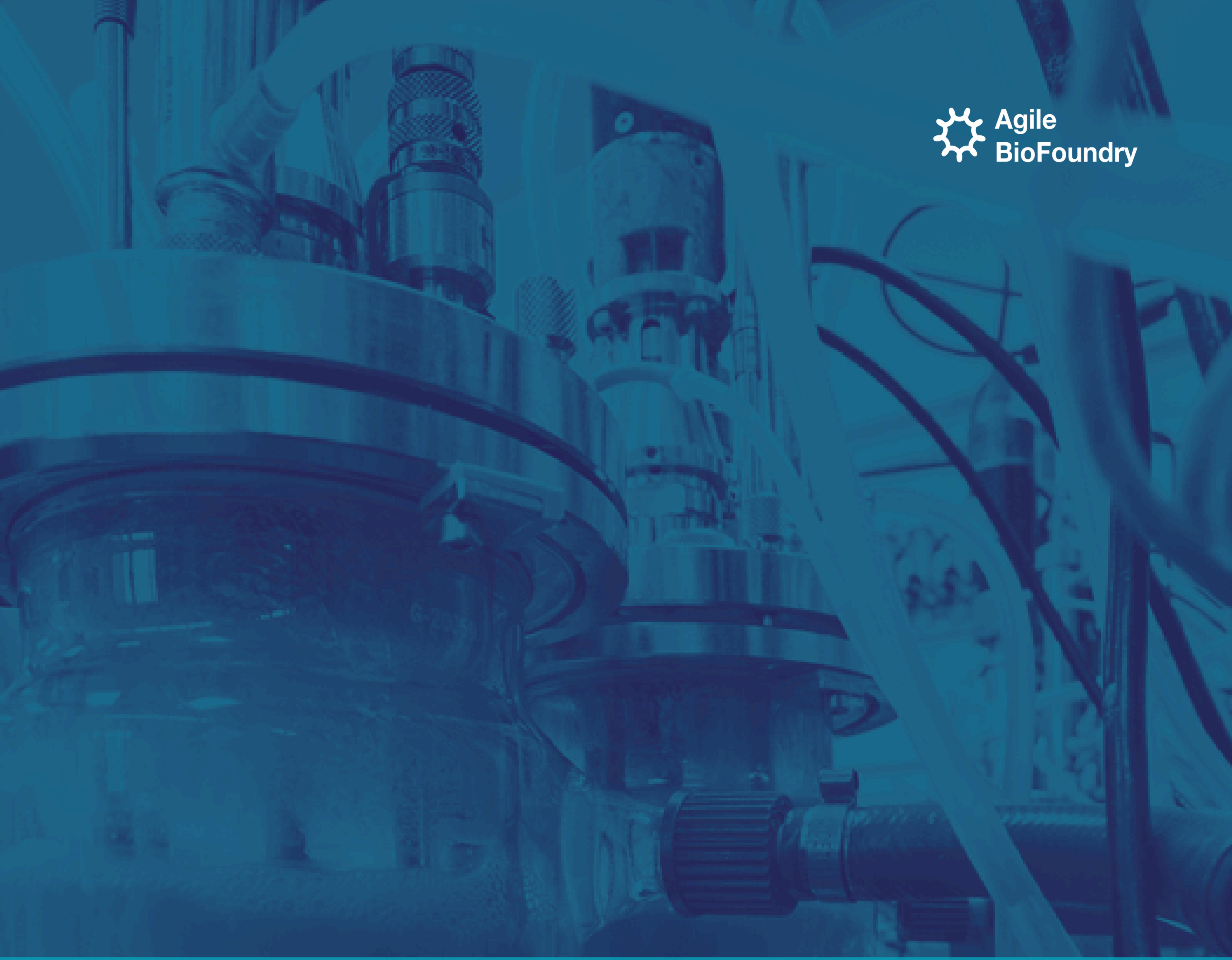


Agile BioFoundry

AGILE BIOFOUNDRY

Impact Report

FY17-FY22

Introduction

The Agile BioFoundry (ABF) completed 6 years of operations in 2022, in pursuit of a goal to reduce time to bioprocess scale-up by 50%. During this time, the ABF has developed and deployed processes and technologies that enable commercially relevant biomanufacturing of a range of bioproducts.

This report describes the major technical advances of the ABF in terms of why they are important, how they were achieved, their impact on the biomanufacturing industry, and highlights ABF's other important contributions to the field. The Concluding Remarks section at the end of this report discusses how these advances positioned the ABF for its next phase (2023 and beyond) as well as the key takeaways of these accomplishments in aggregate. The learnings from the time period covered by this report (2016-2022), during which the ABF was largely organized around the engineering biology Design-Build-Test-Learn (DBTL) cycle, served as the foundation for the following years, including ABF's significant restructuring and reorganization in 2023.

The achievements described herein should be judged in the context of the ABF's overall goals and objectives for its first 6 years, which were to develop and deploy technologies to enable biomanufacturing of a range of bioproducts. They reflect advances made via research and development under each of the ABF's 4 major technical tasks, industry engagement and outreach activities, and the management task. Critically, they also all contribute in some way to other major objectives of the ABF, namely to:

- Apply ABF's expertise and experience to accelerate new bioprocess development
- De-risk the scale up of microbial hosts
- Avoid the need for industry to start R&D from scratch, and make industry's pursuit of 'the last mile' more efficient
- Provide access to and fractional use of ABF domain expertise and instrumentation
- Improve accessibility to biologically-derived end-use chemicals, sustainable aviation fuels, performance advantaged bioproducts and other chemicals of interest

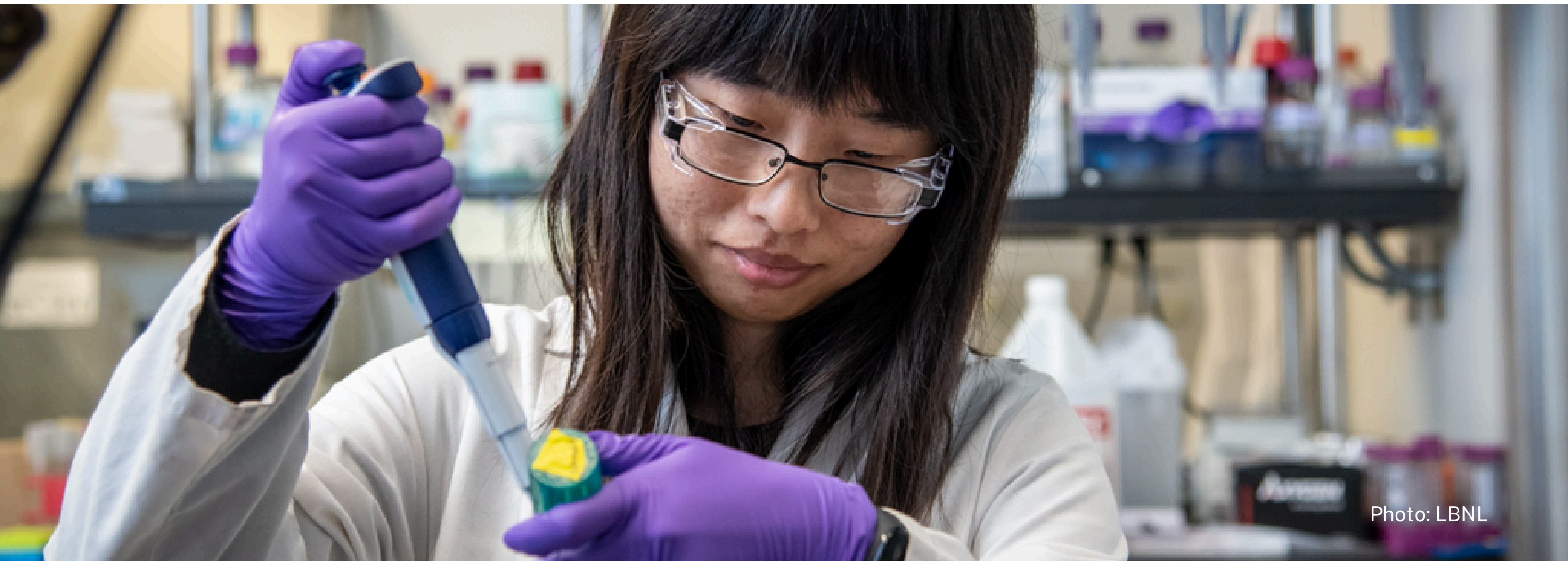


Photo: LBNL

Background

The United States (U.S.) Department of Energy (DOE)'s Office of Energy Efficiency & Renewable Energy's Bioenergy Technologies Office (BETO)-funded Agile BioFoundry was established in 2016 as a distributed consortium that operates as a collaboration of seven U.S. DOE National Laboratories: Argonne National Laboratory, Lawrence Berkeley National Laboratory, Los Alamos National Laboratory, Oak Ridge National Laboratory, Pacific Northwest National Laboratory, Sandia National Laboratories, and the National Renewable Energy Laboratory.

