AGILE BIOFOUNDRY CONSORTIUM

TECHNOLOGY AREA

CONTENTS

INTRODUCTION

The Agile BioFoundry Consortium (ABF) Technology Area is one of 14 related technology areas that were reviewed during the 2019 Bioenergy Technologies Office (BETO) Project Peer Review, which took place March 4–7, 2019, at the Hilton Denver City Center in Denver, Colorado. A total of 10 projects were reviewed in the ABF session by five external experts from industry, academia, and other government agencies.

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately $52,040,000 (Fiscal Year [FY] 2016–2019 obligations), which represents approximately 6.1% of the BETO portfolio reviewed during the 2019 Project Peer Review. During the Project Peer Review, the principal investigator (PI) for each project was given 10–50 minutes (depending primarily on the funding level and priority with respect to near-term goals) to deliver a presentation and respond to questions from the review panel.

Projects were evaluated and scored for their project approach, technical progress and accomplishments, relevance to BETO goals, and future plans. This section of the report contains the results of the project review, including full scoring information for each project, summary comments from each reviewer, and any public response provided by the PI. Overview information on the ABF, full scoring results and analysis, the Review Panel Summary Report, and the Technology Area Programmatic Response are also included in this section.

BETO designated Dr. Jay Fitzgerald as the ABF Technology Area Review Lead, with contractor support from Mr. Clayton Rohman (Allegheny Science & Technology). In this capacity, Dr. Fitzgerald was responsible for all aspects of review planning and implementation.

ABF OVERVIEW

Currently, the industrial biotechnology sector often scales up processes on a case-by-case basis, without tools that can be extrapolated to multiple host organisms, pathways, and applications. The ABF develops and integrates tools, technologies, software, and instrumentation across the DOE national laboratory system for the robust and predictive engineering of biological systems to improve efficiencies in the conversion of biomass to fuels and products. The ABF creates new pathways and organisms engineered to produce biofuels and renewable chemicals from domestic, non-food lignocellulosic biomass. Central to this effort is development of robust host organisms and new microbiology techniques, in conjunction with databases and machine-learning methods, which enable automated design of bioprocesses with predictable performance and scaling, as well as significantly increased conversion efficiency. These efforts are incorporated into a Design-Build-Test-Learn (DBTL) methodology to enable faster, more efficient bioproduct development cycles.

The ABF is a virtual consortium organized around six tasks: Design-Build-Test-Learn, Integrated Analysis, Host Onboarding, Process Development and Scale-Up, Industry Engagement, and Management. To demonstrate the effectiveness of the ABF, strategic target-host pairs with relevance to the bioeconomy are pursued by the consortium in addition to ten industry-led partner projects. The ABF also uses its expertise in host development to contribute to BETO's State of Technology for Biochemical Conversion, in collaboration with the Biological Upgrading of Sugars and Targeted Microbial Development projects.

The ABF session review panel reviewed the six tasks of the ABF, as well as overall direction, management, and industry engagement and partnerships.

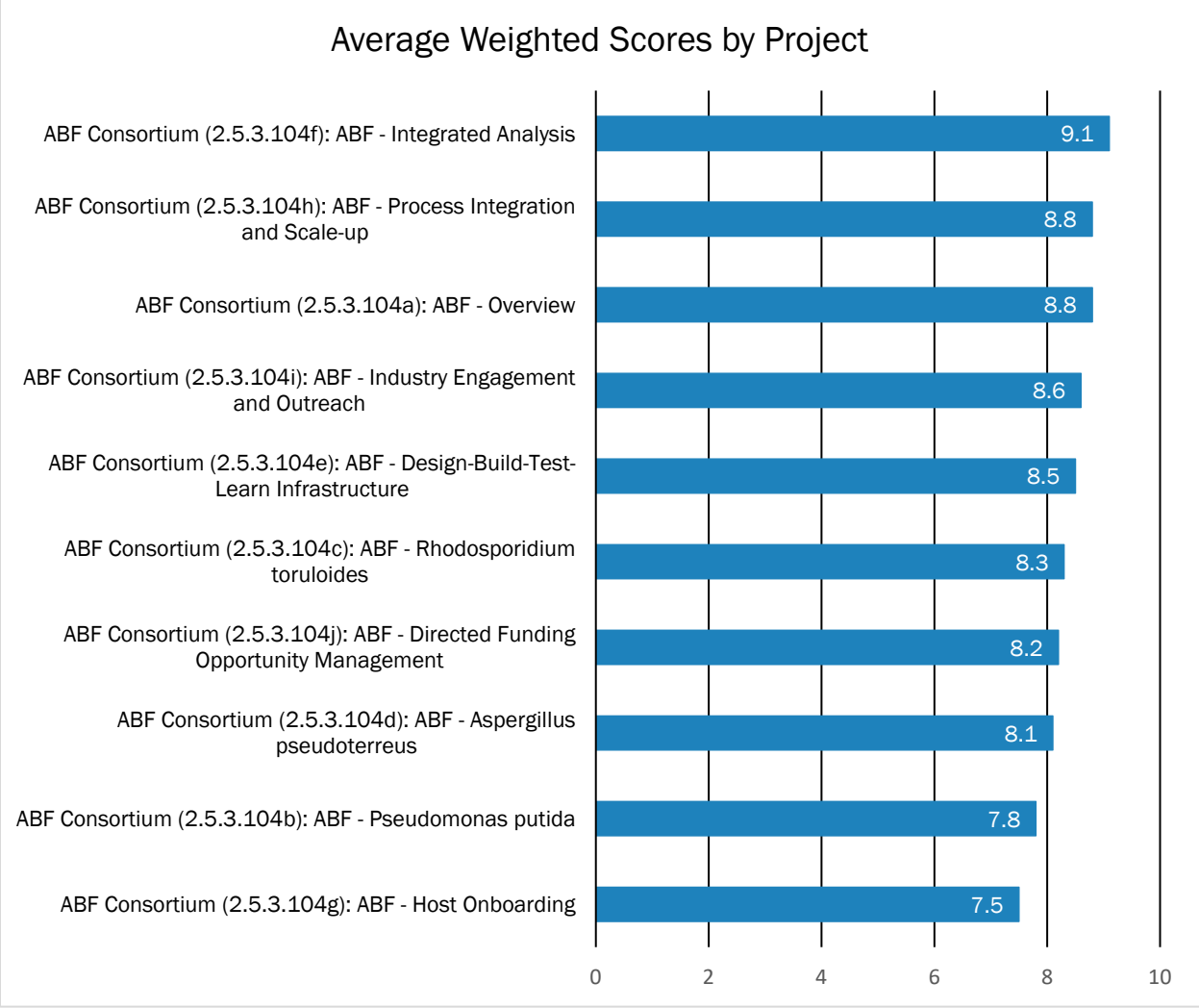
ABF REVIEW PANEL

The following external experts served as reviewers for the ABF Technology Area during the 2019 Project Peer Review:

Name	Affiliation
Ben Gordon*	Broad Foundry
Matthew Tobin	Matthew B. Tobin Consulting
Farzaneh Rezaei	Pivot Bio
Chris Rao	University of Illinois at Urbana–Champaign
Steve Van Dien	Persephone Biome, Inc.

*Lead Reviewer

TECHNOLOGY AREA SCORE RESULTS



ABF REVIEW PANEL SUMMARY REPORT

Prepared by the Agile BioFoundry Review Panel

The ABF aims to reduce the time and cost of commercialization of bioproduction of advanced biofuels and renewable high-volume chemicals through the development of integrated capabilities across the national laboratories. In the peer review, the ABF team presented its approach to this challenge, which comprises three overlapping thrusts: foundational technology development, proof-of-principle projects, and industry engagement.

Technology development is organized around so-called DBTL engineering cycles. It includes innovative work concerning individual technology DBTL components, such as new methods for collecting and analyzing omics data. It also includes efforts toward program-wide technology integration. Importantly, this portfolio of components extends beyond core DBTL technologies to include upstream techno-economic analysis (TEA) and life cycle assessment (LCA), as well as downstream process integration and scale-up.

Proof-of-principle efforts are focused on a number of testbeds, which are structured as microbial production hosts that have been paired with specific chemical targets via TEA/LCA. Three different host-target efforts were presented, and all reported substantial progress enabled by the DBTL integration efforts.

ABF's third thrust aims to ensure the commercial relevance of ABF efforts. To do this, the team has undertaken a multifaceted program for industry engagement that includes collaboration, novel funding, intellectual property (IP) mechanisms, formal surveys, and direct guidance via an industrial advisory board. The review panel evaluated ABF activities both individually and in aggregate. This report summarizes the panel's key impressions of the aggregate effort.

IMPACT

The ABF appears to be on course to fill a critical need not currently addressed by industry or academia—facilitating the growth of the bioeconomy by lowering technical and economic barriers to commercialization of bioproduction, particularly from DOE-relevant feedstocks. As detailed in the sections that follow, individual technical efforts will advance diverse development opportunities by expanding the menu of options for converting feedstocks into commercially viable offerings. For example, by investing in general-purpose infrastructure that can streamline the multistep processes of bringing a new organism online, engineering it to produce molecules at commercially-relevant levels, and piloting scale-up, ABF efforts have the potential to provide an unparalleled mechanism for industrial entities to leverage the national laboratories to de-risk their own internal research and development (R&D). The potential of this impact may be especially high for nascent tech entities. The ABF aims to enable a path to access tools that are not otherwise available without substantial capital investment and/or specialized in-house expertise.

Recognizing that their technology development efforts will only have substantial impact once they are leveraged by industry, the ABF has placed considerable focus on engagement mechanisms. As evidenced by the response to directed funding opportunities (DFOs) and funding opportunity announcements (FOAs), demand and awareness from industry is already strong, and continues to grow. The panel was also particularly supportive of efforts to ensure impact by enforcing TEA/LCA-based prioritization of host-target pairs.

INNOVATION

The ABF is demonstrating impressive innovation in three different spheres: (1) within the core DBTL technologies, (2) in areas extending beyond DBTL, and (3) in technology integration and program management. The demonstrations of core DBTL technologies that were highlighted in peer review were centered around three different target-host pairs, for which the team demonstrated the power of applying iterative rounds of strain construction, screening, metabolic modeling and machine learning, multiomics analysis, and fermentation development. The panel found it noteworthy that the team leveraged its flexibility

by deploying DBTL at different scales on a case-by-case basis. It applied "mini"-DBTL for fewer than a dozen strains, and "full" DBTL when needed for experiments requiring on the order of a hundred strains. In addition, by applying quantitative metabolic and kinetic models, and by combining them with omics data, the team identified nonobvious genetic targets for knockout and overexpression. Some of these have been experimentally validated, but more validation is required (and is reportedly planned). The ABF team also presented highlights of DBTL innovations, which included design and data management software systems, DNA sequencing, new technologies for generating sequence variation, omics, and machine learning. Over the course of the project period, the team also demonstrated improvements in overall DBTL times, though much more improvement is still needed.

The panel also recognized important innovations in areas beyond the DBTL core. The new host onboarding program focuses on de-risking the processes of selecting and adapting new microorganisms for production. This fills an important gap in the field by streamlining access to non-model organisms, and it therefore has the potential to generate significant value and to expand the pool of industrially-relevant hosts and host-target pairs. With respect to process integration and scale-up, novel "round-robin" testing across different facilities validates processes in new ways, and also prepares the team for tech transfer. On the more bureaucratic side, the team has innovated important means to encourage and streamline industry engagement through FOA, DFO, and cooperative research and development agreement (CRADA) mechanisms. As an example, the panel was encouraged by the team's experimentation with a uniform CRADA. This effort was conceived in response to extensive delays that arose from contract negotiations, and it aims to increase contracting efficiency by establishing terms up front via a public disclosure. If it is effective, then it will serve as a model for other efforts.

Finally, the review panel also favorably reviewed the management plan, which innovates by combining collaboration best practices and a dedicated project manager with new domain-specific technologies (e.g., the Design Implementation Validation Automation [DIVA] software platform). The ABF team uses this combination to manage communication and coordination of complex projects involving multiple laboratories, companies, and technologies. In the 2017 Peer Review, reviewers expressed concern that geographic distance would impede integration, but this has apparently not been the case so far.

SYNERGIES

Perhaps uniquely within the BETO portfolio, the ABF is explicitly predicated on cultivating synergies across the national labs. The effort is predicated on the assumption that careful coordination of a portfolio of diverse technologies can bring about a paradigm shift in biotechnology R&D. In this light, perhaps the very most significant achievement of the current performance period is the project's operational status—the ABF platform is up and running! More than a dozen DBTL and mini-DBTL cycles have already been completed, validating the ability of the consortium to leverage synergies across the program. To its credit, the team has also started to incorporate TEA/LCA, host onboarding, and process integration into development cycles; more emphasis in these areas would further benefit the program.

The panel also noted a few synergies with other technology areas within the BETO portfolio. For example, a handful of projects in the Biochemical Conversion Technology Area are already leveraging the ABF to differing degrees. While these engagements should continue to be nurtured, the panel also recommends that the team explore the potential for synergy across several other technology areas, such as algal systems, carbon dioxide, and lignin utilization. Engagement with projects in these technology areas could also serve to provide metrics regarding technology impact, and information flowing back from these activities into the ABF could also provide helpful direction. For example, new technology developments regarding feedstocks, deconstruction technologies, CO_2 recovery strategies, or coproduct processes may change the math regarding target downselection.

FOCUS

The panel was satisfied with the focuses across DBTL, integration, and industry engagement. We note, however, that a comprehensive review of all individual component technical thrusts of DBTL itself was not provided in the peer review due to time constraints. Similarly, the explicit technical connections between individual research project goals and the overarching program-level goal of 10-time improvement in efficiency were not presented. As a result, the panel's assessment of the ABF DBTL focus is limited to the testbeds and highlights that were provided. In these, the panel feels that the ABF is appropriately focused on improving the speed and power of DBTL cycles. That said, the panel also recommends that even more emphasis be placed on TEA, LCA, process integration, and scale-up. The panel recognizes that the program has already placed significant prioritization in these areas, but as they are critical for enabling and de-risking R&D efforts, additional measures should be taken to ensure that they keep pace with all of the emergent technologies in the portfolio.

The review panel also provided feedback on the ABF's prioritization of nonobvious "learn." They agree with the ABF team that the ultimate value of the platform will rest on its ability to provide actionable insights via DBTL efforts. However, it is not yet clear whether novel artificial intelligence and machine-learning algorithms are necessary to achieve this goal. For example, it may be the case that the unique data-generation capabilities of the ABF may produce information rich enough for simple, off-the-shelf, learning algorithms to be sufficient. As such, novel methods will need to demonstrate added value in comparison to standard, well-established methods.

