AGILE BIOFOUNDRY VISION

VISION

The Agile BioFoundry will unite and expand the capabilities of the national laboratories to develop a robust, agile biomanufacturing platform accessible to researchers across the private and public sectors.

MISSION

The Agile BioFoundry will integrate industrially-relevant production microbes, advanced tools for biological engineering and data analysis, and robust, scaled up processes for integrated biomanufacturing.

GOAL

The Agile BioFoundry seeks to develop innovative, open-source, and scalable technologies that will enable a robust bioeconomy by reducing the time and cost of developing bioproducts.

THE NEED FOR A PUBLIC EFFORT

For forty years, researchers have been able to conveniently and precisely manipulate the genome of an organism to create products of interest. In the private sector, many companies have used genetic engineering and synthetic biology technologies to create new bio-based fuels and chemicals that can displace petroleum counterparts, while others have commercialized those underlying technologies. Many of these efforts have been focused on specialization, where a company develops expertise and capabilities for a select host organism and a relatively limited set of products from that host. Due to the competitive nature of the field, much of this knowledge is not transferred outside of the company and other efforts repeat prior work. Improvements in technology, especially computing and laboratory automation, have meant that some of the work to build a strain is quick and relatively easy to accomplish. Despite this, there are still bottlenecks to achieving a robust process that can be implemented at the needed scale for production. Some of the biggest challenges remain around learning from experiments and processes and applying that knowledge to future work to design strains. Some companies are beginning to enter this space, but many develop specific expertise and cannot access complementary technologies that may exist in the private sector.

The national labs have unique and complementary capabilities that can be united to build out a robust biomanufacturing platform that addresses the needs of companies in the bio-based fuels and products sector (and biomanufacturing more broadly). These capabilities include: biological discovery and application of new genes, proteins, pathways, and organisms, facilities for characterization of organisms' genomes, proteomes, and metabolomes for holistic understanding, high-performance computing, and facilities and equipment for integrating bioprocesses and scaling them up. While these capabilities exist in the private sector, they are not always integrated and rarely accessible to others. Additionally, the underlying research and development, capabilities and facilities lie outside the core

missions of most companies. To truly turn biological engineering into biomanufacturing, the expertise and capabilities of the national labs must be united and informed by real-world challenges facing private industry.

KEY STAKEHOLDERS

The Agile BioFoundry considers its stakeholders to include:

- Companies that develop bio-based fuels and chemicals
- Companies that develop instrumentation, equipment, software, and reagents for biological engineering
- Researchers at companies, national laboratories and universities that would like to build new strains
- Researchers at companies, national laboratories and universities that develop new bioproducts
- Researchers at companies, national laboratories and universities that develop new technologies
- Federal agency staff that support bioeconomy programs
- General public interested in a more economically- and environmentally sustainable bioeconomy

FRAMEWORK FOR THE AGILE BIOFOUNDRY

To achieve its mission, the Agile BioFoundry (ABF) will unite the capabilities of the Department of Energy National Laboratories to integrate sophisticated synthetic biology tools including software for biological design, machine learning, high-throughput analytics, techno-economic and life cycle analyses, and expertise, into an agile and dynamic platform for biomanufacturing of microbes for production of bio-based fuels and chemicals. The ABF will focus its efforts on integrating the Design-Build-Test-Learn (DBTL) cycle of biological design while incorporating analysis that guides product/organism fit and selection, techno-economic and life cycle analyses, and process considerations for predictable scaling and process robustness. Figure 1 describes the proposed research and development focus for the ABF. As envisioned, the labs will use the techno-economic and life cycle analyses to develop a set of exemplar molecules, with industry and other stakeholder involvement, that address key barriers for the DBTL cycle and process integration to demonstrate an integrated biomanufacturing platform. These exemplar molecules will allow the ABF to benchmark and measure progress as the platform is integrated and optimized.