Industry identification of shared pre-competitive research challenges befitting the National Lab infrastructure was a primary driver for the establishment of the ABF. Our objective has been to develop and deploy technologies that enable commercially relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts — bacteria and fungi — in support of BETO's goals to decarbonize the transportation sector and other energy-intensive industries. The ABF is a public infrastructure investment that increases U.S. industrial competitiveness and creates new opportunities for private sector growth and jobs.

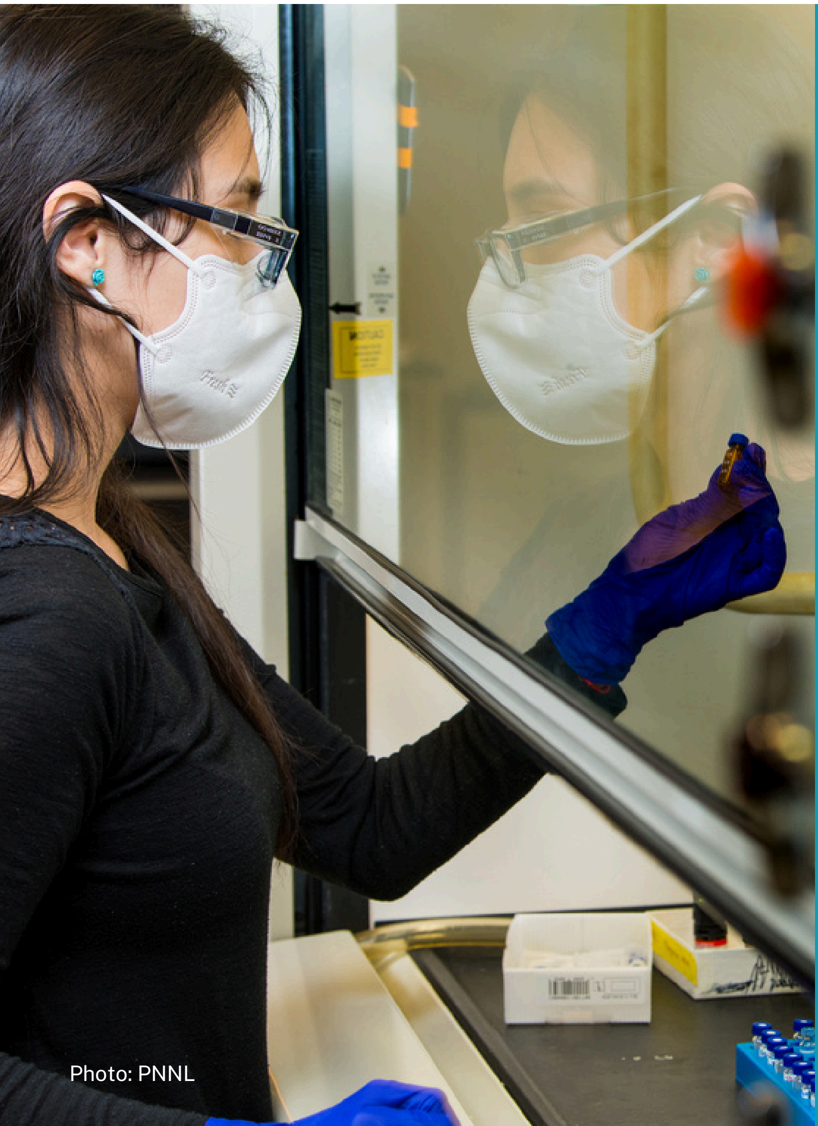


Photo: PNNL

Our Vision

Sustainable biomanufacturing of affordable fuels and chemicals

Our Mission

Develop biomanufacturing tools, processes, and partnerships that enable sustainable industrial production of renewable fuels and chemicals for the nation


Photo: LBNL

Tasks

The operation of the ABF has been divided into the following major tasks:

Management organizes and operates project management, including regular meetings, develops internal communications, supports capital expenditure, and provides deliverables to BETO.

Design-Build-Test-Learn (DBTL) is split into DBTL demonstration projects and DBTL Infrastructure. The demonstration project subtask aims to demonstrate a functional, robust DBTL cycle via work on specific target or beachhead molecules in appropriate host organisms as well as appropriately leverage the power of Learn. The infrastructure subtask develops, operates, and maintains the ABF's biological DBTL engineering cycle infrastructure.

Integrated Analysis evaluates techno-economic and life-cycle analyses (TEA/LCA) for proposed molecules and develops, updates, and improves existing process designs and TEA/LCA models to help guide ABF priorities and highlight important drivers in an integrated process context.

Host Onboarding and Development evaluates host organisms to determine which onboarding criteria are not yet met, fills these gaps through tool development and data collection, and in doing so, elevates hosts within the ABF's internally developed tier system. The task team has two main objectives: 1) onboard additional organisms into the ABF and elevate them within the tier system for use in future DBTL cycles, and 2) develop the HObT (Host Onboarding Tool) web application to house information on hosts available within the ABF.

Process Integration and Scale Up develops scaled fermentations aimed at optimizing titer, rate, and yield, and provides integrated, bench-scale data for TEA/LCA and data for Learn. This task also produces, stores, and ships biomass hydrolysates and compares the performance of these hydrolysates with clean sugar processes.

Industry Engagement and Outreach develops external communications, organizes and facilitates interactions with industry on behalf of the ABF, and provides feedback from the industry stakeholder community to the ABF and BETO that will support decision-making and project-planning activities.

Achievements

Award-winning biosensor accelerates biomanufacturing research

In 2020, the ABF team developed a technology that advances the throughput of screening microbial cells for biomanufacturing. The [Smart Microbial Cell Technology] links the productivity of a cell to an observable phenotype such as fluorescence. Visual performance supports selection of cell colonies, focusing research efforts on only promising variants. Using this approach, the team was able to efficiently create and screen a large library of enzyme variants with reduced product inhibition. Introduction of the improved enzyme in a *P. putida* strain enabled an increase in carbon flux toward the product and a 60% increase in glucose to muconate conversion. The technology can be easily coupled to high throughput flow cytometry.

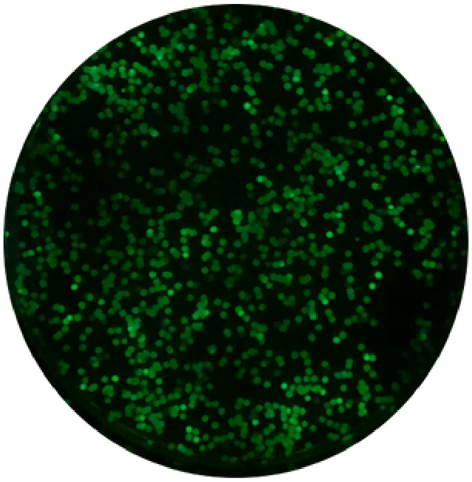


Figure 1. Photo of microbial colonies producing a muconate precursor via conversion of a metabolic intermediate. Brightness is directly correlated with enzyme activity, providing a direct readout of improved function. Image courtesy of Los Alamos National Laboratory.

A microbial population originating from an adaptive laboratory evolution experiment was rapidly sorted based on the fluorescence response from a biosensor, to identify novel mutations. These mutations, when introduced in the base strain, resulted in a 3-fold improvement in productivity. The approach does not require liquid handling equipment and reduces the cost of process consumables. It also significantly reduces the complexity of testing. Overall, it saves time and resources, allowing small resource-constrained companies to conduct their own screening, and larger companies to redirect resources to other pressing challenges.

Furthering impact through collaborations with BETO consortia

Researchers in BETO's Feedstock-Conversion Interface Consortium (FCIC) and the ABF are evaluating the influence of ash, moisture, stover anatomical fractions, and other attributes of harvested corn stover on a microbe's ability to convert hydrolysates of these feedstocks to sustainable aviation fuels. ABF proteomics and metabolomics studies are providing insight into how metabolic activity is affected by these varying conditions. Results of these studies will be added to FCIC's body of work in order to help de-risk the use of such feedstocks for various downstream conversion pathways.