Finally, the panel was very supportive of the ABF's focus on industry engagement. The team's diversified and innovative efforts are on target for an enterprise of this type, and through its interactions it has developed a unique expertise in regarding trends and opportunities. The panel recommends that the team find ways to disseminate this knowledge (e.g., via publication).

TECHNOLOGY DEVELOPMENT PIPELINE

In general, the panel felt that the technologies pursued were very appropriate for the current stage of technology. The panel did note that the current testbed projects may be too early stage to realize (and test) the full potential of ABF's DBTL approach. In order to better validate and showcase the entire workflow, from TEA though DBTL to process development and scale-up, the panel recommends adding projects that are more mature (e.g., that are already 20%–50% of the way towards commercial goals). In this way, elements of the ABF workflow can be exercised without being hampered, for example, by bottlenecks in the development of genetic tools for new non-model organisms. These projects could be industry engagement projects, or projects linked to other technology areas within BETO. Feedback from such projects would provide valuable feedback to guide ABF future technology prioritization.

RECOMMENDATIONS

First, the panel recommends placing an even greater emphasis on production-oriented research; specifically, TEA/LCA and process development. The work products from these thrusts were very well received, but there is a critical need for even more innovation from both. Similarly, where possible, projects should transition from sugars to feedstocks as early as possible. Some of this is happening already. The recommendation in the previous section regarding application of ABF workflow to more mature projects also falls into this category.

Second, the panel has made a number of technology-specific recommendations in the individual project reviews. Overall, these recommendations provide guidance to ensure both that the areas of technical weakness are appropriately remedied, and that outcomes are appropriately benchmarked. As an example area of weakness, the panel noted that although DBTL cycle times are improving over time, they are still slow. One emergent bottleneck was "Test," indicating an area that ABF should focus efforts to increase efficiency. Similarly, regarding benchmarking, the panel noted that novelty in "Learn" algorithms may not be a firm requirement for ABF success as described above. At the program level, there needs to be clearer, direct

connections made between project-level aims and milestones and the overall 10-times efficiency improvement goal.

Third, as the ABF matures, the panel felt strongly that its program management, governance structures, and business model will also need to mature. First, as mentioned above, concerns from the 2017 Peer Review regarding distributed operations have so far been successfully addressed via a number of mechanisms to facilitate collaboration. In the present review, however, the panel is concerned that these mechanisms will not scale as ABF's purview and portfolio of projects grows. It will therefore become important to institute tools and additional layers of technical project management and coordination, both in terms of personnel and in terms of additional formal mechanisms for technical tracking and communication that, for example, may go beyond DIVA and the Office of Energy Efficiency and Renewable Energy annual operating plan (AOP) system. Regarding governance, the industry advisory board (IAB) would be strengthened and its perspective expanded by adding companies outside of bioconversion and representing more of the country. Moreover, in order to ensure ongoing appropriate focus within the portfolio of foundational ABF technologies, the panel recommends instituting two additional governance structures complementary to the IAB: a scientific advisory board composed of external experts in academia in artificial intelligence, genetics, TEA, etc. to provide a mechanism for deep portfolio review of technical directions at a level of detail not compatible with the biannual peer review process, and a biosecurity advisory board to aid in the evaluation of dual-use technologies and other potential risks. The intention here is to help the leadership avoid allowing the shape of the portfolio to be determined by inertia rather than by careful, strategic assessment and reassessment. Finally, as the ABF transitions into a national resource, it should continue its ongoing work to develop a clear business model that includes long-term sustenance, IP strategy, and additional mechanisms to streamline engagement with third parties.

ABF PROGRAMMATIC RESPONSE

OVERVIEW

The program would like to thank the reviewers for their time and thoughtful comments throughout the review process. The program responses to reviewer recommendations are found in the following section.

Recommendation 1: Focus on mature projects.

The Panel recommends placing greater focus on production-oriented research using TEA/LCA tools and real feedstocks as soon as possible within a project. We agree that utilizing TEA/LCA tools to prioritize targets will be key to achieving industrial relevance. The budget for TEA/LCA activities has been doubled moving into FY 2020 to increase the scope of activities, as well as to keep up with the additional new target and host demonstration projects being undertaken. Part of the selection process for new demonstration projects will involve an initial assessment of TEA/LCA, and R&D will continue to be guided by the findings of TEA/LCA sensitivity analyses. Metrics have been selected for target-host pair demonstrations that are >50% of the way toward commercial goals, and selection criteria will be developed to emphasize projects that are 20%–50% of the way toward commercial goals in projects from competitive solicitations. The ABF will continue to use deacetylated, mechanically refined, enzymatically hydrolyzed corn stover provided to each ABF R&D project as a standard, real-world feedstock. Starting in FY 2020, this material will also be pre-filtered and clarified for ease of use both internal to the ABF and for partner projects.

Recommendation 2: Reduce DBTL cycle time.

The panel observed that cycle times for DBTL were improving over time but were quite high, particularly for "Test." In addition, the panel felt that more direct connections needed to be made between individual projects

and higher-level goals as the project matures. We agree that a focus on cycle time will be critical to the success of the ABF. Several milestones have been set for early FY 2020 to create a better definition of cycle time and to characterize cycle time for all ABF unit operations individually. New "Test" capacity is being brought online in the form of BioLector and ambr systems as well as DNA sequencing capacity, gas chromatography/mass spectrometry and high-performance liquid chromatography capacity, and proteomics capacity. In addition, we are creating a new set of milestones to address connectivity of individual projects to the overall ABF DBTL efficiency improvement goals. We will be using project management tools, such as Gantt charts and connectivity maps, to help ensure that dependencies are addressed clearly.

Recommendation 3: Management during growth.

The panel noted that as the ABF matures and creates more partnerships, program management and governance structures must also mature. We agree with the panel that more external projects will require additional resource management on the part of the ABF. We are exploring the option of recruiting a coordinator for external projects who would be responsible for ensuring that equipment and personnel resources are properly managed to meet external obligations as well as increase internal capabilities. The panel also recommended that a scientific advisory board be formed to ensure that the technical direction of the ABF is being guided by the latest developments in the fields of synthetic biology, machine learning, and biomanufacturing. We agree that an external advisory group in addition to the IAB aimed more at scientific direction would have value and we are exploring options, such as adding additional scientific experts to the existing IAB, in this space.

ABF – OVERVIEW

Agile BioFoundry Consortium

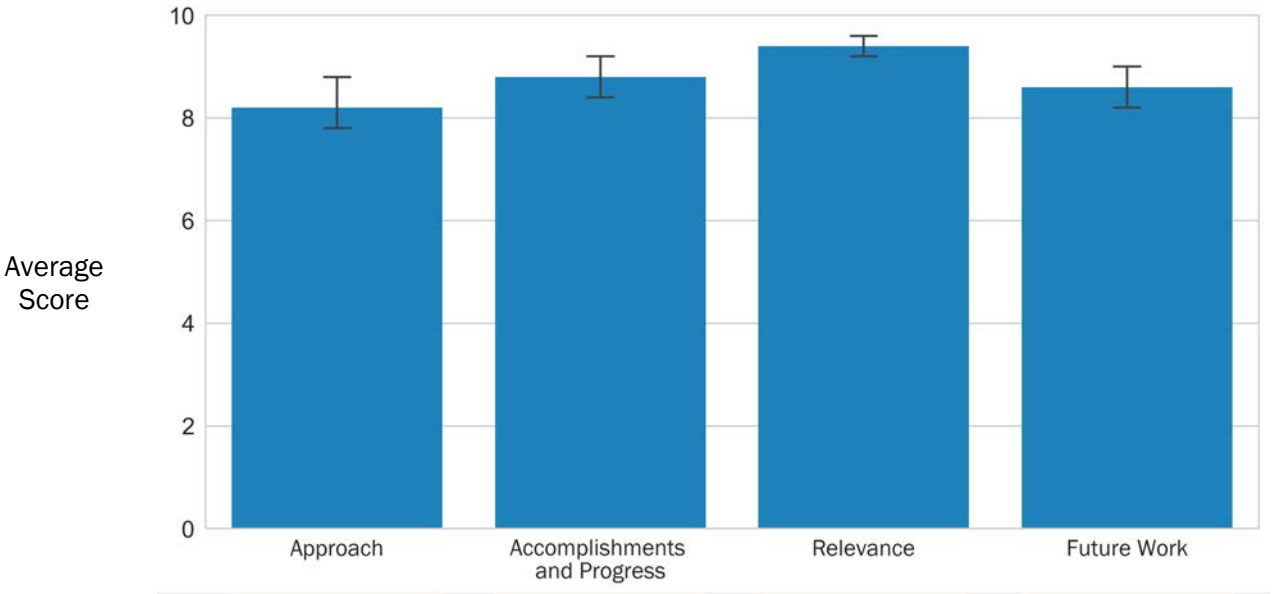
PROJECT DESCRIPTION

WBS:	2.5.3.104a
CID:	NL0030036a
Principal Investigator:	Dr. Nathan Hillson
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$4,400,000
DOE Funding FY16:	$300,000
DOE Funding FY17:	$1,300,000
DOE Funding FY18:	$1,700,000
DOE Funding FY19:	$1,100,000
Project Status:	Ongoing

The overarching goal of DOE's Agile BioFoundry Consortium (ABF) is to 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 biofoundry that produces synthetic biology. Towards achieving this goal, the ABF has brought together domain expertise and infrastructure that is distributed across eight U.S. national labs: Lawrence Berkeley National Laboratory, Sandia National Laboratories, Pacific Northwest National Laboratory, National Renewable Energy Laboratory, Argonne National Laboratory, Oak Ridge National Laboratory, Los Alamos National Laboratory, and Idaho National Laboratory. This talk provided an overview of the ABF by setting the stage for the subsequent ABF presentations and provided some R&D highlights, including work with industry, from the first 2.5 years of the ABF's operations.

Weighted Project Score: 8.8

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



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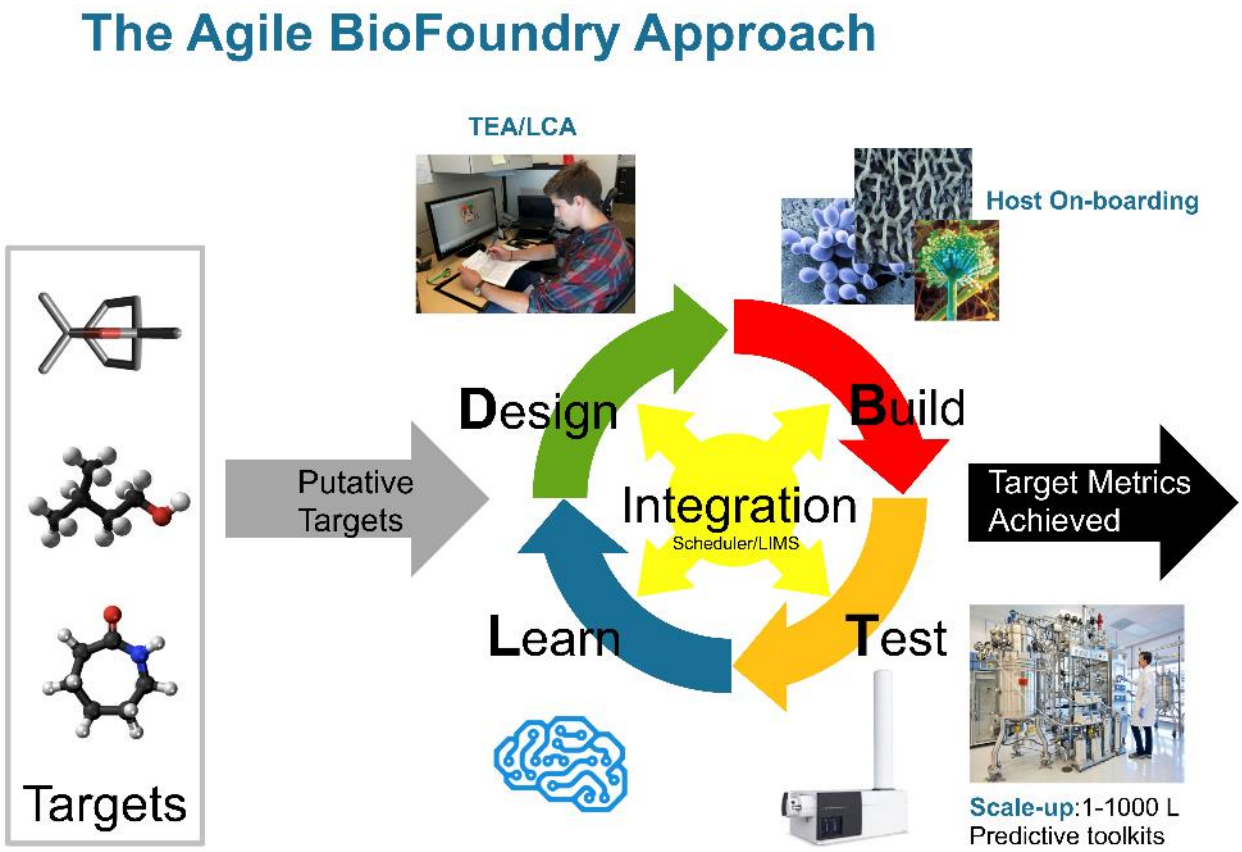


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- The overall goal of the ABF is to reduce the time and cost of commercialization through the development of a distributed foundry. The foundry is built around the DBTL paradigm. The team is focusing on the Learn component, which is the most challenging step in this process. They are placing particular emphasis on nonintuitive Learn predictions, which really amounts to showing that the Learn step provides concrete value. Overall, the vision is very compelling. Scale-up and TEA provide the main differentiators from other efforts along with the distributed nature of the foundry. Reasonable milestones are provided though they are somewhat vague. More concrete milestones would strengthen the project. Also, it is not clear how the the team will achieve their stated efficiency goals. Will it be through improved process knowledge, eliminating bottlenecks, better operational management, or simply increasing capacity?

- The ABF is well situated to lower technical and economic barriers faced by industry in developing new bioproducts, attempting to increase speed, and decrease risk. It is an optimal platform for integrating and accessing the potential of the national laboratories, synergizing strengths across sites with the right work, being done by the right lab, with the right team. There appears to be significant progress in national laboratory integration from previous reviews. Critically, the ABF is focused on alignment with industrial needs and realities—a must-have for its relevance and to achieve BETO objectives.

- The ABF was created to develop an infrastructure enabling a rapid DBTL cycle that can achieve a 50% reduction in project development time. The concept is to develop host-target pairs that can be leveraged

by industry to go the rest of the way to commercialization. Functional groups are set up to operate like an industry program. There is a very formal project management structure for tracking tasks and milestones, including a 50% time project manager. The team has met most of the milestones so far, and the accomplishments will be covered in the individual presentations. There has been good industry engagement, and this will be streamlined in the future. Technical aspects of the future work is well defined, though there is some ambiguity around how to measure improvement in DBTL cycle time. TEA work has been good so far for preliminary analysis of the host/target pairs, but will require more emphasis as the projects move forward.