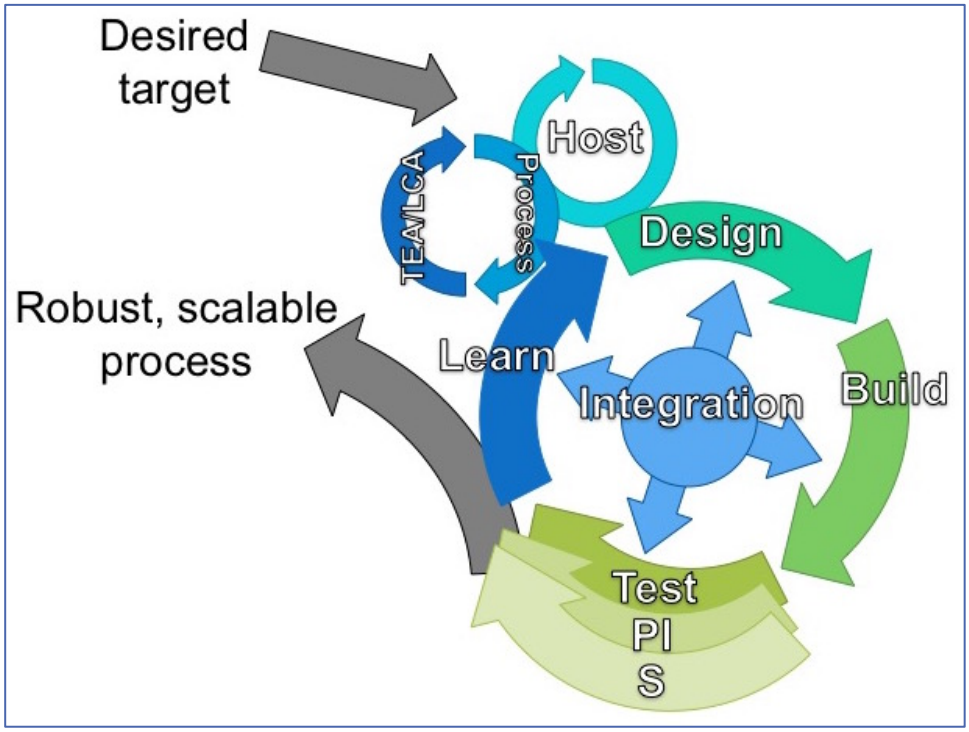

Figure 1. *The platform that the ABF will develop to deliver robust and scalable processes for desired target molecules. The core of the ABF effort will be to develop the integrated Design-Build-Test-Learn cycle, where Design is informed by techno-economic analyses, life cycle assessments, and process considerations that allow for optimum host organism selection. Test is represented by multiple arrows to indicate various dimensions of data gathered for learn, including bench-scale analysis (Test), process integration (PI), and scaling to relevant scales (S) for the bioprocess. Each of these dimensions of Test will provide valuable information for Learn and will be incorporated into the upfront TEA/LCA and process considerations for future rounds of Design.*

LONG-TERM IMPACT AND THREE-YEAR ACHIEVEMENTS

The ABF will broadly enable biomanufacturing across the bioeconomy through the development of new host organisms an integrated Design-Build-Test-Learn cycle that is accessible to industry and to university and national lab researchers. The ABF is envisioned to enable companies to bring new products to market more cost-effectively and efficiently through the reduction of bioprocess design cycle times. With these improvements the ABF, in partnership with academia and industry, has the following long-term aims:

- Decrease the energy intensity of current manufacturing processes by 40% over status quo
- Decrease the carbon intensity of current manufacturing processes by 60% over status quo
- Reduce time to market cycle time by 50% over status quo
- Increase biomanufacturing cycle efficiency (cost, time) >40% over status quo
- Develop new manufacturing technologies, increase US industry competitiveness, and create new opportunities for private sector growth

Progress and success for each target will be measured against an initial project baseline and then measured annually against that baseline. Some metrics that will be considered are: DBTL cycle time to

industrially-relevant titers, rates, and yields; number of cycle iterations needed to reach those relevant titers, rates, and yields; projected reductions in cost and environmental impacts for a target; and the number of person hours needed to achieve targets.