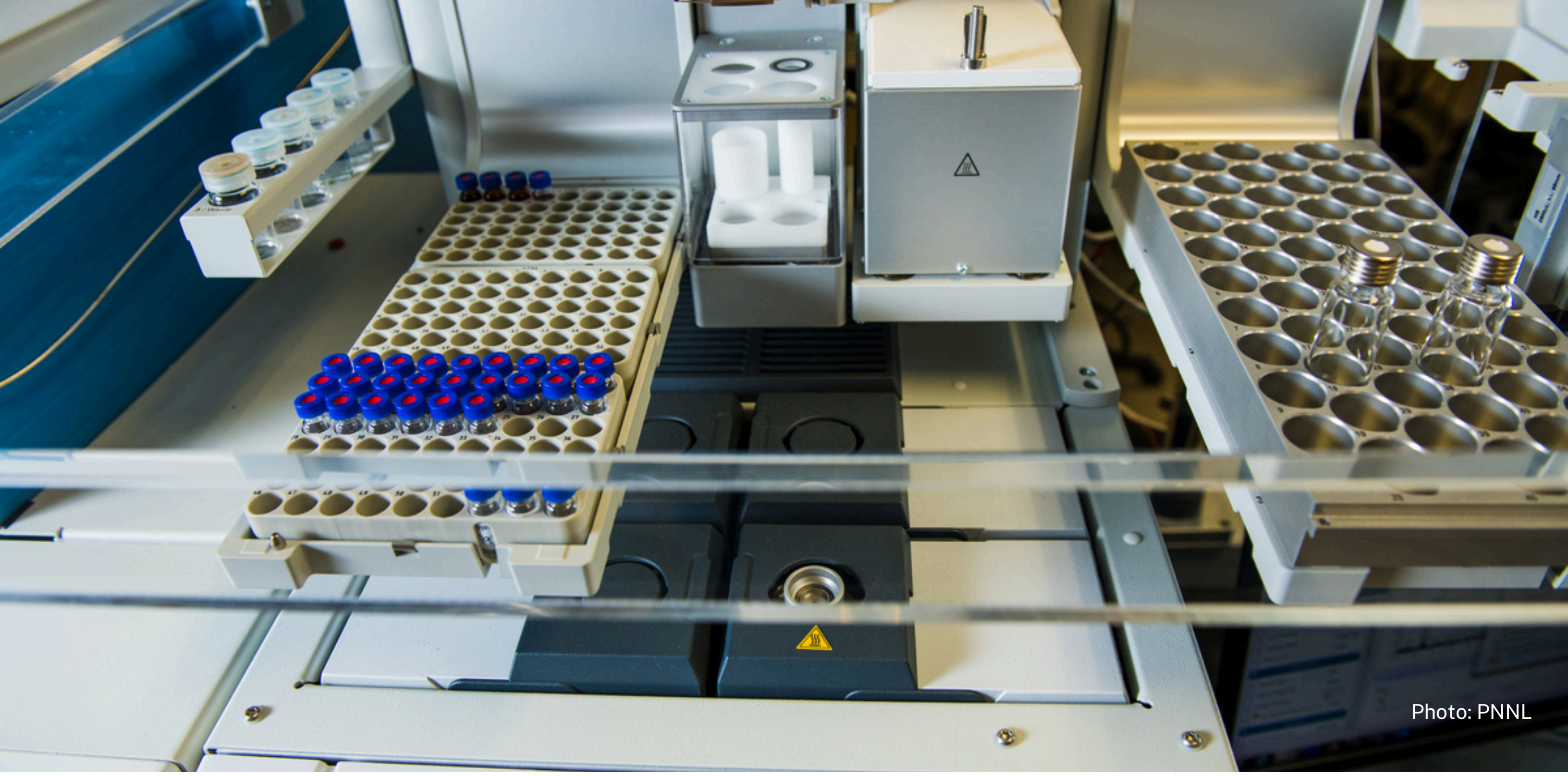


Photo: PNNL

Capacity building gives industry access to key capabilities

The ABF has built capacity across capabilities spanning the engineering biology cycle to reduce biomanufacturing commercialization timelines. This capacity building has been achieved through integrating off-the-shelf commercial solutions, and through developing new prioritized capabilities that address unmet needs. This constitutes a significant accomplishment, in that the ABF now acts as public biomanufacturing infrastructure investment, providing the private sector access to capital-intensive expertise, instrumentation, and capabilities that are unavailable elsewhere.

Biofoundries are fundamentally investments in enabling-infrastructure relating not only to physical instrumentation and equipment, but also to software, data repositories, trained models, methodologies including standard operating procedures, repositories of microbial hosts and DNA, organizational processes and practices, and a workforce spanning a diversity of domain expertise. The ABF manifests as a multiplicative return on investment in that it enables many companies to forgo the cost of having to repetitively and independently re-develop the same infrastructure from scratch.

The ABF has realized at least 2X and up to 10X overall efficiency gains in transferring existing capabilities (5 tools and 2 metabolic pathways) to additional organisms, relative to their initial development. This demonstrates that the ABF can adequately support companies needing capabilities related to what the ABF has already established. These capacity building accomplishments are a foundation that will be leveraged in future ABF collaborations with industry, and in the ABF's own future efforts toward sustainable aviation fuels and renewable biochemicals.

Establishment of routes to beachhead molecules and TEA/LCA exemplar methodologies

To support rapid access to a broad range of biochemical product space, the ABF has strategically developed routes through metabolic beachhead molecules (intermediates enroute to downstream targets of interest to industry) across a variety of host organisms. The ABF has also developed TEA/LCA methodologies that assess the strategic value in establishing a given beachhead over another.

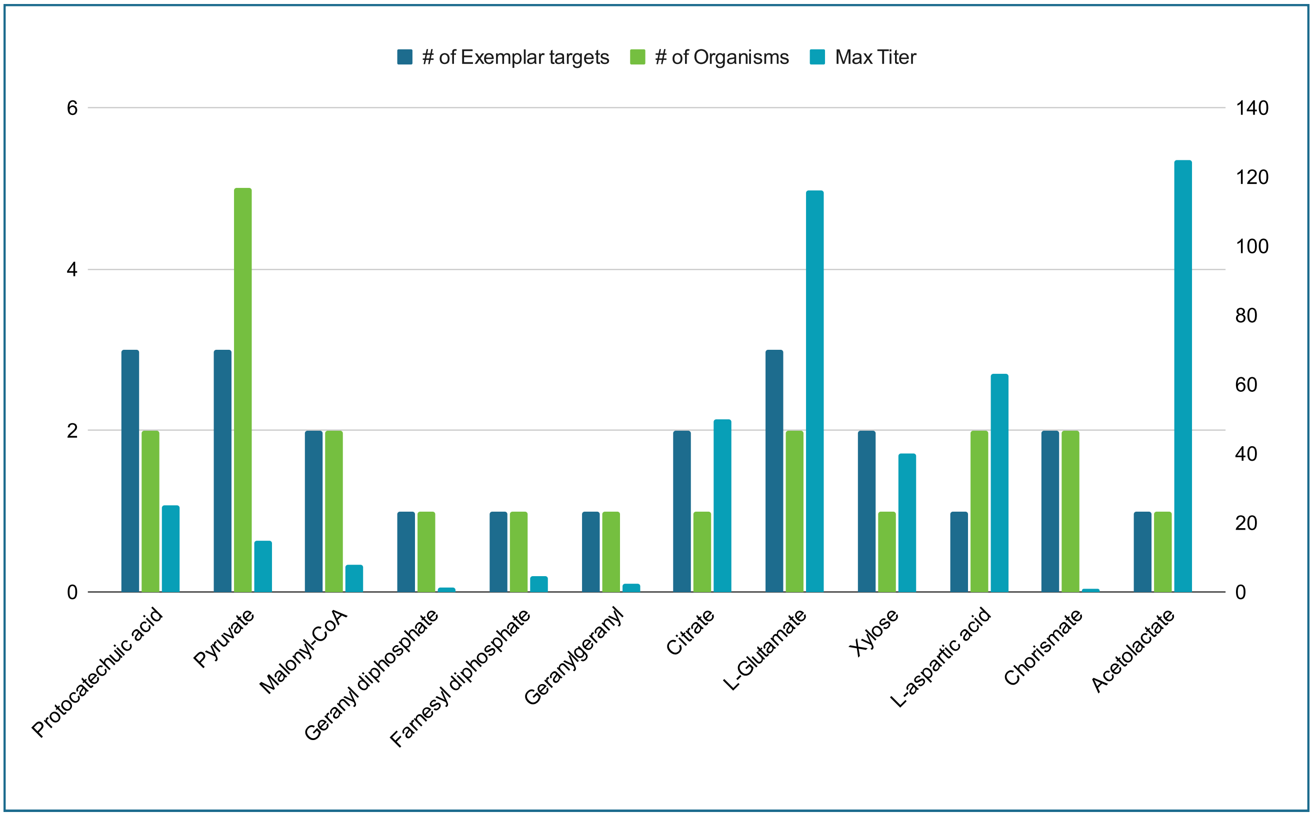
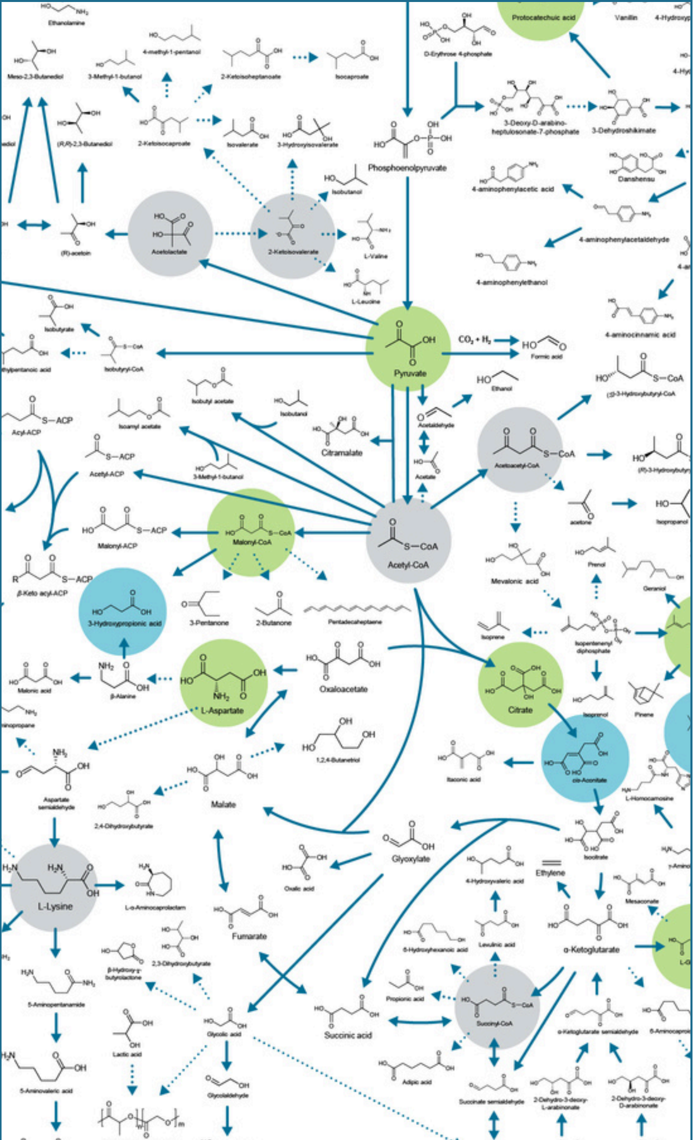