- This is a well organized and clear project despite complexity of the project in regards to having multiple teams across the United States involved. As with the previous ABF project, the PIs are encouraged to expand on how their approach will help reduce development cycle by 10 times as this does not seem to be clear in any of the ABF projects.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- The ABF overview presentation included substantially simplified milestone language. For example, slide 36 listed a FY 2019 Q2 milestone as "Deep Learning nonintuitive predictions." However, the actual language for this particular milestone is "Deep Learning from multiomics data sets for one or more Crop 1 or Crop 2 targets leads to a set of actionable genetic and/or process modifications (each individually or in combination predicted to increase titer, rate, or yield by 20% or more) to be implemented and evaluated for FY 2019 go-no-go decision." As such, the actual milestones are more concrete than the simplified versions presented to the reviewers. Regarding the achievement of our stated (10 times) efficiency improvement goals, as the reviewer notes, efficiency is a product of multiple factors, and as such, there are multiple facets through which efficiency could be improved. Whereas in the presentation we did discuss several of these facets (e.g., number of cycles required to obtain a given level of performance, strains/designs needed per cycle, personnel/instrumentation resources used per cycle, cycle time), the reviewer is correct in that we did not specify precisely through which facet(s) the efficiency goals would be achieved. We have been pursuing an all-of-the-above strategy, including, for example, emphasis on the Learn component, to not only reduce the number of cycles and strains required per cycle, but also to enhance operations toward reducing resource requirements and cycle times. For the ABF's second three-year AOP cycle, we will quantitatively evaluate efficiency improvements and analyze which facet(s) prove to be the best opportunities for further efficiency gains.

- The reviewer is correct in stating that there has been ambiguity in how we measure improvements to the DBTL cycle time. For our presentation during peer review, we had qualitative but not quantitative definitions of what constitutes a full DBTL (vs. mini-DBTL) cycle. For example, that DBTL requires a minimum set of unit operations, but what that minimum set is had not yet been specified. As such, without a concrete definition, it had not yet been possible to unambiguously measure DBTL cycle time (and as a consequence, improvements thereof). For the ABF's second three-year AOP cycle, we will have the concrete DBTL specification in place, and we will work towards increasingly and automatically capturing cycle time metrics in our workflow-supporting software infrastructure (e.g., DIVA), which will facilitate and standardize how cycle times (and their improvements) are measured. For the ABF's second three-year AOP cycle, we will place increasing emphasis on TEA and LCA as the project moves forward.

- A minor correction to the reviewer's comment. One outcome of the ABF will be to increase engineering cycle efficiency, not the cycle (time) exclusively. See above regarding the multifaceted approach to increasing efficiency.

ABF – *PSEUDOMONAS PUTIDA*

Agile BioFoundry Consortium

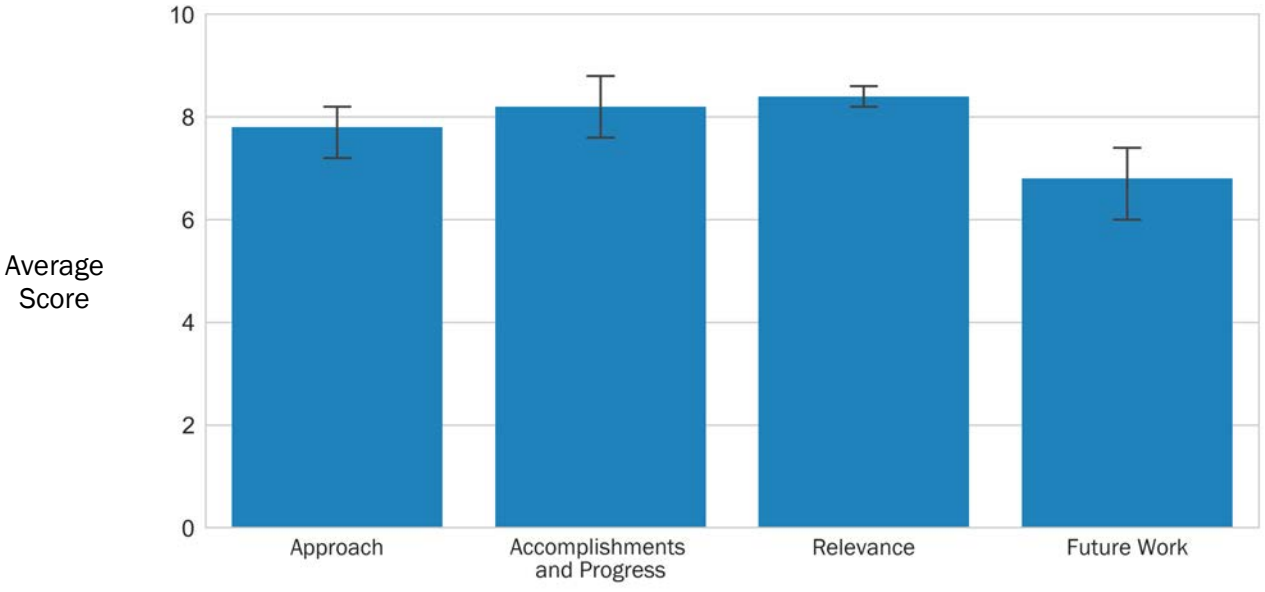
PROJECT DESCRIPTION

WBS:	2.5.3.104b
CID:	NL0030036b
Principal Investigator:	Dr. Gregg Beckham
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$10,300,000
DOE Funding FY16:	$500,000
DOE Funding FY17:	$3,800,000
DOE Funding FY18:	$3,600,000
DOE Funding FY19:	$2,400,000
Project Status:	Ongoing

The goal of the target-host pairings in the ABF is to employ the DBTL cycle to engineer promising microbial hosts to produce target molecules of interest to the bioeconomy. This will aid in the establishment of the distributed foundry, highlight key bottlenecks in the DBTL process, and enable the accomplishment of critical milestones toward the foundry concept. *Pseudomonas putida* KT2440 has been selected as one of three hosts in the ABF because it is a readily engineered, Gram-negative, fast-growing bacterium that is well known to exhibit high toxicity tolerance to many established microbial inhibitors found in biomass-derived streams and for which reasonable genetic tools exist. Moreover, from a product perspective, it has both robust aromatic-catabolic pathways (relevant to producing shikimate-derived products) and naturally produces polyhydroxyalkanoates (PHAs) under nutrient-limiting conditions. For Target 1 of the ABF, *cis,cis*-muconic acid (and other C6 diacids in the same pathway) was selected as the primary target, which can be converted into adipic acid and terephthalic acid, as well as used as a functional replacement platform chemical. For Target 2 of the ABF with *P. putida*, branched-chain PHAs were selected as the primary target compounds, which can be used as a biodegradable and inherently recyclable packaging material.

Weighted Project Score: 7.8

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



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The approach in this task in the ABF closely mirrors that of the other target-host pairings, bringing a multilaboratory team together to implement various aspects of the DBTL cycle and to promote tool development, with the aim of both demonstrating target molecule production and optimizing their titer, rate, and yield. One key focus in this task is to enhance the Learn functionality so that it will be able to effectively analyze key data from Design-Build-Test and make nonintuitive predictions that can be used to improve titer, yate, and/or yield of these crop molecules. The technical accomplishments to date in this task include the development of robust, baseline strains that are able to produce *cis,cis*-muconic acid, which resulted from the ABF pilot project. The strains were examined in the first DBTL cycle with multiomics Test, and nonintuitive predictions from Learn were used to engineer further improvements in strain performance. New DBTL cycles in this vein include "Learn-friendly" Test experiments that are ongoing now to more comprehensively characterize sugar utilization in this strain. In several mini-DBTL cycles, the project has employed DBTL cycles to accomplish direct adipic acid production in *P. puitda*, expanded the strain to be able to rapidly consume xylose and arabinose (in the presence of glucose), and developed multiple *in vivo* and *in vitro* biosensors for detecting key intermediates and products (to accelerate the DBTL cycle). Overall, these efforts have shown that the ABF concept works, and, most importantly, have identified areas where improvements can be made to increase process efficiency and decrease cycle time, both metrics that will enable the ABF to reach its primary goal of stimulating the bioeconomy by reducing time and cost for deployment of bioproducts into the market.

Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- As a testbed for the ABF effort, this team reports an impressive number of *P. putida* developments and innovations. Through a combination of different kinds of DBTL and mini-DBTL cycles, they have enhanced production of two target compounds, improved sugar utilization in the host, and made inroads into taking greater advantage of omics data, machine learning, and biosensors to further enhance development and to expand into new target compounds. It will be important to evaluate the impact of these latter efforts as they are completed during the next phase of the performance period.

- This subproject is focused on producing C6 diacids and branched-chained PHA in *Pseudomonas putida* to demonstrate the ABF framework. The team is mostly focused on improving rates based on TEA. Overall, the team is making good progress, in particular with regards to generating data sets for the Learn step. All of the proposed projects are commercially promising. The biosensor work is also very promising.

- With less than a year remaining in this project, future work seems ambitious. Prioritizing tasks and research to support key outcomes is needed.

- Development of a nontraditional and industrially important bacterial host in *P. putida* is of high interest, and this project has a good start, especially regarding muconic acid production. The use of a TEA to focus rate is a good sign that TEAs are being employed to make project decisions. The project appears to have discovered some nonintuitive predictions, a highly important validation from omics-driven Test-Learn.

- *Pseudomonas putida* is being developed as a host, with the model products being C6 diacids and branched-chain polyhydroxyalkanoates. Actual hydrolysate is being used to evalate performance. The overall objectives are to develop a robust bacterial host while demonstrating improvements to the DBTL cycle time and generating data sets to be used in modeling and learning. The team has demonstrated that the DBTL cycle can be applied iteratively to overcome bottlenecks and make improvements. Omics and computational work led to specific targets. However, improvement so far seems to be a result of improved sugar uptake alone, rather than any pathway enhancements. It may be that enzyme engineering is needed to improve pathway flux. In addition, the DBTL cycle is also quite slow. There are inherent limitations in the speed of conducting genetic manipulations and other tasks, but more workflow management could streamline progress.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewer for the positive feedback. We completely agree that we need to evaluate the impact of the tool development as part of the DBTL cycle and overall ABF concept, and we are actively doing that now. We note, for example, that the biosensor work has already led directly to improved muconate strains, so investment in those tools are generating improvements and accelerated DBTL progress.

- We agree that the future work is indeed ambitious, but we have an aggressive timeline and a large team working in close coordination to be able to be successful in our objectives.

- We thank the reviewer for the positive feedback, and we agree that the initial DBTL cycle timelines were very long. This was the case mostly because we were setting up the tools, workflows, etc. We anticipate that the timelines will be reduced significantly in future DBTL cycles.

ABF – *RHODOSPORIDIUM TORULOIDES*

Agile BioFoundry Consortium

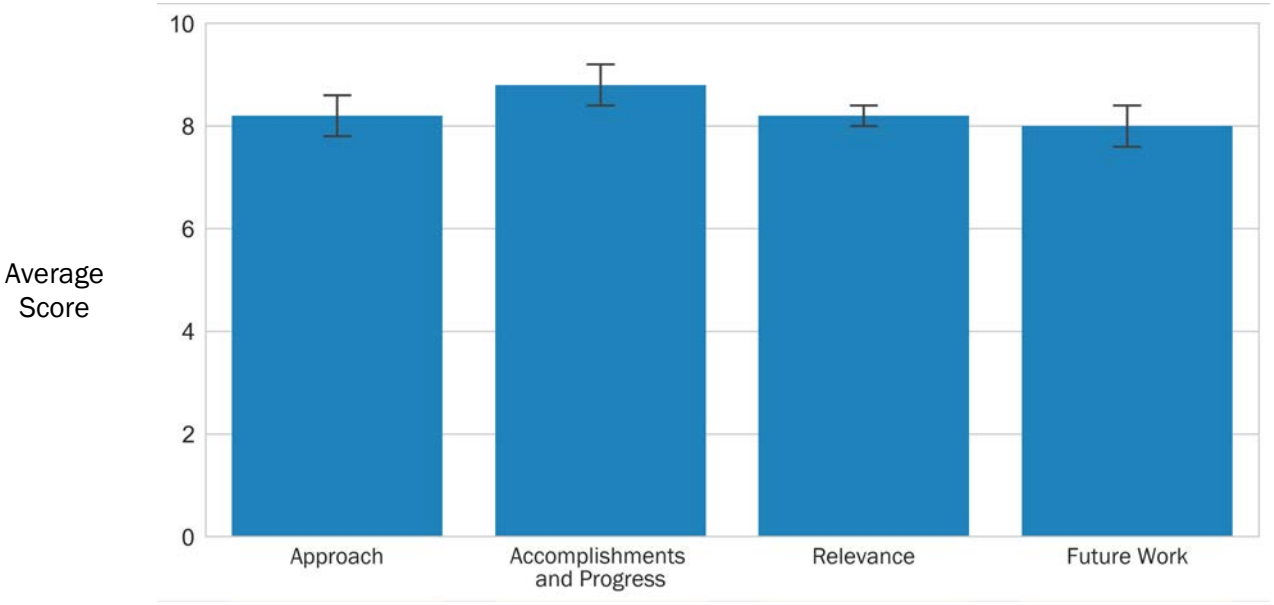
PROJECT DESCRIPTION

WBS:	2.5.3.104c
CID:	NL0030036c
Principal Investigator:	Dr. John Gladden
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$4,620,000
DOE Funding FY16:	$20,000
DOE Funding FY17:	$1,100,000
DOE Funding FY18:	$1,600,000
DOE Funding FY19:	$1,900,000
Project Status:	Ongoing

The goal of the target-host pairings in the ABF is to employ the DBTL cycle to engineer promising microbial hosts to produce target molecules of interest to the bioeconomy. This will aid in the establishment of the distributed foundry, highlight key bottlenecks in the DBTL process, and enable the accomplishment of critical milestones toward the foundry concept. *Rhodosporidium toruloides* IFO0880 has been selected as one of three hosts in the ABF because it is a readily engineered, fast-growing basidiomycete yeast that is able to grow on lignocellulose-derived hexose and pentose sugars as well as lignin-derived aromatics. It is also carotenogenic and oleaginous, indicating that it has high flux through two biosynthetic pathways that can be leveraged to produce a myriad of biofuels and bioproducts. For Target 1 of the ABF, four terpenes were selected (cineole, bisabolene, farnesene, and kaurene), each with applications as biofuels or bioproducts (e.g., adhesives, polymers, etc.). For Target 2 of the ABF, fatty alcohols were selected as the primary target compounds, which have many applications, such as lubricants and detergents.