At the end of the initial three years of funding, the ABF will:

- Demonstrate an integrated Foundry platform, as measured by markedly increased titers, rates, and yields for production of 3-5 exemplar molecules over the initial baselines with reduced person-hour contributions to the DBTL cycle
- Deliver a public set of tools and technologies for rapid and facile engineering of biological organisms
- Make available publicly-available databases and software to improve biological design with an advanced starting point for production of targets over the current state of the art
- Offer genetically-tractable, industrially-relevant host organisms for bio-based fuel and chemical production
- Partner with industry and other collaborators to develop pathways for products of interest in relevant host organisms (initial partnerships to begin in year 2, fully integrated development by the completion of year 3)

Within five years, the ABF will be able to develop more than 100 concurrent targets in their corresponding host organism, with a cycle time of less than three months. At year 5, the targets will include some retrosynthetic pathways (i.e. targets for which no existing pathway has been identified) and some targets of opportunity (i.e. beachhead molecules that have the potential for further transformation into more than 30 end products). By year 10, we envision that most ABF targets will be designed through biological retrosynthesis and that the ABF will have a full complement of targets of opportunity in multiple host organisms. Table 1 outlines some target performance metrics for the first five years of the ABF.

	FY17	FY18	FY19	5 years
Number of ABF hosts in operation	5	7	10	>20
Number of concurrent target/host combinations per year	5	15	30	>100
DBTL cycle time	9 months	8 months	6 months	<3 months
Strain samples analyzed per year	35,000	50,000	75,000	>100,000

Table 1: *Cycle time and capacity targets for the ABF*

ELEMENTS OF THE ABF CORE R&D MISSION

DESIGN-BUILD-TEST-LEARN CYCLE

Design is the ability to design bioprocesses for desired target, including the necessary workflows to build out pathways in a host organism and the needed testing to understand performance. R&D for design includes the development of computer assisted design software for biological pathways, databases of known expression systems, and the genetic tools available to build organisms. Design is further informed by iterations of techno-economic

analyses and life cycle assessments combined with overall process consideration that allow for ideal host organism selection.

Build encompasses the organismal chassis for the designed pathways, liquid handling and other automated methods of assembling the genetic systems for the pathways, and transformation capabilities to insert pathways into the organismal chassis.

Test includes the assays, instrumentation, and equipment necessary to understand how a designed pathway behaves in a host organism. While test is often thought of as assaying the performance of an organism under specific growth conditions, the ABF will incorporate process integration and scaling into Test. Just as it is important to understand how a pathway performs in its host, it is also important to understand how that designed organism fits into an integrated process, including the feedstocks used and upstream and downstream unit operations, including product isolation/separation. Additionally, understanding the performance at increasing scales is critical to understanding an organism's performance in the environments of fermenters or other reactors.

Learn is central to the activities of the ABF. One of the critical barriers still unaddressed in biological engineering is the ability to completely rationally improve upon design based upon data gathered in Test. Through machine learning, sophisticated statistical modeling, and metabolic flux analysis, this data will be translated into predictions that can be combined with TEA and LCA, process considerations, and host organism parameters to improve and predict the Design of future pathways and processes. As currently envisioned, the databases and information created through Learn will be made accessible to the community to share the knowledge gained and advance biomanufacturing broadly.

REQUIRED CAPABILITIES TO IMPROVE DBTL FOR INDUSTRIAL PROCESSES

Techno-economic analyses (TEA) and life cycle assessments (LCA) enable researchers to understand impacts beyond the organism on process considerations. These analyses define criteria around pathway and host organism selection that allow for improved economic feasibility and minimized environmental impacts for a bioprocess and help direct research towards elements of a process that most influence cost or environmental performance.

Process considerations are essential for the design of a robust bioprocess. The selected process includes many points where understanding the host organism and designed pathway are critical. Unit operations such as fermentation conditions and separations technologies are determined understanding how a particular pathway will behave in a selected host. Design can take potential contaminants, toxic byproducts, or any other number of factors into consideration to reduce the challenges inherent in integrating a process.

Process integration and scaling are critical for understanding strain performance in context of an overall bioprocess and its potential translation to an industrial setting. Distinct from just understanding how the process considerations influence Design, process integration and scaling test a process with all the relevant unit operations in sequence and at increasing scales. During process integration, the strain is grown at the appropriate scale using the relevant feedstock that has been formatted for the bioprocess and subjected to the appropriate pretreatment and saccharification process steps. Additionally, the downstream processing steps

including product separation, purification, and upgrading are incorporated to understand and identify problems, as well as to provide data for analysis activities.