Figure 2. Established ABF beachhead molecules.

This innovative strategic effort by the ABF is consistent with a public infrastructure investment (akin to building a subway system) and can be applied to industry collaborations. Most metabolic engineering projects work toward increasing a bioprocess' titer, rate, yield, and scalability with TEA/LCA metric guidance for a specific end product (with a market price and volume). In contrast, the goal is to increase the flux through (but not the accumulation of) a beachhead, and TEA/LCA assessments can not be made for the beachhead itself, as there is often no established market for it. Through the pursuit of representative downstream target "exemplar" molecules, the ABF has developed methods and metrics for prioritizing beachhead development, and for determining when a beachhead has been successfully established.

The ABF has now established more than 10 beachheads, several of which have been demonstrated in multiple host organisms and through multiple exemplar molecules (Figure 2). These beachhead accomplishments have already been leveraged in industry collaboration projects, and will continue to be a valuable asset for future projects.



Visualizing bioproduct accessibility through a metabolic map

The ABF adapted an existing metabolic map[1] to visually communicate the ABF's areas of expertise to potential industry and academic partners, in terms of pathway development and evaluation. This map has been used in various ABF presentations and marketing materials. Metabolic pathway maps are commonly used to illustrate how catalyzed reactions convert substrates into products, and compilations of these illustrate how the pathways are related to one another and represent a more holistic view of metabolism and other complex bioprocesses. The ABF map identifies the locations of its established (and in development) beachhead molecules , which offer potential partners a head start to achieving a route to their desired target molecule. A full version of the map is included at the end of this report.

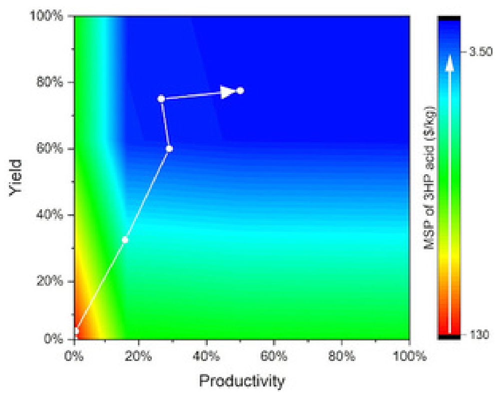
[1] Adapted by permission from Springer Nature Customer Service Centre GmbH: Nature, Nature Catalysis, A comprehensive metabolic map for production of bio-based chemicals, Lee, S.Y., et al., © 2019

International leadership amongst public biofoundries

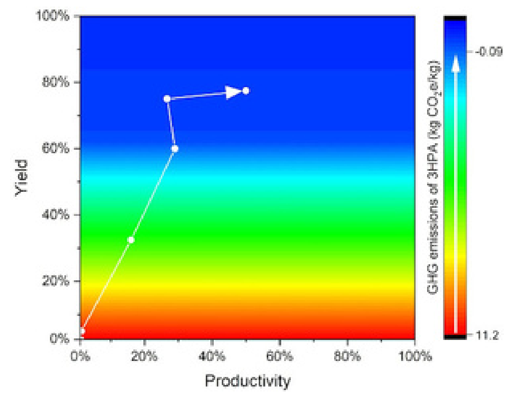
In 2019, the ABF co-led the establishment of the Global Biofoundries Alliance (GBA) as a founding member. The GBA has since grown to 33 non-commercial biofoundries worldwide that collectively share experiences and work together to overcome shared challenges and unmet scientific and engineering needs. Through its participation, the ABF is contributing to U.S. leadership in biofoundries and biomanufacturing, including the development of international standards. As one of the oldest and largest non-commercial biofoundries, the ABF is looked to as a model of success to be emulated. Participation in the GBA has allowed ABF to exchange staff, transfer and replicate technology and methods across facilities, (e.g. automation of *A. niger* transformation in collaboration with co-member VTT Technical Research Centre of Finland), and co-business development. These benefits contribute to the ABF's capability capacity building as a public infrastructure investment, and also in its impact on industry reducing biomanufacturing commercialization timelines.

Capabilities enable progress on flagship biomanufacturing targets

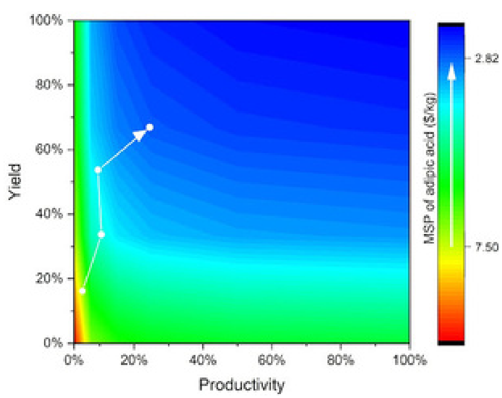
By applying tools and technologies developed by the ABF, we have demonstrated improvements in biomanufacturing in terms of increased speed and efficiency of strain development. Advances targeting products such as 3-hydroxypropionic acid (3HP), which can be used to make acrylic acid-based polymers, and muconic acid, which can be used to make adipic acid, a nylon precursor, have led to a progressive improvement of product yields and productivities. Leveraging these metrics to TEA and LCA demonstrates how these improvements have affected the minimum selling price and the greenhouse gas emissions associated with production.