Weighted Project Score: 8.3

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



I One standard deviation of reviewers' scores

The approach in this task in the ABF closely mirrors that of the other target-host pairings, bringing a multilaboratory team together to implement various aspects of the DBTL cycle and to promote tool development, with the aim of both demonstrating target molecule production and optimizing their titer, rate, and yield. One key focus area in this task is to enhance the Learn functionality so that it will be able to effectively analyze key data from Design-Build-Test and make nonintuitive predictions that can be used to improve titer, rate, and/or yield of these crop molecules. The technical accomplishments to date in this task include the development of robust, baseline strains that produce either terpenes or fatty alcohols, most of these molecules at g/L titers. The bisabolene strain was examined in the first DBTL cycle with multiomics Test, and several nonintuitive predictions from Learn are currently being tested in the second DBTL cycle for their ability to improve in strain performance. New DBTL cycles in this vein include "Learn-friendly" Test experiments for both terpenes and fatty alcohols, designed specifically for machine learning, with the goal to optimize either pathway dynamics or growth conditions toward increased titers, respectively. In several mini-DBTL cycles, the project has identified key Design-Build strategies for rapid optimization of terpene production and for fatty alcohols, and identified several key host genes to modulate for enhanced production. Overall, these efforts have shown that the ABF concept works, and, most importantly, have identified areas where improvements can be made to increase process efficiency and decrease cycle time, both metrics that will enable the ABF to reach its primary goal of stimulating the bioeconomy by reducing time and cost for deployment of bioproducts into the market.

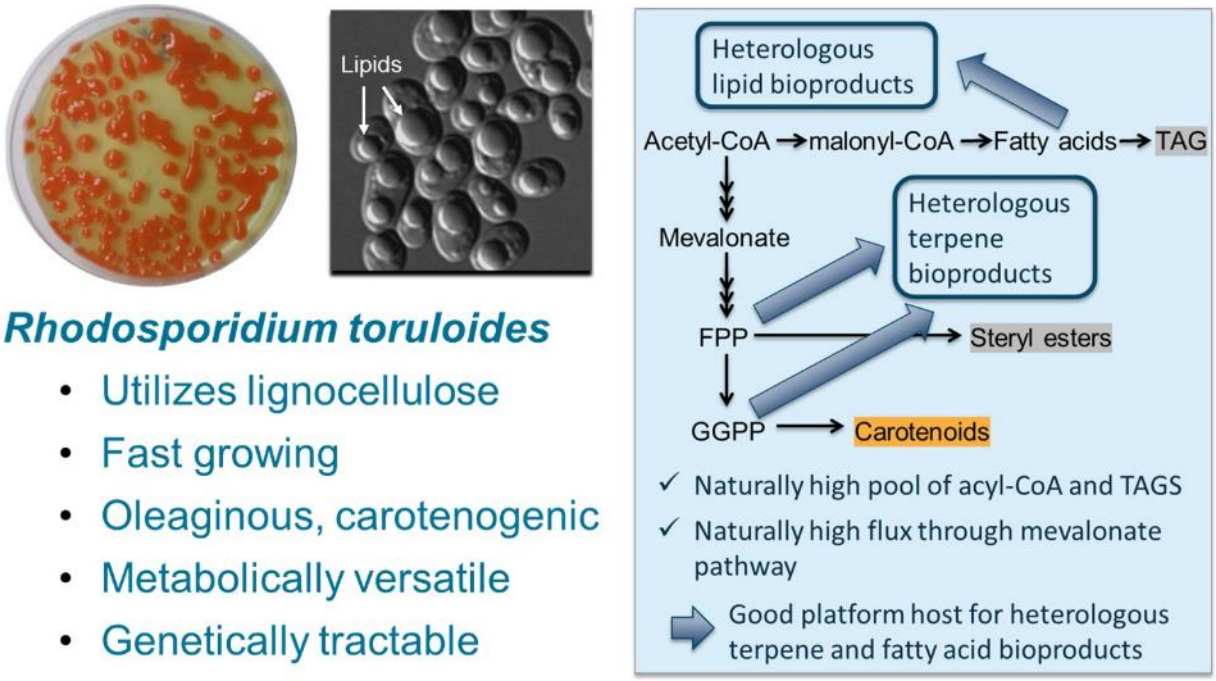


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- As a testbed for the ABF, this team reports significant process in using DBTL to aid in the development of the expression of terpenes and fatty alcohols in *R. toruloides*. Through large-scale testing (100–200 strains constructed), they have shown improvements in production of both of these target compounds. Particularly noteworthy are the impacts of computational methods on their progress; simple regression models validate predictions regarding the impact of expression level on yield and by developing new, quantitative metabolic models and kinetic models for this organism and combining them with omics

data, they have shown that they can identify nonobvious genetic targets for optimization, some of which have been validated experimentally.

- This goal of the subproject is to produce terpenes and fatty alcohols in *Rhodosporidium toruloides*. This is a great target organism, as little is known about its metabolism. The team is making good progress. The dynamic proteomic data sets will be very useful for the Learn step. The initial Learn prediction regarding transcription factor overexpression seems promising.

- Great progress so far. Same as other subsections in ABF projects, it would be great if the PI can expand on time reduction (10 times) and their proposed approach claiming to achieve that reduction. Overall, subprojects are missing the connection to their project to the overall goal/target in a clear way.

- *Rhodosporidium toruloides* is a fungal host being developed for terpenes and long-chain fatty alcohols. The organism readily consumes not only C5 and C6 sugars, but also aromatics; thus it can be used in lignin conversion. This project showcases several capabilites of the ABF:

    - Iterative rounds of strain construction and screening
    - Metabolic modeling and machine learning
    - Multiomics analysis
    - Fermentation development.

  One particular novel application of machine learning was the "kinetic learn" method, using protein time series data in conjuction with a metabolic model to predict metabolite levels. The first DBTL cycle was completed for both products, and used to inform designs for the next cycle. Mini cycles were also used to screen strains and conditions prior to a full experiment where omics data were collected. It is still uncertain how useful the machine-learning approach was compared to rational intuition and the metabolic model alone. This will be clearer once the recommended modifications are tested and evaluated.

- *R. toruloides* is a good host choice for ABF objectives (e.g., for DBTL demonstration) and complements other host choices across the ABF. Target choices are also sensible and reflect pathways that lead to target-rich classes of compounds (terpenes and fatty acids) with wide commercialization potential.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Thanks for your comments. The ABF has certainly accelerated development of this organism.

- Thanks for your comments. We agree that this is a great target organism for the ABF.

- Our ultimate goal within the ABF is to reduce the time to bioprocess scale-up by 50% through improvements in DBTL cycle efficiency. Reduction of DBTL cycle time is one of several metrics that will contribute to increased DBTL cycle efficiency. One of our goals for the initial DBTL cycles implemented for each target/host pair was to identify areas where efficiency gains can be made. This exercise has allowed us to identify many areas for improvement. Specifically regarding time, improvements can be made in Design through the assembly and validation of a library of engineering parts, which can then be more rapidly refactored into new Designs with a greater likelihood of being functional. Within Build, improvements can be made with streamlined transformation and screening protocols as well as development of high-throughput plate-based protocols to enable examination of a greater number of samples in a shorter timeframe. For Test, now that analysis of baseline samples has been conducted and protocols validated for this host, sample analysis can be expediated. In addition, reliance on mini-DBTL Test analysis (high-performance liquid chromatography, gas

chromatography/mass spectrometry, etc.) can be expedited by high-throughput Testing of strains constructed in the aforementioned plate-based Build efforts. For Learn, we now have a metabolic model to put our multiomic data into context, which can be analyzed both manually and computationally. We are in the process of assessing several machine-learning platforms for data analysis, which should also expediate Learn and lead to better predictions for improvements to be made in subsequent DBTL cycles, reducing the time it takes to optimize titer, rate, and yield.

- Regarding connection to the broader ABF milestones, while not specifically called out in our presentations, all the ABF hosts have multiple specific milestones within the ABF. To assess the overall progress of the ABF, these milestones were specified to be met through the combination of all three ABF host organisms and their targets. So, rather than host-specific milestones, we aggregated host milestones. The milestones defined both the number of target molecules selected to be engineered into these host organism and specific titer, rate, and yield targets.

- While we are very excited about some of the machine-learning Learn methods, we are still in the early phases of validating and optimizing these approaches in *R. toruloides*. The ABF offers a great testbed for these approaches, and as time goes on, will provide the large data sets some of these methods require. We are in the process of dedicating a significant portion of our Test and Learn resources to generate those data, in consultation with the Learn team experts. We are also assessing less data-intensive Learn approaches in parallel to ensure we have multiple routes to success. Where possible, we will leverage the same data sets for different Learn approaches but will also generate tailored data as needed. We are in the process of vetting different Learn approaches, and will focus resources toward those that are determined to be the most promising after this initial vetting phase. We are excited to experimentally validate these approaches in upcoming DBTL cycles.

- Thanks for your comments. We agree that this is a good host to help us meet our ABF objectives.

ABF – *ASPERGILLUS PSEUDOTERREUS*

Agile BioFoundry Consortium

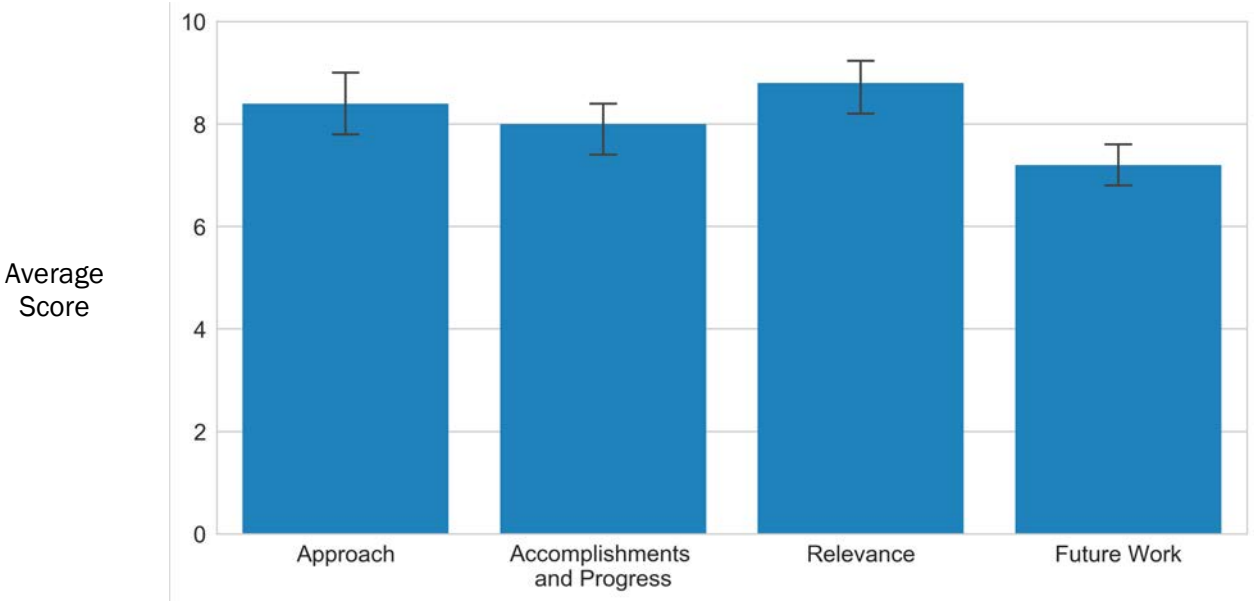
PROJECT DESCRIPTION

WBS:	2.5.3.104d
CID:	NL0030036d
Principal Investigator:	Dr. Jon Magnuson
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$5,720,000
DOE Funding FY16:	$20,000
DOE Funding FY17:	$1,900,000
DOE Funding FY18:	$2,000,000
DOE Funding FY19:	$1,800,000
Project Status:	Ongoing

The goal of the target-host pairings in the ABF is to employ the DBTL cycle to engineer promising microbial hosts to produce target molecules of interest to the bioeconomy. This will aid in the establishment of the distributed foundry, highlight key bottlenecks in the DBTL process, and enable accomplishment of critical milestones toward the foundry concept. *Aspergillus pseudoterreus* has been selected as one of three hosts in the ABF because it is a readily engineered, highly acid tolerant, and filamentous fungus that is used in industry for the production of native organic acids in their free acid form. It can grow on a wide variety of sugars, as well as oligosaccharides and polysaccharides with inorganic sources of other nutrients. For Target 1 of the ABF, 3-hydroxypropionic acid (3HP) was selected. This molecule can be converted into a major commodity chemical, acrylic acid, by technology existing within the ABF partner laboratories to serve as a direct replacement molecule. For Target 2 of the ABF with *A. pseudoterreus*, aconitic acid from the six-carbon tricarboxylic acid family, which includes citric and isocitric acids, was selected as the primary target compound. This family of six-carbon tricarboxylic acids are used as acidulants and chelators in the food, cement, and other industries.

Weighted Project Score: 8.1

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



I One standard deviation of reviewers' scores

The approach in this task in the ABF closely mirrors that of the other target-host pairings, bringing a multilaboratory team together to implement various aspects of the DBTL cycle and to promote tool development, with the aims of first demonstrating target molecule production and then optimizing their titer, rate, and yield. A key focus in this task is to enhance the Learn functionality so that it will be able to effectively analyze key data from Design-Build-Test and make nonintuitive predictions that can be used to improve titer, rate, and/or yield of these crop molecules. The technical accomplishments to date in this task include the development of acid-tolerant (pH 1–3) baseline strains that are able to produce 3-hydroxypropionic acid via the beta-alanine pathway. Strains containing one or two copies of the pathway at different locations in the chromosomes were examined in the first DBTL cycle with the multiomics Test and both traditional and nonintuitive Learn approaches. These have identified gene targets for another DBTL cycle in FY 2019 aimed at improving titer, rate, and/or yield of the target 3HP. Multiomics testing has also identified a list of candidates potentially involved in 3HP degradation that will be targets for deletion to increase net titer, rate, and yield. Similarly, transporter genes that may be involved in increasing aconitic acid transport between cell compartments and exports from the cell have been identified and will be the subject of another DBTL cycle for this target. Overall, these efforts have shown that the ABF concept works, and most importantly, have identified areas where improvements can be made to increase process efficiency and decrease cycle time, both metrics that will enable the ABF to reach its primary goal of stimulating the bioeconomy by reducing time and cost for deployment of bioproducts into the market.