Host organism selection is a key step in designing a bioprocess. Beyond impacting key production metrics like titer, rate, and yield, organism selection also affects pathway and process design. Organisms that perform well at high-temperature can offer savings on the energy required to maintain low temperatures in fermenters. Organisms that perform well at low or high pHs can tolerate production of specific molecules. While there are well-developed industrial host organisms, there is still a great need for further development, including genetic manipulation systems and growth conditions, of a range of host organisms that can act as chassis for a variety of diverse bioprocess pathways. The host selection for the ABF will focus on covering a range of product spaces through a diverse slate of organisms that have the potential for wide use in industry (or are already widely used but can use improvement).

Integration of these activities is key to the mission, goal, and success of the ABF. Currently, Design, Build, and Test are implemented and integrated at varying levels in industry depending upon the mission, business model, and resources of a company. Learn still remains a critical barrier, with only a few companies investing in technologies to use machine learning or statistical methods to predict better organism and pathway design. A truly integrated DBTL platform that incorporates an understanding of overall process design, TEA/LCA, and scaling does not yet exist in private industry. The ABF will leverage and unite capabilities to offer an integrated platform that is accessible to researchers at companies, in academia, and at the national labs for rapid and efficient engineering of biology.

R&D FOCUS OF THE ABF

The ABF platform will need to address the critical barriers that industry faces in order to develop a user base. To understand what these barriers are, as well as what and when they should be addressed by R&D within the ABF, the national labs involved in the consortium hosted an Industry Listening Workshop on March 15, 2016 in Berkeley, CA. Here we outline the R&D that the ABF will undertake, some of which was identified at the workshop. The full report from that workshop can be found in Appendix A. The R&D highlighted in the section will primarily be developed such that it can be integrated into the ABF platform. Whenever possible, the ABF will draw upon existing technologies and capabilities within the national labs.

DESIGN TECHNOLOGIES

Design begins with target selection, a process that, beyond chemical and biological expertise, includes market, techno-economic (TEA), and life-cycle analysis (LCA) as a means of developing a robust and prioritized list of molecular targets for the ABF to pursue. Since underlying datasets change over time, closely integrating and refreshing market analysis, TEA, and LCA together with bioprocess design is necessary. In collaboration with Integrated Analysis, Process Integration/Scaling, and the Industry Partnership Team, Design will demonstrate a design toolchain software architecture that enables push notifications (as proposed in the National Academy's Industrialization of Biology Roadmap), alerting the ABF as well as industrial partners when a bioprocess becomes viable. Many bioprocesses could achieve the same target. Beyond upstream (e.g., feedstock characteristics) and downstream (e.g., purification) possibilities, there are multiple conversion systems to consider, including different hosts, pathways, expression configurations, and bioreactor conditions. Design will demonstrate a design

toolchain software architecture that enables end-to-end bioprocess design (as proposed in the Industrialization of Biology Roadmap), to anticipate and exclude subcomponent designs that diminish overall bioprocess performance.

The proposed Design tasks will leverage and integrate unique and existing capabilities and software tools (including SMRC (Species Manipulation Relation Cultivation – Host Onboarding), ICE (Inventory of Composable Elements – Design/Build), DIVA (Design Integration Validation Automation – Design/Build), j5 (DNA assembly design automation – Design/Build), SPL (Sequence Polishing Library – Design/Build), SynTrack (Build workflow tracking), EDD (Experiment Data Depot – Test/Learn), and ArrowLand (multi-omics/flux visualization – Test/Learn/Design)), to build the design toolchain software architecture. A large portion of each Design task will continue to require hands-on contributions from domain experts until their knowledge is systematically codified into design software in later years. In collaboration with Learn, Design will support Build and Test by prioritizing designs that facilitate, maximize success rates, and minimizing the time and cost requirements of build and test procedures.