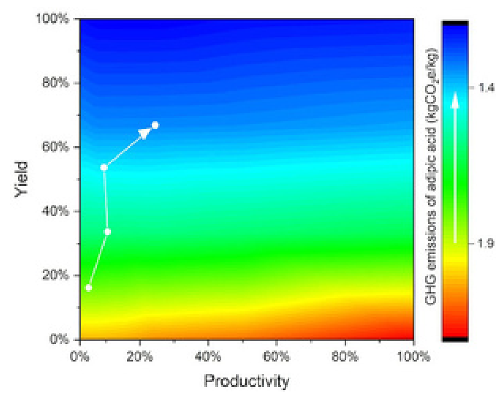
Work on 3HP has progressed to reduce the bio-derived minimum selling price, by 97%, achieving approximately $3.50/kg, compared with $1.40-$2.42/kg for the fossil fuel-based product.



Development of bio-derived 3HP would deliver estimated greenhouse gas emissions of 0.09 kg CO2 e/kg, a reduction of 99% versus the fossil fuel equivalent (6 kg CO2 e/kg), and also to negligible levels versus its starting point as an ABF target, of 11.2 kg CO2 e/kg.



For adipic acid, the ABF has reduced the minimum selling price of the bioproduct by 38% to $2.82/kg, compared with $1.14-$1.73/kg for the fossil fuel equivalent.



Development of bio-derived adipic acid would result in estimated greenhouse gas emissions of 1.4 kg CO2 e/kg, representing a reduction of 85% versus the fossil fuel equivalent (~9.4 kg CO2 e/kg). ABF work has reduced the carbon footprint of the bio-derived product by >25%, from 1.9 kg CO2 e/kg.

Although these bio-derived products are not yet cost-competitive with the fossil fuel incumbent, in each case we have identified paths towards significant further price reduction for both target products, and progress towards chemical replacement. In a mature carbon market, the greenhouse gas reductions would further contribute to cost competitiveness.

Photo: NREL

Host onboarding accelerates strain development efforts

The ABF has onboarded and further developed 15 microbial host organisms, along with resources and tools that enable their use via a process described as tier elevation. The tools acquired or developed include advanced genetic tools for high-throughput (HTP) Build and genome-scale libraries for HTP Test, metabolic models, omics and physiological datasets, and a variety of protocols, which collectively enable potential project partnerships in a diverse range of applications. The ABF created the publicly accessible Host Onboarding Tool to help communicate the technical depth and resources made available to industry via this effort. This work is collectively impactful in that it has broadened the range and increased the engineerability of host organisms (and in turn bioprocess conditions, feedstocks, and classes of target molecules) that are accessible to industry as they seek to reduce their commercialization timelines and costs.

Cross-lab experiments provide insight into commercialization challenges

The ABF has successfully replicated complex scale-up experiments at multiple facilities within the consortium. This is a significant accomplishment, as bioprocessing is a vastly multi-parametric field, with myriad complex variables impacting outcomes. The team also discovered that a difference in altitude may present a hitherto unexpected barrier to technology transfer. Our multi facility experiments in Emeryville, CA and Golden, CO (with an altitude differential of approximately 5,700 feet) yielded different results. Higher oxygen levels at sea level were associated with higher feedstock consumption and lower production volumes. This underlines the challenges associated with the technology development and commercialization process, which by definition needs to be broadly applicable, in contrast with the attention to detail needed when developing experimental methods and processes — in this case the detailed monitoring of process environmental conditions.

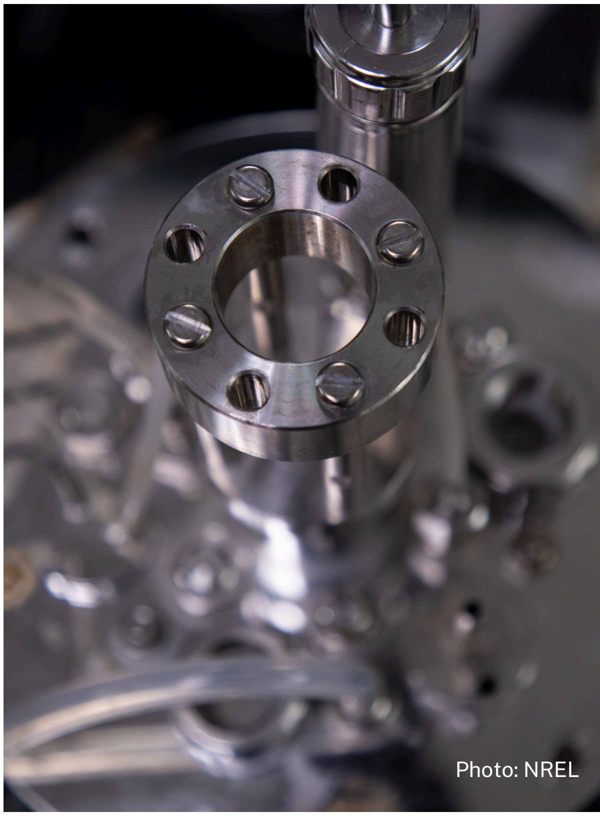


Photo: NREL

Understanding industry needs through active engagement

The goals of ABF's Industry Engagement and Outreach team are to facilitate interactions between the ABF and external stakeholders, enable responsiveness to industry needs, and communicate ABF capabilities. One important way it does this is by gathering feedback from industrial biomanufacturing stakeholders on the ABF and the biomanufacturing industry at large and using this information to support decision-making and project-planning activities.

Photo: NREL

These insights are collected via surveys and by engaging biomanufacturing stakeholders in one-on-one interviews aimed at gaining a deeper, more nuanced understanding on topics of interest. To date, 34 surveys have been returned and more than 90 interviews have been conducted. The ABF also holds quarterly meetings with its Industry Advisory Board to regularly engage its members around scientific successes, possible future directions and priorities, and efficiency of industrial interactions.

Machine learning tool systematically guides research

The ABF has developed and deployed the Automated Recommendation Tool (ART), an effective tool for guiding metabolic engineering that does not require a full understanding of the biological system of interest. ART creates a predictive model from experimental data through the use of machine learning, and provides recommendations for the next round of experiments that are predicted to improve results. The ability to predict outcomes with incomplete information means that project teams are able to focus on gaining insight into a smaller set of unknowns when designing their experiments, which in itself saves time and money. In doing so, projects can see targeted rapid improvements in results over several experimental rounds. ART will lead to highly accurate predicted results for the designed experiments, and as a result, much shorter strain development times (with associated cost savings) and more attractive propositions for further R&D funding or technology development rounds. ART's real world impact is well summarized by the fact that its design recommendations led to a >2X increase in tryptophan versus the state-of-the-art technology, utilizing model training data that represented only 3% of the total possible pathway combinations.

Interagency partnerships bring new opportunities

The ABF works to continually improve its responsiveness to the needs of the biomanufacturing community. Partnering with other U.S. agencies and organizations is proving to be an attractive approach to leveraging other funding pools, gaining new inroads to the research community, and fulfilling DOE's commitment to the power of diversity, equity, and inclusion to drive cutting-edge science. Recent examples include joint National Science Foundation (NSF)/ABF funding opportunities, and the ABF's funding of efforts within the Minority Serving Institution (MSI) Science Technology Engineering and Mathematics (STEM) Research and Development Consortium (MSDRC).

The ABF has also had exploratory conversations with the BioMADE manufacturing innovation institute regarding similar opportunities. NSF/MSI, ABF, and BioMADE sit along a gradient of technology and manufacturing readiness levels, and these collaborations are impactful in that they contribute to bringing new capabilities and workforce (from NSF/MSI) into the ABF, and to the graduation on and off-ramping of ABF efforts.

Photo: ORNL

Improving management of collaborations with industry and academia

The ABF has procured a software package designed specifically for grant management. The software will greatly increase the efficiency of workflows, centralize application-related information, and improve the reliability and ease of use for managers, applicants, reviewers, and other stakeholders. The data repository and its encoded forms, workflows, and visualization tools will save significant time spent in organizing all funding opportunity-related data. Therefore, staff may dedicate more time to building relationships across the community and tracking the full impact of BETO-funded and funds-in research.

The acquisition of professional-grade grant management software is one of dozens of process improvements to arise from the learnings of past funding opportunities, strategic working groups, and leadership meetings.

Alumni pursue careers in bio-related industries

The ABF has become a contributor to a pipeline of talent and resources that will serve the growing U.S. bioeconomy over coming generations. Over 90 ABF alumni, many of them postdoctoral researchers, have gone on to pursue careers in a variety of fields in biotechnology and biomanufacturing. As the need grows for highly trained scientists and engineers in these fields, the ABF will continue to contribute to creating a diverse workforce that will have an impact on the U.S. bioeconomy.

Publications and inventions showcase innovation

In its first six years of operation, the ABF has established a strong scientific publication record, generating 65 publications that have garnered over 1,900 citations.