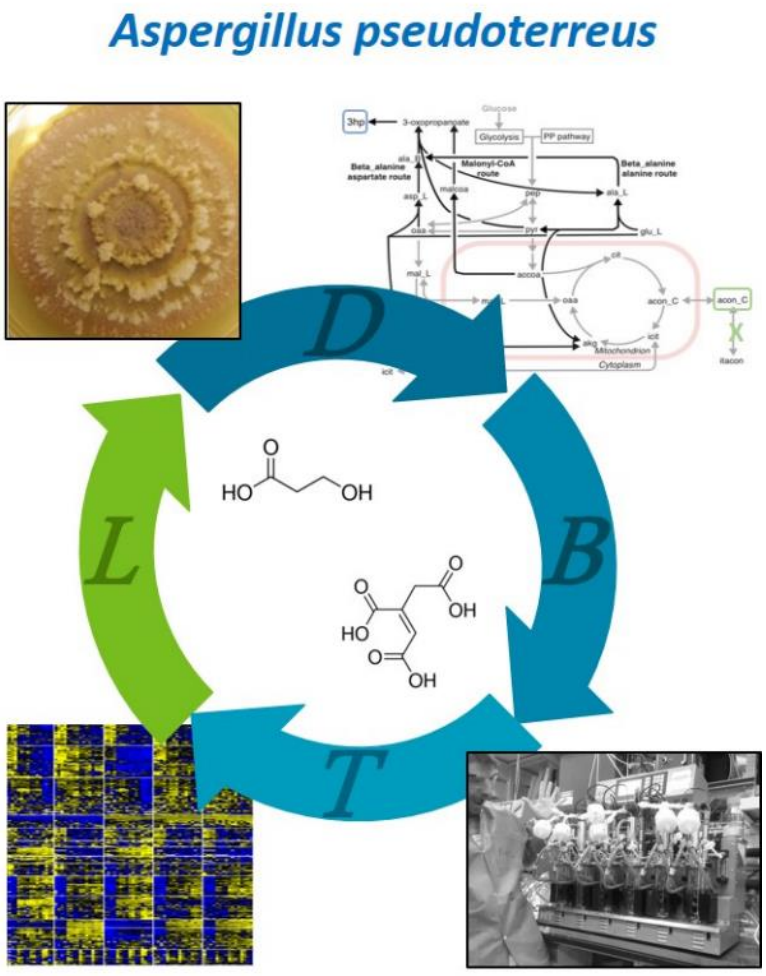


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- The goal of this subproject is to produce 3HP and aconitic acid in *Aspergillus pseudoterreus*. The inclusion of a filamentous fungus provides a useful test for the ABF. The team is making solid progress. It is difficult to evaluate the Learn component of this project at this stage, though it is clearly an important step. More details should be provided given the focus on Learn. While clear milestones are presented, the project would benefit from competitive benchmarks, particularly with regards to 3HP.

- Overall, significant portions of work were done (more than 80%), however future work still pictured significant research, which does not seem to be achievable within less than a year left in the project. IP was mentioned in the first slide, so if there is any IP associated with this project, the principal investigator would need to touch on potential IP.

- As a testbed for the ABF, this team reports in-process results in applying DBTL to aid in the development of the expression of organic acids in an industrially relevant fungus, *A. pseudoterreus*. Initial mini-DBTL cycles have established baseline strain performance of 1.3 g/L and 10 g/L for respective targets, and they have started to analyze omics data in order to guide genetic modifications for subsequent design cycles. The group has also started to develop new genetic constructs in order to streamline strain construction.

- *A. pseudoterreus* is a fungal host related to strains historically used for enzyme expression and production of citric acid. Due to its acid tolerance, it is a suitable host for producing organic acids in the free acid form. Here, it is being considered for aconitic acid and 3HP. The pathways to these molecules are well established, so the team is focused on solving the key challenges of improving timelines for genetic manipulation and reducing byproduct formation. For the latter, they have leveraged a variety of omics techniques in conjunction with metabolic modeling and machine learning. This has led to identification of targets for knockout or overexpression, many of them not intuitive. However, the value of these techniques cannot really be evaluated until these manipulations are made and results provided. The team has also greatly reduced the amount of time needed for testing strains, and has taken steps to improve translation and reproducibility from shake flask to fermentation.

- The selection of a filamentous fungus, especially *Aspergillus*, is an excellent inclusion in the host-target development programs due to industrial relevance and as an opportunity to optimize a DBTL cycle. This organism has inherent handling and growth challenges that impose time and throughput limitations, which perhaps can be overcome with improved Learn (including deep-Learn) capabilities that seem to be the focus. In this respect, it may be a good example of how DBTL can be flexible to garner improvements by emphasizing certain elements of the cycle because of limitations in others. Progress has been good and the outcome of potentially exciting unintuitive learnings is pending. It might be of benefit to develop relationships with external entities who are developing (other, but related) strains and tools for similar hosts to move towards high-throughput use.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We believe filamentous fungi are very important, as evidenced by their wide use within industry. "Traditional" Learn techniques using metabolic models as a framework for analyzing omics data and to support flux balance analysis for identifying bottlenecks and targets has been a major emphasis and has resulted in both intuitive and nonintuitive targets. Advanced Learn techniques, such as Artificial Neural Networks (ANN), have been employed in the last year to identify nonintuitive targets, and this effort will expand in the future with increasing numbers of large Test data sets. A number of targets already suggested by Learn remain to be Designed, Built, and Tested. We will be able to address the highest-priority targets in FY 2019. Milestones pertaining to all of the targets have been satisfied, but we realize we are still well short of handing off the 3HP strain for commercialization.

- The demarcation between work to be accomplished in the remainder of FY 2019 and moving beyond into FY 2020 could have been clearer. A provisional patent was filed, and a full patent will be filed before the end of April. Since this was a public presentation, few details were discussed.
- We agree with this assessment and we have a significant number of high-priority targets in regard to improving titer, rate, and yield for both target molecules. The highest-priority targets will be constructed and tested before the end of FY 2019 (see response above). We are excited to have the opportunity to report on those results in the literature and at peer review two years hence.
- We appreciate the guidance with regard to exploring external collaborations for development of high-throughput tools for these challenging but highly useful hosts. We also have work in a project outside of BETO exploring high-throughput methods for transformation that will be leveraged within the ABF.

ABF – DESIGN-BUILD-TEST-LEARN INFRASTRUCTURE

Agile BioFoundry Consortium

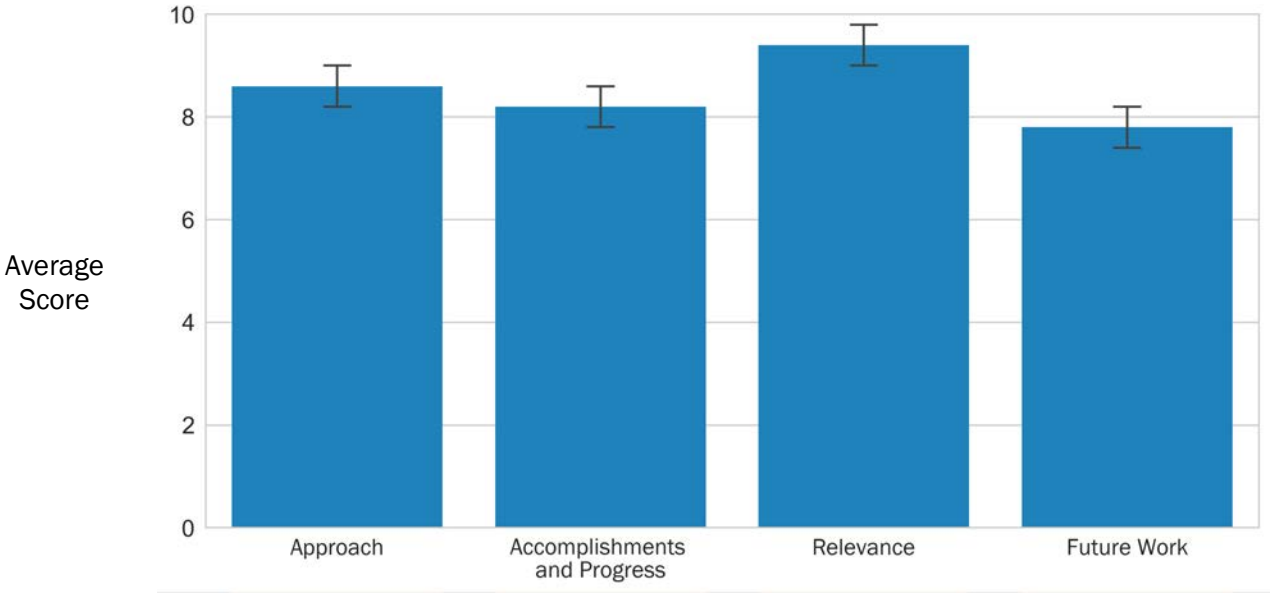
PROJECT DESCRIPTION

One of the key tasks of the DOE ABF is to design, implement, operate and maintain DBTL infrastructure that enables the efforts of the ABF, as well as industry and other BETO projects and consortia. This talk will provide an overview of the ABF's DBTL infrastructure (in a more detailed and deliberate fashion than in previous or subsequent ABF presentations), and provide some DBTL infrastructure R&D highlights from the first 2.5 years of the ABF's operations.

WBS:	2.5.3.104e
CID:	NL0030036e
Principal Investigator:	Dr. Nathan Hillson
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$15,200,000
DOE Funding FY16:	$1,000,000
DOE Funding FY17:	$5,000,000
DOE Funding FY18:	$4,500,000
DOE Funding FY19:	$4,700,000
Project Status:	Ongoing

Weighted Project Score: 8.5

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



I One standard deviation of reviewers' scores

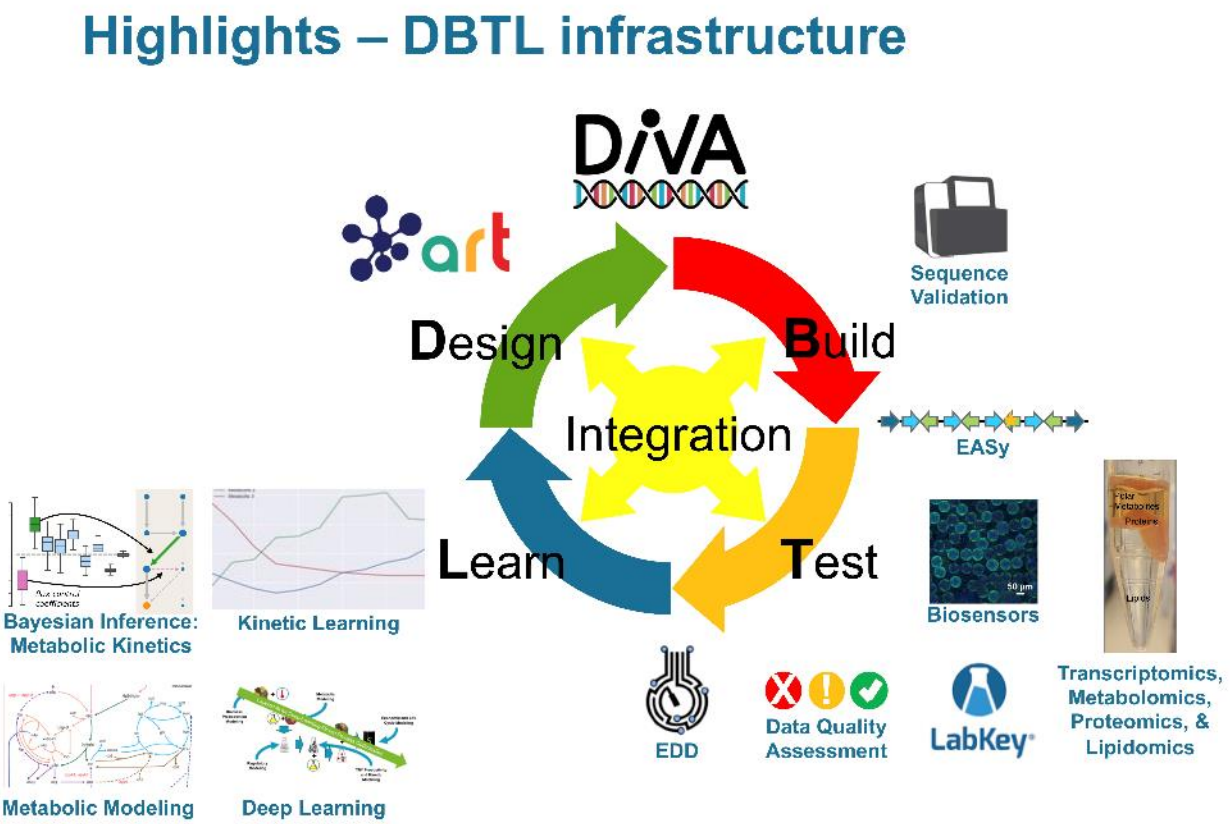


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- This subproject is focused on developing the DBTL infrastructure within the ABF. The main contribution is bringing together multiple tools into a common platform. The focus on reproducible unit operations across different institutions is a valuable goal and key strength of the foundry.

- Great management. It is great to see an overview of the projects and how each has contributed to the DBTL cycle in detail.

- The team presented highlights of over a dozen novel capabilities under development under the ABF umbrella. These efforts span the entire DBTL cycle and include at least eight different software projects, in recognition of the importance of establishing high-quality maintained software as an enabling capability for the community. Layered on top of these technology development efforts are strategic plans to ensure their relevance, including multifaceted industry engagement (especially including CRADAs), TEA-driven project prioritization, and a commitment to wide dissemination of the software.

- The DBTL infrastructure is the core of the ABF and supports all other tasks. Having this infrastucture in place and streamlined is critical for reducing both DBTL cycle time as well as overall project timelines. This is dependent on having a good software platform to maintain data and enable Learn activities, and the team has built or aquired a number of tools. These include DNA construct design and assembly, various types of metabolic modeling, deep-learning algorithms, and laboratory information management systems for data storage and sharing. New Build tools are next-generation sequencing to verify construct accuracy and a novel method for gene evolution based on duplication and recombination. Sample processing for omics analysis has also been streamlined. The DBTL cycle time has been improved, but is

still too long. Now that all the computational and experimental tools are in place, effort should be spent on developing a streamlined workflow. Also, the current ABF projects may be too early to really gain full benefit from DBTL. As a test case, it would be useful to apply this to a mid-stage project with an organism with well established genetic tools.

- Development of the DBTL infrastructure to realize efficiency gains in project execution and delivery is at the heart of the ABF engine design room. The software, tools, processes, and other assets combine to optimize the project development cycle and can greatly enhance productivity and success at the ABF and, if made available, to external stakeholders such as industry and academia. It will be important to identify the appropriate business model to achieve this.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We agree that our DBTL cycle time (as of the 2019 BETO Peer Review) is too long. We are now quantitatively defining what constitutes a DBTL cycle (vs. mini-DBTL) beyond the qualitative definitions provided at peer review. We will be working towards increasing the coverage and granularity of our cycle-time metrics capture, and use the resulting data to prioritize our DBTL workflow streamlining efforts. We agree that some ABF projects may be too early stage to benefit from DBTL (which might otherwise be better served by mini-DBTL); finding the transition point (in terms of project maturity needed in order to benefit from DBTL) is a good idea.

