challenges to scale *C. necator* production of non-native bioproducts from H_2/CO_2

Agilent CRADA

- ABF PI: Jon Magnuson
- Projected Start Date: 03/11/19
- Projected End Date: 02/28/21
- Percent Completed: 0%

	Year 1	Year 2	Total Funding
DOE Funded	$400k (PNNL $200k, NREL $124k, SNL $76k)	$400k (PNNL $200k, NREL $124k, SNL $76k)	$800k
Cost Share	$120k	$120k	$240k

Technical Approach

- Project coupoles two powerful mass spectrometry (MS) platforms; (1) PNNL's Drift Tube Ion Mobility Spectrometry (DTIMS) with Time of Flight MS (TOF-MS) and (2) PNNL's and LBNL's triple quadrupole (QQQ) MS with Agilent's prototype system to process hundreds of metabolomic and proteomic samples per day via automated analytical workflows (AAW) and Ultra-High Performance Liquid Chromatography (UHPLC).
- This project will fulfill a need for high-throughput analytical workflows that reduce time and resource needs, thus increasing the number of samples analyzed for a fixed resource allocation.
- Apply ABF Machine Learning to data sets generated.

2018 BEEPS FOA

Lygos

- $2M award
  - SNL $300k
  - LBNL $875k
  - PNNL $225k

- PI: Jeff Dietrich, Lygos

- The objective of this project is to implement a high-throughput microbial engineering DesignBuild-Test-Learn cycle incorporating transcriptomic, metabolomic, and proteomic analyses along with machine learning.

- In collaboration with the Agile BioFoundry, Lygos will implement this high-throughput DBTL workflow to improve production of the target biochemical in *Pichia kudriavzevii*, an acid tolerant strain of yeast.

- Planned strain improvements include overexpression of pathway proteins and reduction or deletion of native yeast metabolic genes and pathways. The performance of the engineered strain in fermentation will be assayed using cellulosic sugar as a raw material.

ZymoChem

- $1.3M award
  - ORNL - $326k
  - LBNL - $458k

- PI: Harshal Chokhawala, CEO, ZymoChem

- The objective of this project is to aim to develop *Bacillus* as an industrial host for making an environmentally-safe, non-toxic, biodegradable biopolymer from lignocellulose-derived C5 and C6 sugars.

- ZymoChem will work with the Agile BioFoundry to leverage key capabilities within ORNL (genetic transformation and tool development) and LBNL (–omics capabilities, process integration, and bioprocess scale-up).

- The successful completion of the project will greatly accelerate ZymoChem's endeavors toward commercializing a low-cost bioprocess for producing bio-based polymers that offer significant environmental and performance benefits.

University of California – San Diego

- $2M award
  - PNNL - $185.75k
  - LBNL - $185.75k

- PI: Stephen Mayfield, UCSD

- The objective of this project is to develop develop novel algae platforms for the production of polymer precursors, while simultaneously developing basic tools to enable improved algal production systems that will accelerate the process from initial concept to market supply.

- UCSD will work with the ABF to design and construct advanced synthetic biology tools, including promoter libraries optimized through machine learning, and expand our current metabolic models of algal growth to better direct carbon flow to enhance production of target chemicals.

- These optimized strains will be field tested for target yields greater than 20 g/L under photosynthetic growth conditions, as well as under heterotrophic conditions in a fermenter using cellulosic-derived sugar feedstocks.

4 – Relevance

As this task is responsible for organizing and facilitating industry interactions and agreements, the value of this effort lies in tracking, facilitating and improving the protocols associated with the management and execution of those opportunities, including support of all decision-making and project-planning activities. The efficient execution of these CRADA agreements will enable the development, optimization and transfer of ABF technologies to the marketplace and benefit the growth of the bioeconomy.

5 – Future Work

- **Continue tracking progress made on establishing new CRADAs as needed for awards made under BEEPS FOA**
- **Strive to improve CRADA process efficiency**
  - **Revised uniform ABF CRADA agreed to by DOE and the ABF Labs and made public on on ABF website (https://agilebiofoundry.org/work-with-us/) and emailed to industry listserv**
- **Work with ABF team and BETO on future funding opportunities in FY19 and beyond**

6 - Summary

- **ABF has generated significant industry interest**
- **BETO issued two strategic funding opportunities in FY17 and FY18 that promote ABF industry interactions and collaborations**
- **ABF DFO and BEEPS FOA executed successfully, with 10 ABF proposals awarded $10.3M in DOE funds**
- **ABF has established robust management and review protocols in place for opportunities it manages (DFO)**
- **DOE BETO has established robust management and review protocols in place for opportunities it manages (FOA)**
- **All of these projects are entered into the DOE AOP system to monitor progress, spending and impact**
- **Significant process has already been made in streamlining how ABF can execute future CRADAs and improve transparency and process efficiency**