The ABF has generated several innovations and inventions through both its core research and cooperative R&D it has conducted with partnering companies and institutions. This includes the invention of a wide variety of tools, biological constructs, and software packages for advancing new technologies toward commercialization, to either support the production of specific sustainable aviation fuels and chemicals or to facilitate related research in synthetic biology.

Disclosures	Patent applications	Software copyrights	Licenses
33	16	4	5

Industry Collaborations

Working with industry to elevate and promote growth of the U.S. bioeconomy has been a central focus of the ABF since its inception. The ABF works proactively with industry to accelerate the innovation and adoption of new biomanufacturing approaches that will foster growth of the bioeconomy.

Over the past six years, the ABF has been involved in three rounds of proposal calls to industry to join forces with the ABF to improve specific aspects of the DBTL cycle. This has generated 37 collaborative projects with 24 companies and 13 universities that represent over $41 million in funding to date. These projects cover the entire science and technology spectrum that is needed to significantly improve the DBTL cycle and advance the state-of-the-art in synthetic biology and biomanufacturing.

Enabling Scale-Up of Lygos' Sustainable Bioproduct

In 2018, biotech company Lygos teamed up with the ABF to increase production of organic isobutyric acid, and scale up its production to commercial relevance. Isobutyric acid is a chemical compound with a wide range of applications, from flavoring to solvents and even as a building block of plexiglass. Applying ABF's Design-Build-Test-Learn capabilities, this two-year collaboration resulted in a 20-fold increase in production of isobutyric acid.

ABF's proteomics and metabolomics capabilities were key to identifying and resolving bottlenecks in the process. "The combination of the multi-omics dataset and ABF's expertise, tools, and technologies have enabled us to further accelerate the strain engineering design, build, test, and learn cycle," said Andrew Conley, Vice President of Biology R&D at Lygos.

"Thanks to this collaboration with the ABF, Lygos is now one step closer to our goal of replacing another toxic industrial chemical process with a multi-functional biobased form of isobutyric acid that offers compelling performance, economic and environmental advantages over the traditional industrial alternative," Conley said.

Photo: PNNL

Producing High-Value Chemicals from Renewable Sources

In order to combat the costly industrial processes to develop bioproducts, ABF and biotech company Visolis teamed up to explore new feedstocks and microbes that are cheaper, more reliable, and perform well. The teams set out to develop a system that can produce an industry-relevant platform molecule that can be used in fuels and as high performance polymers. This system would use waste CO2 combined with aerobic fermentation at a low pH to decrease costs.

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

The teams demonstrated that this system could produce promising amounts of Visolis’ platform molecule. ABF’s team developed techno-economic models to evaluate the technology’s economic potential.

Visolis will be developing this technology further, either internally or in collaboration with ABF scientists. Dugar said the proof of concept achieved through this project is crucial for Visolis, allowing them to present their technology to investors and attract further funding.

A Bright Idea for Plastic Upcycling

Terephthalic acid, or TPA, is a commodity chemical and precursor to poly-ethylene terephthalate (PET), a petroleum-based polymer that is used in plastic bottles, clothing items, and packaging. Despite being recyclable, most items that contain PET end up in landfills or leak into the environment. Traditional PET recycling approaches are only able to break PET down into products that aren't as valuable, as key properties are lost during each reprocessing cycle.

As a result, there's a lot of interest in researching how plastic waste can be broken down into the individual molecules it's made of — such as TPA — so that these molecules can be converted into new products. Only a few bacteria have been studied for their ability to utilize TPA. Another challenge is that TPA isn't able to diffuse or be transported through the cell membrane, which is necessary for it to be taken up by the cell and further broken down. Transporter genes that can help TPA move through the cell membrane are required.

To address this need, researchers at the ABF and the University of Georgia engineered the soil bacterium *Acinetobacter baylyi* ADP1 to both transport and detect TPA. By combining technologies from each lab, the researchers were able to identify a transporter gene that could import TPA into the bacteria.

In addition, the researchers developed a fluorescent biosensor that confirmed the transport of TPA in the bacteria. The custom-designed biosensor detects intracellular TPA and activates a fluorescent reporter gene, allowing scientists to quickly identify successful bioproduction of TPA as well as successful degradation of PET into TPA.

"This project has given me a glimpse into the interface of fundamental and applied research," said Ellen Neidle, professor at the University of Georgia. "It has led me to see how a concerted effort funded at the national level can take these ideas and possibilities and turn them into reality."

Machine Learning Opens New Doors in Bioproduct Development

In 2019, biotech company Lygos teamed up with the ABF to optimize strain performance through ABF's multi-omics and machine learning capabilities. Machine learning can speed up the bioengineering process dramatically, transforming large amounts of data into predictions that effectively guide the development of bio-based products.

After generating over 80,000 data points to train machine learning algorithms, ABF researchers developed artificial neural networks to leverage this data and provide Lygos with actionable recommendations to increase strain performance through Design-Build-Test-Learn cycles.

The project is impacting Lygos' overall approach to synthetic biology, particularly when it comes to their interest in producing malonic acid, which can be used in plastics, food additives, and more. "Our platform for malonic acid at Lygos is quite well established. Because of that, we've gone down many scientific avenues to improve performance," said Mark Held, senior scientist at Lygos. "When you do that for a long time, you start running out of rational places to further engineer. That is where this multi-omics and machine learning technology shows its strength. It has the ability to find things we can't easily predict."

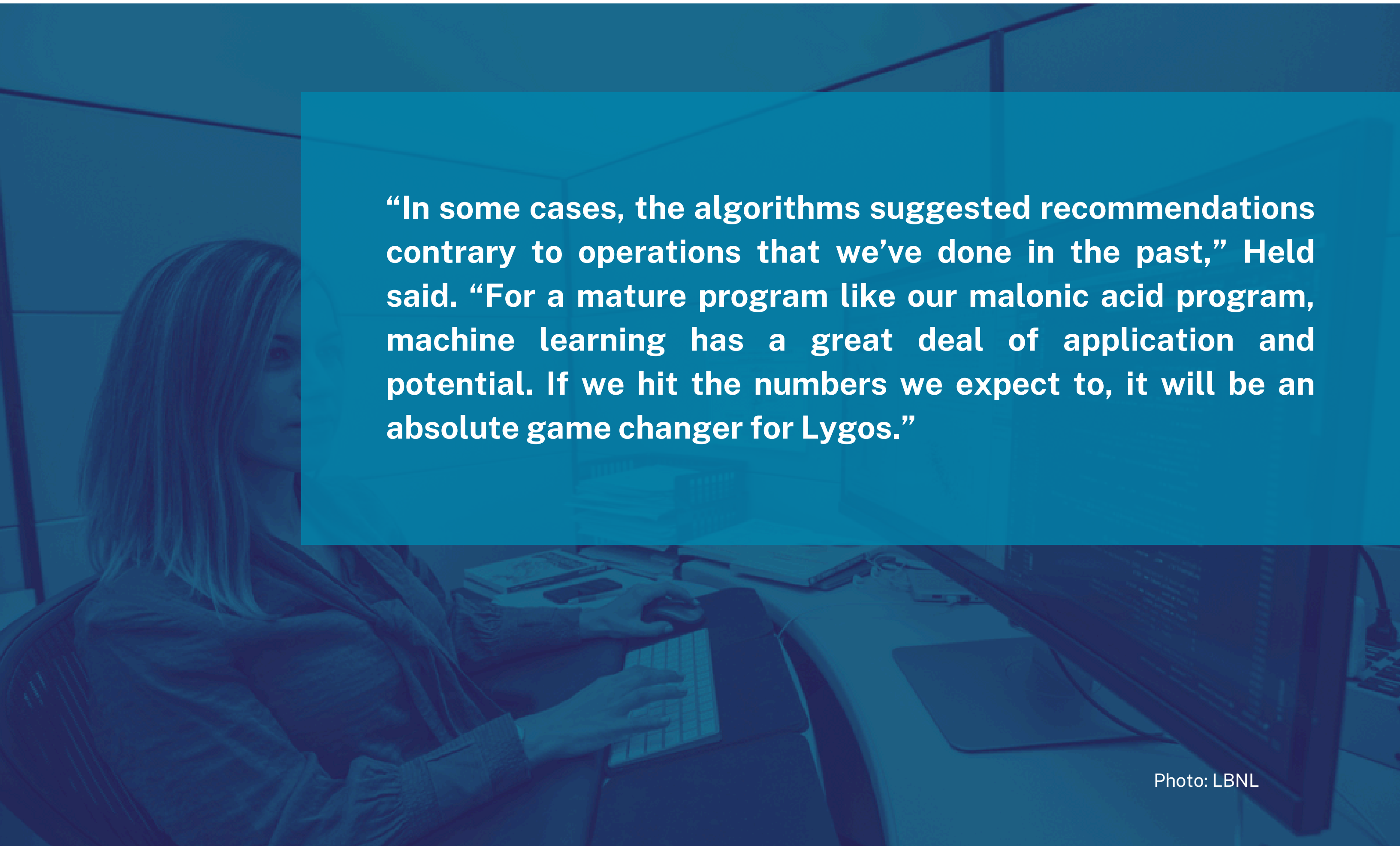


Photo: LBNL

Collaboration Highlights

Agilent

Developed the Automated Method Selection software tool, coupling novel liquid chromatography (LC) and powerful mass spectrometry platforms to predict the best LC-method for analysis of any new molecule of interest.