- The ABF's philosophy is to use methods, instruments, software, etc., that are accessible (and develop those that will be accessible) to industry and academia, either through commercial vendors or through licensing from the ABF itself (via the national labs). This enables our industrial and academic collaborators to practice these same methods, instrumentation, and software behind their own corporate or institutional firewalls without persistent reliance on the ABF. There are established licensing models and mechanisms (e.g., exclusive in a field of use or nonexclusive, freely open source) that enable this, with the general broad objective to maximize impact and market transformation (which determines the licensing mechanism). For the ABF in particular, the nonexclusive (including freely open source) mechanisms are strongly preferred (so that multiple companies and academic groups can benefit from them) with the exception of exclusive licenses to technology platform companies that will make the technologies broadly accessible. Part of the sustainable business model for the ABF, then, is to incentivize its collaborators to opt for nonexclusive licensing options in CRADAs, and we plan to explore these options in the next phase of the ABF.

ABF – INTEGRATED ANALYSIS

Agile Biofoundry Consortium

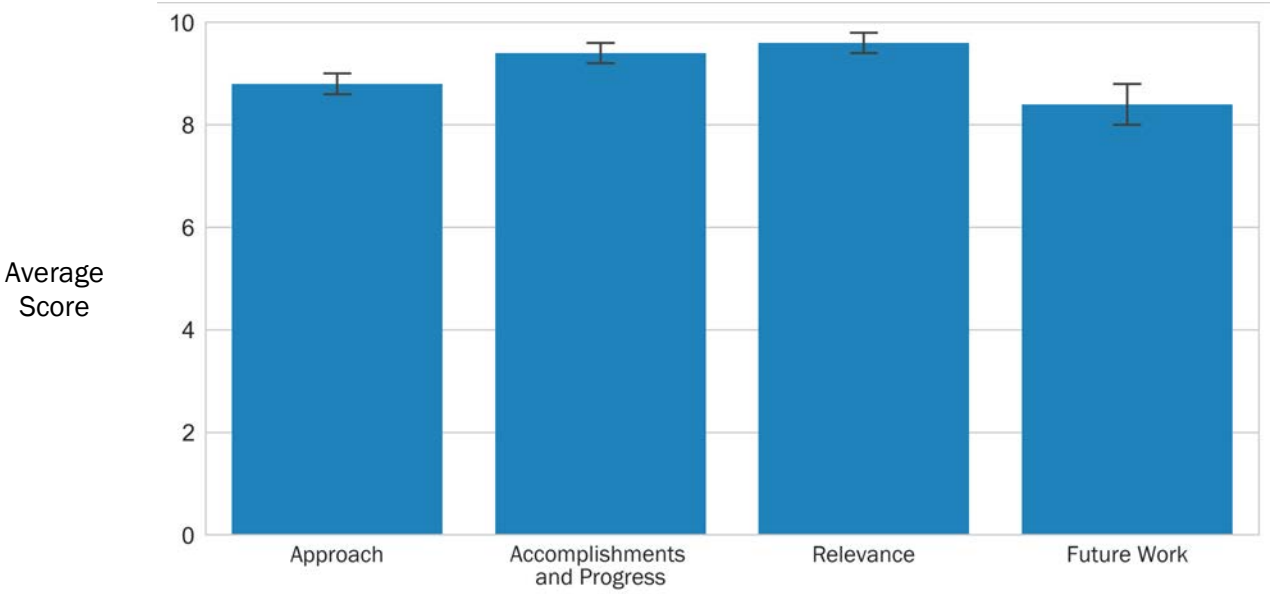
PROJECT DESCRIPTION

The Integrated Analysis task is incorporated within the DBTL approach with a goal of providing an analysis-based foundation to the science and research of the ABF. The collaborative team develop TEA and LCA for the pairs of chemical targets and host microbes being pursued. To date, the team has performed analyses on over 10 different target chemical host-microbe combinations. This talk will summarize the ongoing analysis work and will detail how TEA and LCA have been integrated to support ABF goals with a focus towards developing bio-based products that are sustainable and economically viable. Future work plans for the Integrated Analysis project also will be outlined.

WBS:	2.5.3.104f
CID:	NL0030036f
Principal Investigator:	Dr. Mary Biddy
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$500,000
DOE Funding FY16:	$100,000
DOE Funding FY17:	$200,000
DOE Funding FY18:	$100,000
DOE Funding FY19:	$100,000
Project Status:	Ongoing

Weighted Project Score: 9.1

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



I One standard deviation of reviewers' scores

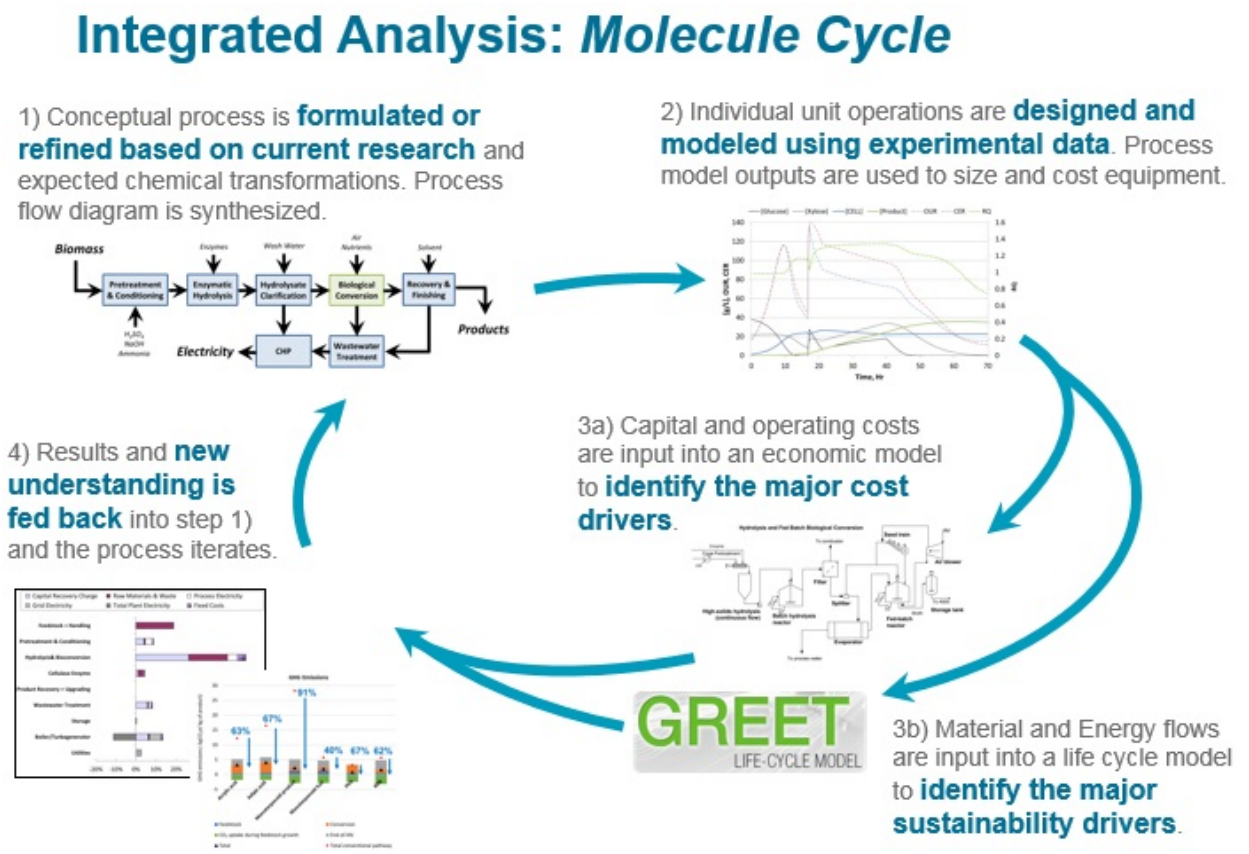


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- The goal of this subproject within the ABF is to provide TEA and LCA in order to evaluate the economics and sustainability of proposed molecules. In addition, they will develop conceptual process designs. Such analysis will be useful for determining the feasibility of different host-target pairs. This is an essential service and should be a central activity. The budget is small and likely insufficient to support these critical activities.

- The project and projects similar to these are well needed to provide clear understanding of manufacturability, cost, and LCA of the biological product. The principal investigators did a great job creating clear analysis for a variety of products and it would be great to see more of these analysis/projects in the future.

- The Integrated Analysis team has provided critical guidance to the ABF project through careful techno-economic and sustainability analyses. This work helps ensure that ABF efforts attain relevance to the bioproducts industry and by prioritizing targets for testbed, help bolster relevance even for projects still at the proof-of-principle stage. These continuing efforts are vital for ABF success.

- The goal of this function is to assess economic and sustainability drivers for the ABF work, analyzing individual host-target pairs. This provides a consistent, unbiased framework for evaluating and prioritizing processes, and is thus critical to choosing successful projects. In addition, it can help drive decisions during a development project to determine which metrics have the strongest impact on cost. The team completed TEA and LCA on 10 host-target pairs and observed clear trends on the factors

influencing both economics and sustainability. In the future, these models will be refined with data gathered from the projects.

- Meaningful TCAs and LCAs are the currency of fact-based decisions for downselection and prioritization within and across projects. The value that this program is bringing is critical to the success of the ABF, provided that inputs are sound and that the results and recommendations are acted upon by those making program decisions. This program is not only important, but should probably be expanded.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their helpful and supportive feedback. We will work to incorporate these suggestions in future analysis efforts.

ABF – HOST ONBOARDING

Agile BioFoundry Consortium

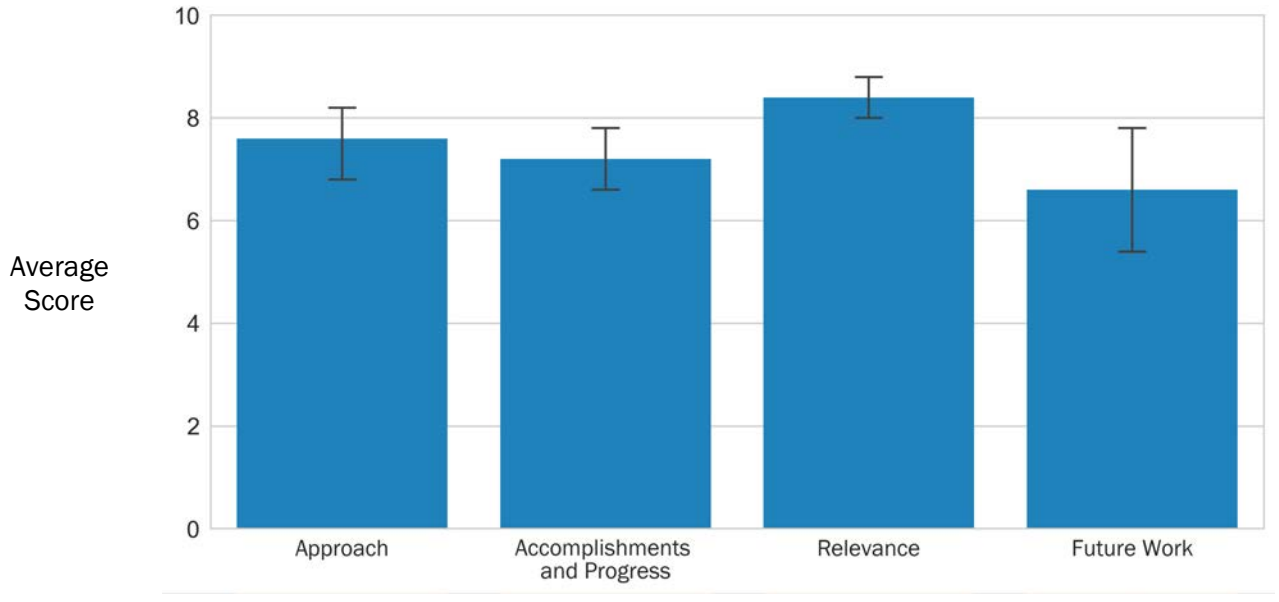
PROJECT DESCRIPTION

WBS:	2.5.3.104g
CID:	NL0030036g
Principal Investigator:	Dr. Adam Guss
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$3,500,000
DOE Funding FY16:	$100,000
DOE Funding FY17:	$1,200,000
DOE Funding FY18:	$700,000
DOE Funding FY19:	$1,500,000
Project Status:	Ongoing

Non-model microorganisms often have advantageous physiological traits that could be leveraged for advanced bioprocessing, such as the ability to thrive at low pH or to utilize uncommon substrates such as syngas or oligomeric sugars. However, a lack of genetic tools and fundamental knowledge about these organisms hinders strain development. The role of the Host Onboarding team is threefold: (1) evaluate proposed hosts for new host-target pairs within the ABF, (2) develop genetic tools that allow new hosts to be used for DBTL cycles within the ABF and by outside stakeholders, and (3) improve genetic tools for BETO "State Of Technology" (SOT) organisms to increase DBTL cycle efficiency across the BETO portfolio. In this talk, the Host Onboarding team will discuss the development of a "Tier System" to evaluate the readiness of an organism for DBTL cycles, as well as current progress on developing new hosts such as *Bacillus coagulans* and *Clostridium carboxidivorans*. Future work on these organisms and SOT organisms such as *Zymomonas mobilis* and *Clostridium tyrobutyricum* will also be discussed.

Weighted Project Score: 7.5

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



OVERALL IMPRESSIONS

- The goal of this subproject is to develop new non-model organisms for chemical and fuel production. A key step involves developing a ranking system for evaluating the potential of new non-model organisms. Overall, this project addresses an important problem. Many tasks are proposed. The project would benefit by clearly ranking the importance of these tasks and explaining how effort will be allocated toward achieving them. Otherwise, it is difficult to evaluate progress.

- This project aims to fill important gaps not addressed elsewhere in the BETO portfolio regarding host onboarding by developing analytical tools for pragmatic, data-driven downselection of non-model organisms with new and useful phenotypes and developing genetic manipulation tools to provide access to these phenotypes. The team has successfully developed a principled prioritization system and applied it to identify three non-model organism targets, for which it has now started the process of developing genetic tools.

- This group prioritizes new host organisms for bioprocessing, and develops genetic tools and basic knowledge to make them tractable. Novel host organisms can extend the range of feedstocks and products due to the ability to utilize novel carbon sources and tolerate inhibitors or extreme conditions. In order for these host organisms to find applications, genetic tools must be developed so they can be manipulated. It is good that these tools are being developed early, so they are ready when needed. The team created a ranking system for organisms to help prioritize, and also a tier system to determine which organisms are ready to enter the DBTL process. Furthermore, a web portal will give protocols and lab contacts for methods of each organism. They developed a methodology to overcome host restriction modification systems, and have made progress on two difficult organisms.