BUILD TECHNOLOGIES

Build will translate Design into biological reality, including DNA construction, the introduction of DNA into the host, and forward genetic screens to further modify target pathways and host metabolism toward higher titer, rate, and yield. Build will obtain information pertaining to host transformation and cultivation, developed by Host On-boarding and housed in the SMRC database, and use this information to engineer the selected host. Build tasks will include both genome modification of the host organism and introduction of the target bioproduct biosynthetic pathway. DNA construction will rely on Design to determine the optimal DNA construction strategy. Correctly assembled DNA constructs will be used for host engineering.

Build will use information provided by Design and Host Onboarding to develop, optimize, and implement standardize workflows for transformation and screening. Protocols for transformation and integration will be standardized across strains using landing pad technologies. Build will use the Integration systems scheduler to coordinate Build material exchange with Design and Test as well as coordinate builds that require sequential modification, i.e. genome modifications required before the target pathway can be introduced into the host. Build will coordinate with Test to supply strains and biomass for analysis. Since Build will inherently have established the capabilities for small scale cultivation, it will produce and supply material for initial Test validation of target molecule production, transcriptomic, proteomic, and metabolomic analysis. Once Test has validated the production of the target, Build can further modify the host genome and bioproduct pathway via forward genetic screens, developed by the Host Onboarding Team.

TEST TECHNOLOGIES

Test will assess the performance of newly built organisms to identify specific improvements that can be made in subsequent DBTL cycles, as well as provide data for TEA and LCA. Culturing options will be guided by maximum throughput and minimum sample size consistent with the need of subsequent analyses (e.g., BioLector platform) to potentially larger scale for second or third iterations of specific Crop testing. Test will conduct transcriptomic, proteomic and metabolomic analysis, applied in concert or individually, depending on the data needs of Learn. Transcriptomics will be used to quantitatively assess gene expression across stages (time points) of a bioprocess or between different conditions. Proteomics will quantify target pathway enzymes, and identify post-translational modifications. Metabolomics based on LC-MS, GC-MS, MALDI, NIMS and NMR techniques will be used to identify carbon flux bottlenecks. The majority of these capabilities are already in place and running at the

partner labs. In addition to multi-omic analysis, a number of specific assays for metabolites and specific enzymatic activities will be used *in vitro* or *in situ* for strain screening and selection. Moreover, we will leverage high throughput, whole cell selection and biosensor-based assays, coupled with high throughput metabolic flux perturbation approaches, to optimize metabolic flux toward high titers, rates, and yields. These assays will be based on different types of detection, sample volumes, and throughput.

Imaging capabilities will complement omics analyses and targeted assays. A vast array of light, spectroscopic imaging and particle based imaging capabilities are available at the national labs and provide the ability to examine organisms cultivated over a range of scales and different conditions. For some pathways, targeting enzymes to the proper cell compartment is crucial and imaging enables the assessment of expression and localization of enzymes. Imaging is also valuable for examining critical cell morphologies, and for detection and examination of contaminant organisms in bioprocesses. These imaging techniques are to be used only as needed for troubleshooting targeting or morphology issues.

LEARN TECHNOLOGIES

Learn will leverage Test phenotypic data to direct Design towards increased product titer, rate, and yield. This process will be carried out through statistical and/or mechanistic modeling, as determined by effectivity vs. time requirements (e.g., mechanistic modeling takes longer but may be required when statistical methods plateau). The Experiment Data Depot (EDD) will store and provide standardized data that can be fed into a variety of algorithms and improve current models. Statistical modeling will focus on pathway improvement by using machine learning algorithms applied to multi-omic Test data. Scikit-learn package algorithms will be used to predict production. We will then use these models to predict protein expression profiles that would increase production and work with biological part information to instantiate these optimal proteomics profiles. Mechanistic modeling will focus on intracellular flux analysis and pathway kinetic modeling. Flux analysis will elucidate carbon redistribution in the full host metabolism and identify bottlenecks that are dependent on the host, rather than the pathway. We will use ^{13}C labeling experiments to obtain accurate flux profiles and constrain genome-scale models. We will use these accurate profiles with COBRA algorithms to inform which host reactions are limiting production. Given the nascent nature of methods for predicting biological behavior, a significant fraction of Learn will be spent in leveraging Test data to improve current models. The large amounts of multi-omics data generated for the statistical approach will be used to parameterize ensemble kinetic models and provide part characterization for Design. In parallel, we will use the prior knowledge in the mechanistic models to constrain the feasible space for statistical models.