LanzaTech

Combined comprehensive and unique fermentation datasets with new, advanced artificial intelligence methodologies to create predictive models of fermentation outcomes.

KIVERDI

Developed a synthetic biology toolkit for *Cupriavidus necator* H16, aiding in the production of 1-dodecanol, a byproduct of H2/CO2 bioconversion.

teselagen BIOTECHNOLOGY

Enhanced software tools to enable more effective engineering of biological systems for the production of renewable biofuels and other bioproducts.

ZYMOCHEM

Developed genetic tools for *Bacillus licheniformis* and bioinformatic approaches to enhance polyglutamic acid production from lignocellulosic hydrolysates, an attractive carbon source for bio-based fuel and chemical production.

Industry Advisory Board

The ABF formed an external panel of experts to inform strategies, tactics and decision making for the consortium. The Industry Advisory Board (IAB) is composed of representatives from industry and other entities in synthetic biology, biomanufacturing, industrial biotechnology, and related fields. IAB members are not required to sign an NDA, therefore ABF data and information shared with them is not confidential or proprietary.

The IAB was charged with the following tasks:

- Assist the ABF consortium in maintaining an industry-relevant focus to current and future research and development activities;
- Contribute knowledge of recent (non-proprietary) technology advances and challenges that may impact ABF research directions and decisions; and
- Advise the ABF on effective industry stakeholder engagement strategies and public outreach efforts.

In particular, the IAB has influenced ABF perspectives and decision making via feedback on the following topics:

- Overall approach
- Operational methods and processes
- Industry-relevant metrics
- Appropriate milestones and outcomes
- Target/host selection
- Outreach activities
- Maximizing overall impact

Current IAB Members:

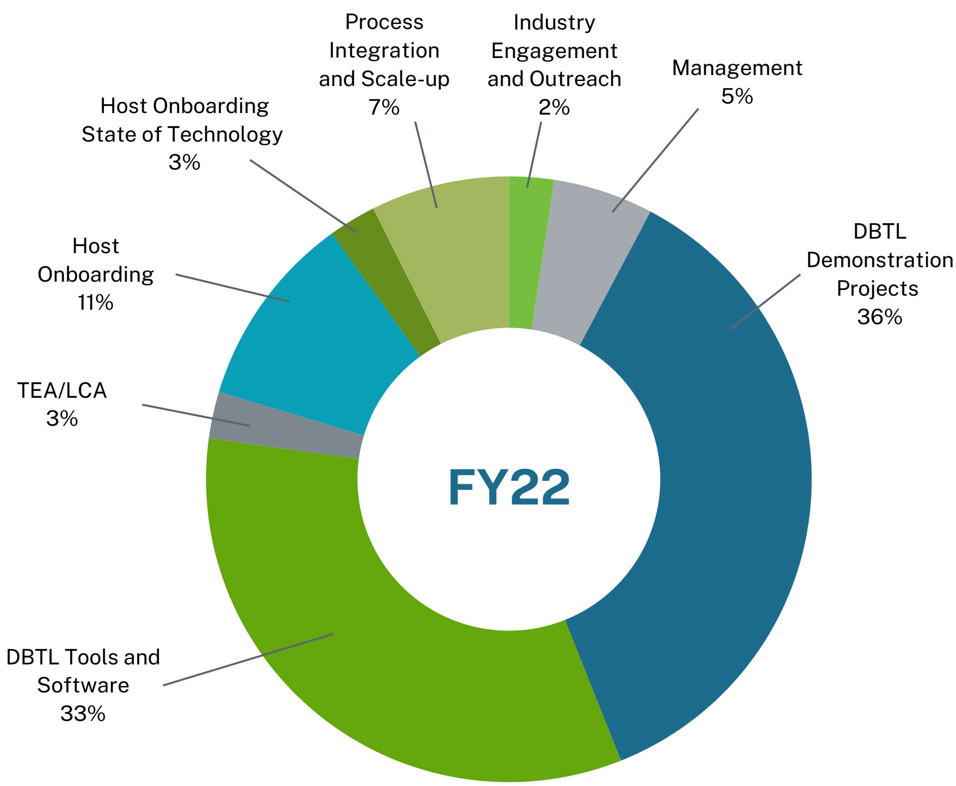
Eli Groban, Thermofisher
Kedar Patel, Zymergen
David Anton, Trelystech, Independent Consultant
Mike Fero, Teselagen
Doug Freidman, Engineering Biology Research Consortium
Nate Tedford, Gingko Bioworks
Derek Abbott, Amyris
Lauren Junker, BASF

Funding Summary

Core

Major Task Topic	FY17	FY18	FY19	FY20	FY21	FY22	Total
DBTL Demonstration Projects	$6,336,000	$7,475,000	$7,285,000	$5,745,000	$4,951,000	$5,433,000	$37,225,000
DBTL Tools and Software	$3,414,000	$4,030,000	$3,925,000	$4,666,000	$5,404,000	$4,979,000	$26,418,000
TEA / LCA	$675,000	$320,000	$150,000	$375,000	$375,000	$375,000	$2,270,000
Host Onboarding	$900,000	$970,000	$654,000	$1,053,000	$1,417,000	$1,560,000	$6,554,000
Host Onboarding - State of Technology			$917,000	$877,000	$582,000	$382,000	$2,758,000
Process Integration and Scale-up	$1,125,000	$660,000	$1,140,000	$1,109,000	$1,109,000	$1,109,000	$6,252,000
Industry Engagement and Outreach	$300,000	$390,000	$470,000	$360,000	$360,000	$360,000	$2,240,000
Management	$2,250,000	$1,155,000	$1,107,000	$815,000	$802,000	$802,000	$6,931,000
Annual Budget	**$15,000,000**	**$15,000,000**	**$15,648,000**	**$15,000,000**	**$15,000,000**	**$15,000,000**	**$90,648,000**

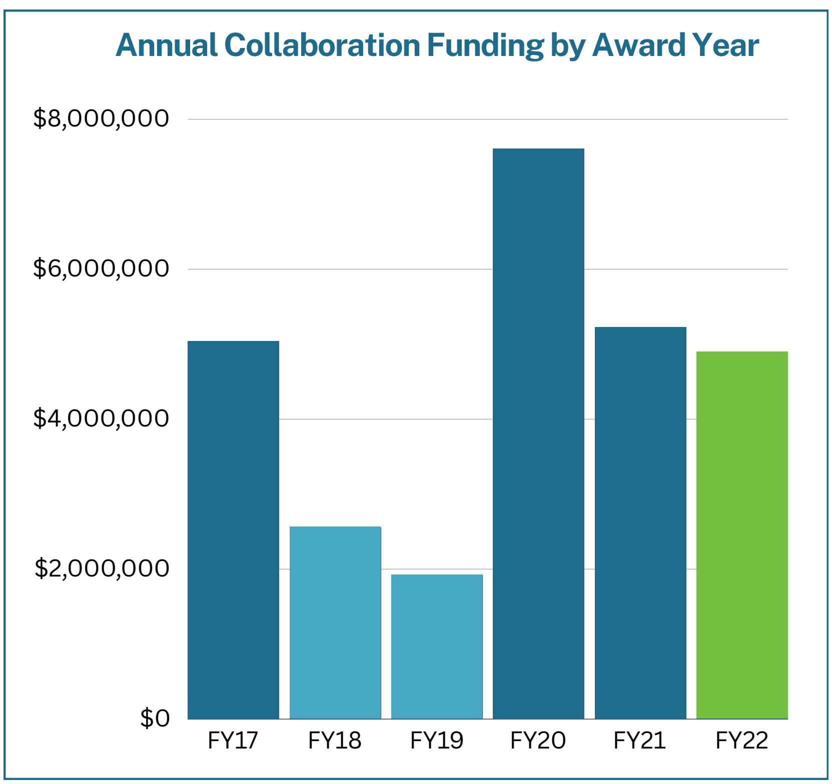
Funding Breakdown by Major Task



Funding Summary

Partnership Projects

Major Task Focus	FY17	FY18	FY19	FY20	FY21	FY22	Total
DBTL Tools and Software	$ 4,217,000	$ 2,003,500	$ 1,922,900	$ 6,951,900	$ 5,230,000	$ 3,864,670	$ 22,267,240
Host Onboarding	$ 725,000			$ 250,000		$ 141,670	$ 1,116,500
Process Integration and Scale-up	$ 100,000	$ 552,000		$ 405,000		$ 325,000	$ 1,382,000
TEA/LCA				$ -		$ 566,670	$ 566,670
Annual Lab Awards	**$ 5,042,000**	**$ 2,555,500**	**$ 1,922,900**	**$ 7,606,900**	**$ 5,230,000**	**$ 4,898,000**	**$ 25,332,400**



Note: FY18 and FY19 were FOAs, not usual DFOs. In those cases, only a portion of the overall available funding came to the Labs.