- The new host onboarding program is creating significant value and ideally will expand the pool of industrially relevant hosts (and host-target pairs). A sensible tiered set of criteria to downselect to approprite selections has been developed and a good, diverse set of initial host targets have been put into play. Consideration toward upgrading biosafety/regulatory criteria to the selection process, as well as a biosafety board, should be considered.

- Good to see the principal investigator mentioned where this project fits in the overall ABF goals. New hosts and new tools seem ambitious. Can the principal investigator select one (either a new host or new method) and focus on and move to the next after success in the first one?

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Thank you for the useful comments. We feel that the both (1) the web portal detailing the ABF capabilities, protocols, and data sets; and (2) the development of new host organisms for use in the ABF and the community at large are of equal importance, and so we are devoting substantial efforts on both fronts to make sure each task is accomplished. To facilitate this, Oak Ridge National Laboratory has taken the lead on developing the genetic tools, which is a core area of expertise for the lab. Similarly, Los Alamos National Laboratory has taken the lead on finalizing and making public the tier system. For onboarding new hosts in FY 2020, we are targeting the development of genetics for a targeted number of targeted microbes (*Bacillus coagulans*, which catabolizes hydrolysate very well, and *Clostridium carboxidivorans*, which catabolizes sugars and syngas) to expand the types of hosts available within the ABF. We are also targeting improvement of genetic tools in organisms that are important for other BETO projects like *Zymomonas mobilis* and *Clostridium tyrobutyricum*. In future years, we will identify new hosts that would bring unique capabilities to the ABF for targeted development.

- Thanks for these comments. We do have a biosafety plan in place, wherein any new organism that is used needs to be evaluated by our home institutions, which have rigorous regulations. We also utilize Lawrence Berekely National Laboratory-developed software called BLiSS to identify biosecurity/biosafety concerns of synthetic DNA in advance. Our current goal is to only use Biosafety

Level 1 organisms, and so the biosafety risk is inherently low. However, the reviewer's point is an important one, and we will work on formalizing a process within the ABF itself, particularly taking into consideration how biosafety might affect use of such organisms by industry.

- Thank you for the comment. Given the importance of developing new organsims and the scope of the ABF, we feel that it is important to develop multiple hosts and methods in parallel. We think there is synergy in parallel development, where new learnings in one system can help accelerate development in all systems.

ABF – PROCESS INTEGRATION AND SCALE-UP

Agile BioFoundry Consortium

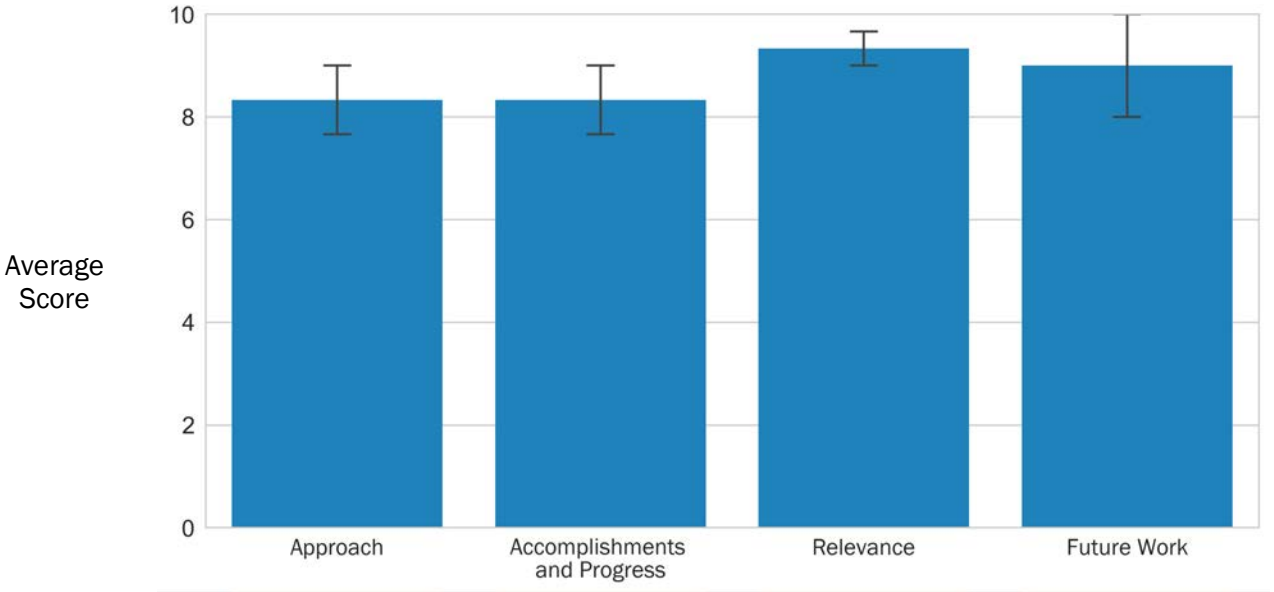
PROJECT DESCRIPTION

WBS:	2.5.3.104h
CID:	NL0030036h
Principal Investigator:	Dr. Deepti Tanjore
Period of Performance:	10/1/2016 - 9/30/2019
Total DOE Funding:	$1,900,000
DOE Funding FY16:	$0
DOE Funding FY17:	$400,000
DOE Funding FY18:	$400,000
DOE Funding FY19:	$1,100,000
Project Status:	Ongoing

The aim of the ABF Integration and Scaling task within the overall project is severalfold: (1) to produce a consistent sugar hydrolysate for the ABF to use across the target-host pair demonstration and scaling efforts, (2) to conduct hydrolysate-based cultivations for the target-host pair teams to provide feedback to the DBTL efforts and to meet critical project milestones related to strain performance, (3) to expand Test capabilities via the onboarding and maintenance of new bioreactor capacity, (4) to conduct pan-scale DBTL efforts with the target-host pairs, and (5) to provide process data to the Integrated Analysis team for TEAs and LCAs to inform DBTL priorities. Overall, this task leverages BETO investment in scale-up and scale-down activities at the Advanced Biofuels and Bioproducts Process Development Unit (ABPDU) and the Integrated Biorefinery Research Facility (IBRF), as well as other partner institutions with existing bioprocess development capabilities (e.g., bioprocess development with filamentous fungi at Pacific Northwest National Laboratory). Additionally, the Integration and Scaling task acts as a liaison to other BETO-funded consortia, such as the Bioprocessing Separations Consortium, the Chemical Catalysis for Bioenergy Consortium, and the Consortium for Computational Physics and Chemistry.

Weighted Project Score: 8.8

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



I One standard deviation of reviewers' scores

Technical accomplishments to date include two pilot-scale runs of the deacetylation, mechanically refining, and enzymatic hydrolysis process at the IBRF to produce a self-consistent, highly characterized hydrolysate stream for the ABF, the majority of which has been distributed to the partner institutions for use in DBTL and scale-up experiments. Ongoing work in hydrolysate production includes leveraging new separations techniques being implemented at the pilot scale to be able to produce highly concentrated, clarified sugar streams for the ABF partners. For all three target-host pairs, the Integration and Scaling task has conducted bioprocess development in direct support of DBTL efforts to improve strain performance and to benchmark the difference between shake flasks and bioreactor cultivations. Additionally, a critical round-robin experiment was conducted between the ABPDU and IBRF using the *Pseudomonas putida*-muconic acid target-host pair as an initial example. These results highlighted good agreement between the facilities, indicating that the ABF is able to produce internally consistent results at different institutions, even with some differences in equipment configuration. Additional round-robin experiments are planned in FY 2019 for the *Rhodosporidium toruloides*-terpene target-host pair and the *Aspergillus pseudoterreus*-3-hydroxypropionic acid target-host pair. In FY 2019, a pan-scale test was also initiated to inform DBTL efforts using the *P. putida*-muconic acid target-host pair as an initial example. Lastly, onboarding new bioreactor capacity has significantly increased the Test capability of the ABF by multiple folds.

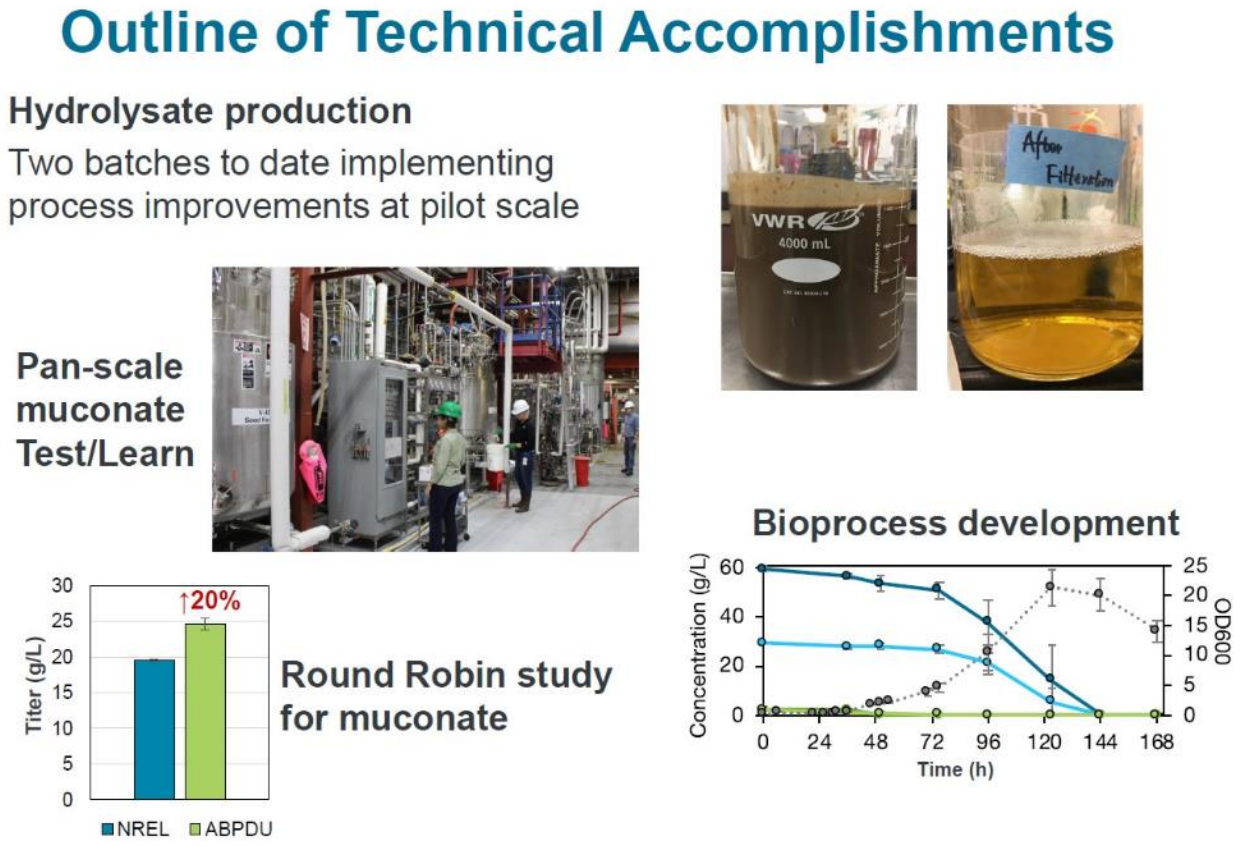


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- This subproject focuses on process scale-up and providing standardized deacetylation and mechanical refining hydrolysates for members and external partners. Overall, the team is making good progress. A

major suggestion would be to develop a more systematic procedure for facilitating scale-up. In particular, the team should focus on identifying the key factors involved in scale-up and optimization in order to accelerate this process and improve the broader impact of this work.

- This group focuses on providing hydrolysate feedstocks, testing ABF strains at various scales, and providing fermentation data to the Learn team. Scaling up a process is an important step in de-risking, and collecting fermentation data at bench scale is important for guiding project decisions. The team is making a strong effort to generate high-quality data and fully characterize the system; for example, omics analysis at multiple scales, closing the carbon balance, and cross-site validation. They are working on a better understanding of the impact of different scales on cell physiology, and are implementing new small-scale culturing techniques. Improvements could be made by defining clear goals and integrating this team better into the overall DBTL workflow.

- Process and integration is an underserved area and often an afterthought in new projects and products. In particular, a lack of scale-up and scale-down consistency is often hard to come by. This project is developing a core competency by standardizing substrates (especially relevant to biomass-derived complex substrates) and pan-scale test methods. So-called "round-robin" testing across sites is helping to develop what is more broadly known to industry as technology transfer expertise, which is how success (or failure) is often determined (e.g., for milestone testing, go-no-go decisions). In a multisite environment, this is an absolute must-have competency for measurable and fact-based success. As more projects come online, more host-target pairs and substrates will need to be put through this process, so it is likely this area will emerge as a priority for additional funding and effort.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewer for the positive and constructive feedback. We agree with the reviewer that there should a systematic procedure for facilitating scale-up. At this time, as we are working with non-canonical hosts, we are still in the process of developing unique cultivation protocols for the scale-up of each target-host pair and at economically viable titers, rates, and yields. Our end-of-project goal is to "Demonstrate target-host pair production of at least 3 molecules at 10 g/L, 100 mg/L/hr, at 40% of theoretical yield from deacetylation and mechanical-refining and enzymatic hydrolysis (DMR-EH) at 10 L." During the process of achieving this goal, we will be learning many nuances of scale-up, some of which can be broadly applied to both canonical and non-canonical hosts. We are documenting these results and look forward to publishing them. In FY 2020 and beyond, given continued support, we should be able to develop a procedure that can be adopted widely for many target-host pairs. We are hopeful that the omics approach to identifying variances in microbial culture behavior and thereby performance with scale will be insightful and provide the necessary guidance to design target-host pairs for robustness during scale-up.

- We appreciate the positive feedback from the reviewer. The reviewer identifies a particular aspect of this task to be essential, namely integration with the DBTL cycle. We are in complete agreement with this feedback, and as described during the presentation, we are actively working on this with the pan-scale Test and Learn activities in *P. putida* and *R. toruloides*. At this time, we consider the scale-up task to be a part of the Test arc of the DBTL cycle. Our most recent scale-up campaigns, generating muconic acid (at 600 L) and fatty alcohols (at 300 L), provided us with the samples and necessary omics and fermentation data to engage with the Learn team. This coordination with the Learn team will allow us to integrate better with the Design and Build arcs in the future. We expect to be more fully integrated in the full DBTL cycle by the end of FY 2021, by which point some of the design principles would be based on our learnings from the scale-up studies.