Concluding Remarks

The ABF's FY17-FY22 accomplishments helped advance the biomanufacturing of fuels and chemicals in several ways. The ABF developed and promoted several new and notable conceptual frameworks. These include the development of routes to metabolic beachheads, and in particular how to assess their strategic value through the evaluation of exemplar downstream molecules of known commercial value and how to visualize them through metabolic cartography. The ABF established its host onboarding and development tier system, along with how to track and share the progress of microbial advancement through the tiers. The ABF made early contributions demonstrating that machine learning models that are mechanism/domain-agnostic (but not Large Language Models), could be effectively applied to exploring and exploiting vast combinatorial biological and bioprocess design spaces. The ABF advanced the biofoundry field through its leadership in the Global Biofoundries Alliance, not only in scientific and technical aspects, but also in business and operational models. Entities initiating their own biofoundries have sought ABF's guidance and advice. These include the U.S. National Science Foundation-supported biofoundries, as well as emerging international biofoundries such as the KAIST/KRIBB K-Biofoundry in South Korea, and biofoundries supported by Bioplatforms Australia.

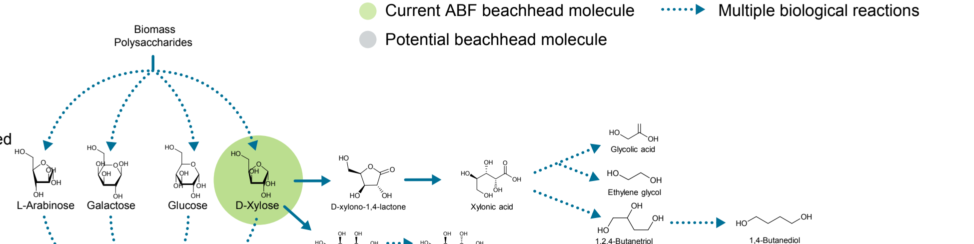
In FY17-FY22, the ABF established a firm foundation of operational infrastructure from which to launch into its next phase. This foundation included established metabolic beachheads, a variety of onboarded bacterial and yeast and fungal hosts, scientific and technical capabilities and domain expertise, datasets, and a process for running ABF-directed funding opportunities and initiating and managing collaboration projects with industrial and academic partners. The ABF established and strengthened foundational relationships within the DOE National Labs, with industry and academic partners — including ABF Industry Advisory Board members — and with other U.S. Federal Agencies and related organizations. These foundational aspects and lessons learned during FY17-FY22, taken together, put the ABF in a strong position from which to transition into its next phase, in which it would emphasize the application of what had been built to industry and DOE mission objectives, toward bioprocess commercialization and impact.

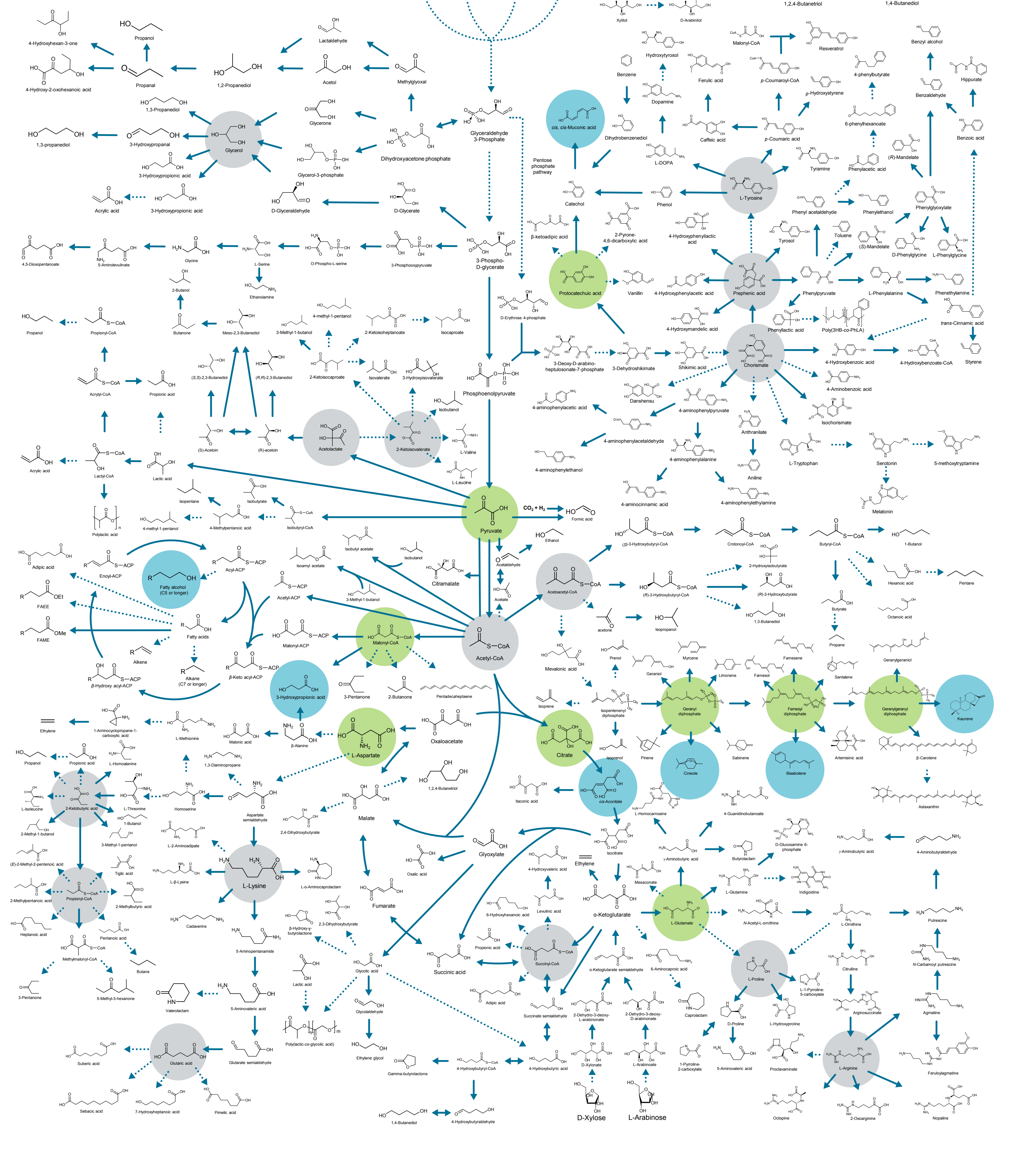
A key takeaway from this report is that public investment in biomanufacturing capabilities and domain expertise, such as the ABF, can be leveraged in partnership with the private sector to positively impact bioprocess commercialization. There are delicate balances between strategic and reactive approaches to biofoundry development, and between infrastructure build-out vs. its application toward commercial impact. It is critical not just to build a capable biofoundry, but to demonstrate that the biofoundry is actually useful and adds value in overcoming real industry challenges. This report in aggregate provides a case study for what types of outcomes and outputs are achievable within the timeframe and resources allocated to the ABF in its first phase.

Agile BioFoundry

- Current ABF target molecule
- Current ABF beachhead molecule
- Potential beachhead molecule
- Biological reaction
- Multiple biological reactions

This map represents a compilation of metabolic pathways that have been described for conversion of biomass-derived polysaccharides to industrial chemicals. Adapted by permission from Springer Nature Customer Service Centre GmbH: Nature, Nature Catalysis, A comprehensive metabolic map for production of bio-based chemicals, Lee, S.Y., et al., © 2019





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