- We thank the reviewer for this comment. We agree with the feedback and will further our round-robin studies by including target-host pairs such as 3-hydroxypropionic acid-*Aspergillus niger* in FY 2020. We will also publish our learnings for industrial and academic entities to benefit from our findings. Further,

the FY 2020 milestone of ABF is being constructed such that reproducibility at separate locations is a prime subject of our studies: “Reproducibility of three distributed Test unit operations, including bioreactor scale-up quantified through comparison of results post data quality assurance for on-site vs. off-site sample analysis.” Future scale-up and round-robin studies will allow us to develop a standard template with necessary know-how that can benefit future endeavors in industrial biotechnology.

ABF – INDUSTRY ENGAGEMENT AND OUTREACH

Agile BioFoundry Consortium

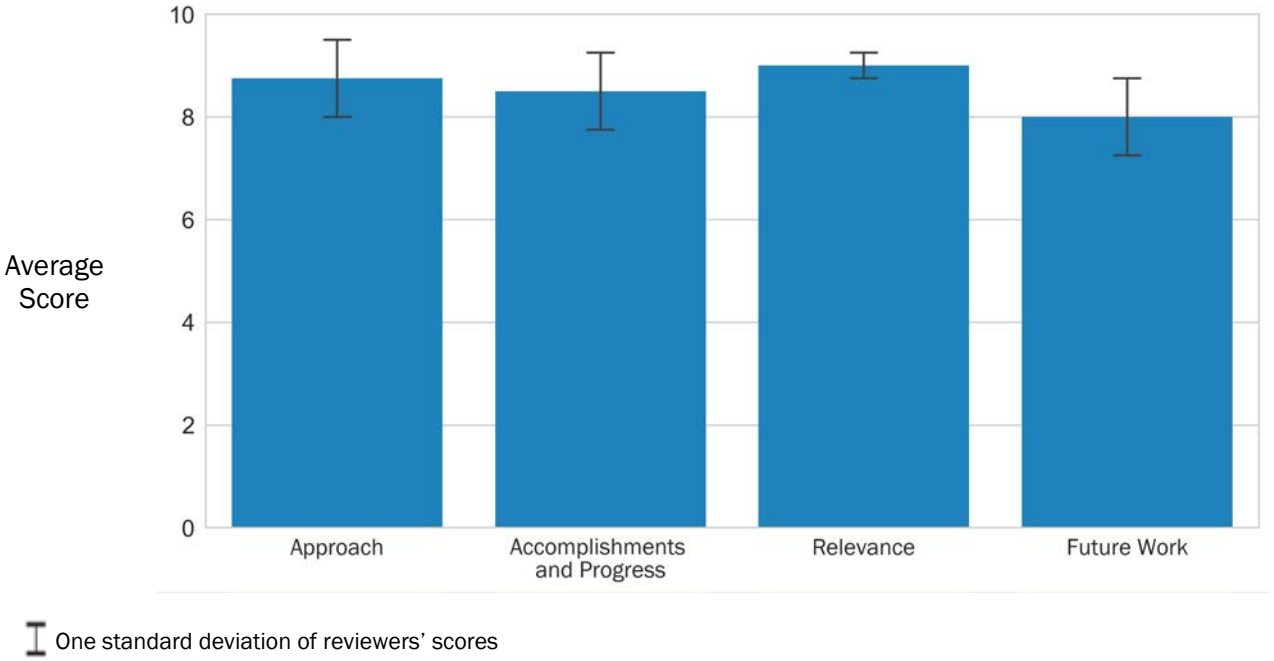
PROJECT DESCRIPTION

WBS:	2.5.3.104i
CID:	NL0030036i
Principal Investigator:	Dr. Phil Laible
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$900,000
DOE Funding FY16:	$0
DOE Funding FY17:	$200,000
DOE Funding FY18:	$300,000
DOE Funding FY19:	$400,000
Project Status:	Ongoing

The objective of the ABF is to develop infrastructure to support industrial biotechnology, while understanding the needs of the industry is critical to achieving this goal. In support of this, the ABF Industry Engagement and Outreach (IEO) team organizes and facilitates interactions with industry, providing feedback from the industry stakeholder community to the ABF and BETO, which supports decision making and project planning. The activities of the team also aim to increase the visibility of the ABF and attract collaborators from academic and industrial communities. The goals of this task are accomplished through the workings of three highly interwoven, strategic focus areas (SFAs): Assessment, Outreach, and Interactions. For the Assessment SFA, an Energy I-Corps approach is used to understand the needs of the biomanufacturing industry by interviewing and surveying its members. In the Outreach SFA, members work to manage the ABF public profile and disseminate ABF information and resources to industrial, academic, governmental, and public stakeholders. For the Interactions SFA, the main goal is the coordination of community-building activities. This includes Industry Days and workshops to ensure research effectiveness and industry responsiveness. This SFA also facilitates interactions with the IAB. The IEO team collectively plans panels and sessions at key industry conferences. Overall, the IEO task contributes to the alignment of

Weighted Project Score: 8.6

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



ABF activities with BETO's milestones and facilitates communication of the ABF value proposition to key stakeholders in industry, R&D organizations, and the public.

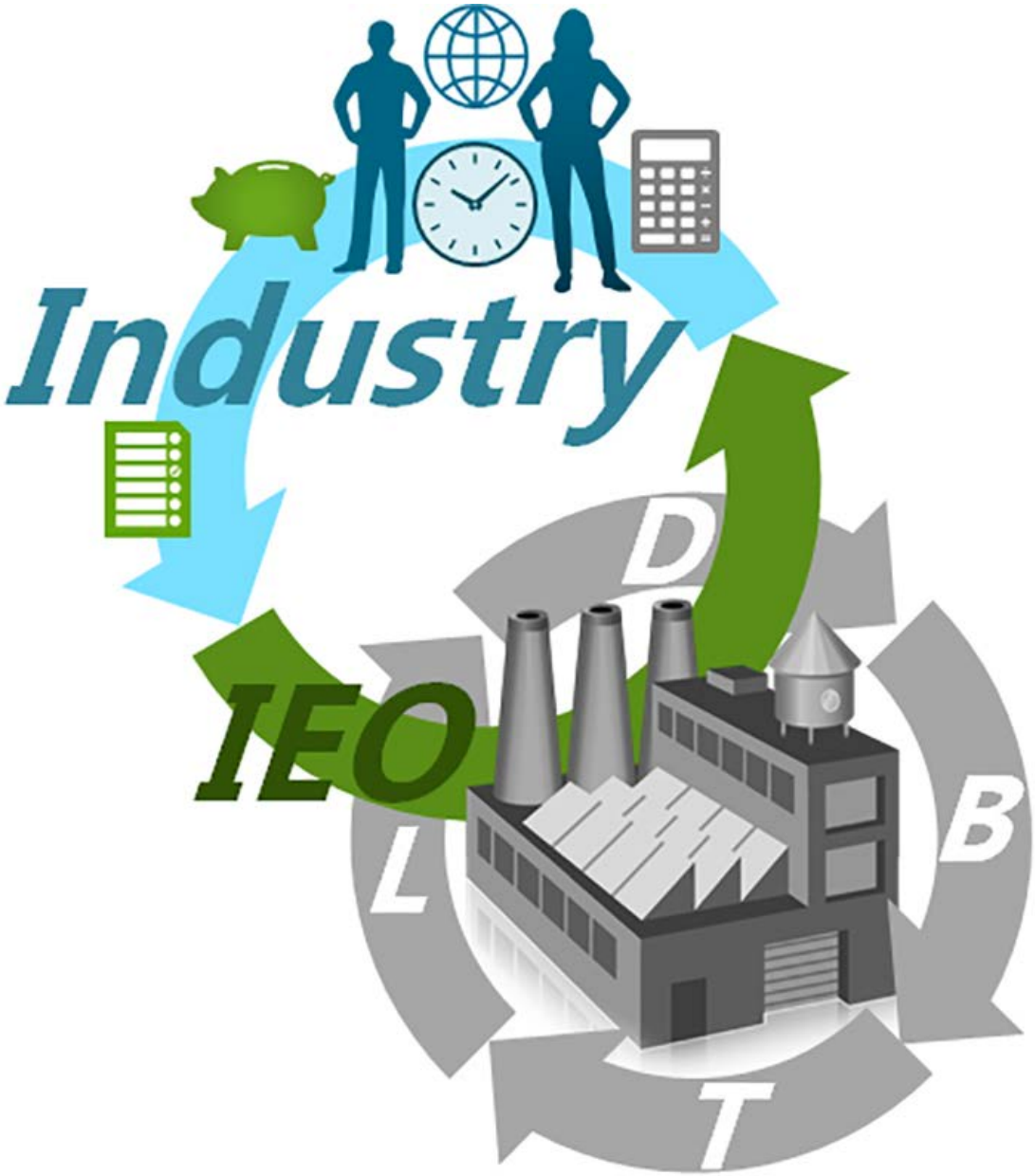


Photo courtesy of Agile Biofoundry Consortium

OVERALL IMPRESSIONS

- The goal of this subproject is to identify and remove obstacles for technology transfer. In addition, they proposed to expand the number of industrial partners. This task is necessary to broaden the impact and relevance of the ABF. Overall, the team is making solid progress. The project would benefit by including explicit milestones in order to evaluate progress and to define success.

- It is heartening to see industry engagement as a "first-class citizen" component of the ABF strategy. The team describes a comprehensive approach to promoting ABF and gaining guidance from industry to ensure future relevance. This approach is built on pillars of passive engagement, active engagement, and third-party assessment and based on quantitative measures of engagement. It appears to be impactful.

- The industry engagement group ensures the ABF is working on projects that are of interest to industry, and that the technologies can be transferred. They are looking to attract academic and industry collaborators and licensees. Knowledge is gathered through interviews, surveys, and listening days. The team is well coordinated at gathering this information, and providing the industry feedback to the experimental groups. More activities are planned for the future, such as hosting panel discussions at conferences.

- Maintaining and refining a customer focus and relevance is a critical objective to be achieved, in part, by gaining highly valuable industry feedback and via IAB interactions. The current effort is doing so with its outreach, interactions, and assessment approach. Real traction will depend on to what extent the results influence stakeholders to evolve/refine the ABF approach and mission.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Regarding milestones, we could have explicitly shown that we have a detailed project plan that includes quarterly IEO task milestones. Each IEO subtask has two or three specific, measurable, attainable, realistic, and time-related (SMART) milestones throughout the year. Joint monthly IEO/management discussions help to keep the subtask on track and accountable. Since these internal milestones might have been confused with formal ABF milestones, we erred on the side of not showing them. They can be made available to the review team upon request.

ABF – DIRECTED FUNDING OPPORTUNITY MANAGEMENT

Agile BioFoundry Consortium

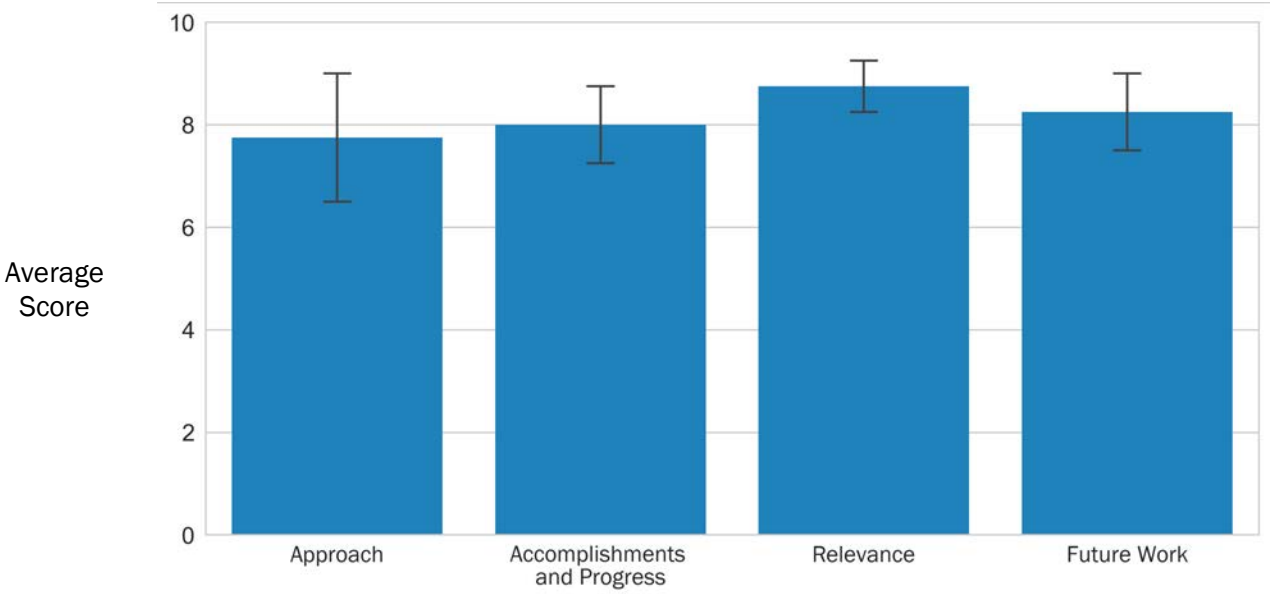
PROJECT DESCRIPTION

This presentation will present a summary of all CRADAs between the ABF and industry as a result of the ABF Directed Funding Opportunity (DFO) and the recent BioEnergy Engineering for Products Synthesis (BEEPS) Funding Opportunity Announcement (FOA). This will include a review of how the ABF leadership team managed the proposal review process for the ABF DFO.

WBS:	2.5.3.104j
CID:	NL0030036j
Principal Investigator:	Dr. Blake Simmons
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$5,000,000
DOE Funding FY16:	$0
DOE Funding FY17:	$5,000,000
DOE Funding FY18:	$0
DOE Funding FY19:	$0
Project Status:	Ongoing

Weighted Project Score: 8.2

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%



I One standard deviation of reviewers' scores

OVERALL IMPRESSIONS

- This subproject focuses on external engagement through partnerships and external funding agreements. The major success was the creation of a template for the CRADA process that works with all of the ABF partners. This resulted in a streamlined process and increased transparency. Overall, these activities are critical to the success of the ABF. In addition, opening up the ABF to external partners though different funding mechanisms is a critical step in broadening the impact of the foundry. Developing robust management and review/evaluation protocols is clearly important.

- ABF/BETO has established FOAs/DFOs in order to establish formal engagements that will be critical 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 incentivizes industry while mainly providing funds within the national laboratory system. This, along with cost share, ensures 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.

- 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 highlights the challenges of working with customers with different business needs. A nonnegotiable CRADA template was developed and may streamline this process in the 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.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for these comments and agree with all of the issues raised.

- We 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.

- We 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 those data.