DBTL INTEGRATION

Integration will leverage and enhance INL's Bioenergy Feedstock Library capabilities to manage and track the logistics of samples shipped between the distributed components of the ABF via global unique identifiers (GUID) and resulting QR (quick response) barcodes. The logistical database will interconnect with other ABF databases including Inventory of Composable Elements (ICE) and the Experimental Data Depot (EDD) via RESTful API interfaces (with appropriate access controls) to connect samples with metadata. This integrated logistical sample management repository will enable distributed ABF operations to scale. To optimize the overall performance of the ABF, resource allocation (bandwidth and temporal ordering) must be under tight control. At the scale of the proposed distributed ABF, an automated solution to dynamic resource allocation is required. Integration will develop a systems-level model of the ABF (e.g., human and instrumentation resource bandwidth and time requirements for each component/operation). Integration will then develop a systems scheduler to automate resource allocation, and thereafter optimize the scheduler to continually improve overall ABF performance.

These performance metrics will be tracked and published as monthly dashboards to the ABF team in order to validate progress, identify bottlenecks, and resource needs relative to productivity.

INTEGRATED ANALYSIS

The ABF will apply an integrated analysis approach coupling market, economic, sustainability and feedstock metrics to evaluate potential target bioproducts and beachhead precursor molecules. Given the broad range of products under evaluation in the ABF effort, market analysis will be pursued to understand the commercial prospects and potential supply chains for the proposed products. This analysis will be critical for proposed beachhead molecules to examine the variety of markets that these platform chemicals could enter. To evaluate the potential impacts of these products, the team will utilize a range of resources for the market analysis including private consultant reports and trade magazines, as well as engaging stakeholders for guidance and reviews of any evaluations and assumptions. TEA will be utilized to identify process metrics and R&D needs for the development of economically viable production pathways for each molecule and will provide key data to support the LCA. These LCA analyses will consider GHG implications for the proposed molecules, as well as explore other sustainability drivers for production of these bio-derived products. Both TEAs and LCAs will be developed using consistent methodologies with other DOE-supported evaluations to ensure the analysis results are comparable and transparent. The Analysis team will work closely with the researchers to provide information needed for the integrated DBTL approach.

HOST ONBOARDING

New, industrially relevant hosts are needed to expand the economical production of target molecules. The Host Onboarding team will leverage national lab expertise in microbiology, molecular and cellular biology, genomics, bioinformatics, metabolic and biochemical engineering, TEA, LCA, multi-omics, and process design to identify new production hosts that have desirable traits and could have a large economic impact on target production and separations. We will also solicit input from industry and academia to identify promising hosts. Examples include organisms that naturally have substantial metabolic flux through critical metabolic pathways, that thrive in conditions that allow new processing conditions (e.g., extreme pH, salinity, temperature), that allow feedstock flexibility including the ability to co-utilize multiple carbon sources (e.g. sugars and lignin) simultaneously, that have diverse oxygen requirements, or that possess other novel and useful traits. Because diverse target molecules and varied processing schemes will require organisms with diverse properties, one of the goals of the Host Onboarding activity will be to cover as much of the relevant biomanufacturing space as possible with a broad pool of host traits.

In order to be fully onboarded into the DBTL pipeline, a certain level of basic tools and knowledge will be required to facilitate the cycle, and the onboarding process will involve meeting these criteria. The general host onboarding process for the first three years of the ABF is described in Figure 2. For each organism, molecular tools will be required, including a sequenced genome, DNA transformation methods, selectable and counter-selectable markers, deletion/overexpression/heterologous expression tools, and high throughput tools for genetic manipulation. The ability to characterize metabolic flux will also be critical, including a complete carbon and electron balance, a metabolic model construction, and ^{13}C fluxomics. This experimental work will primarily be performed within the DBTL task, with the exception of genetic tool development. The above criteria will be organized into tiers, which will describe minimal criteria to be met for onboarding (Tier 1), as well as additional levels (Tiers 2-4) that can be met over time for more advanced use of these hosts in DBTL. Furthermore, all of the information relevant to the onboarding process, including culture conditions, protocols and standard

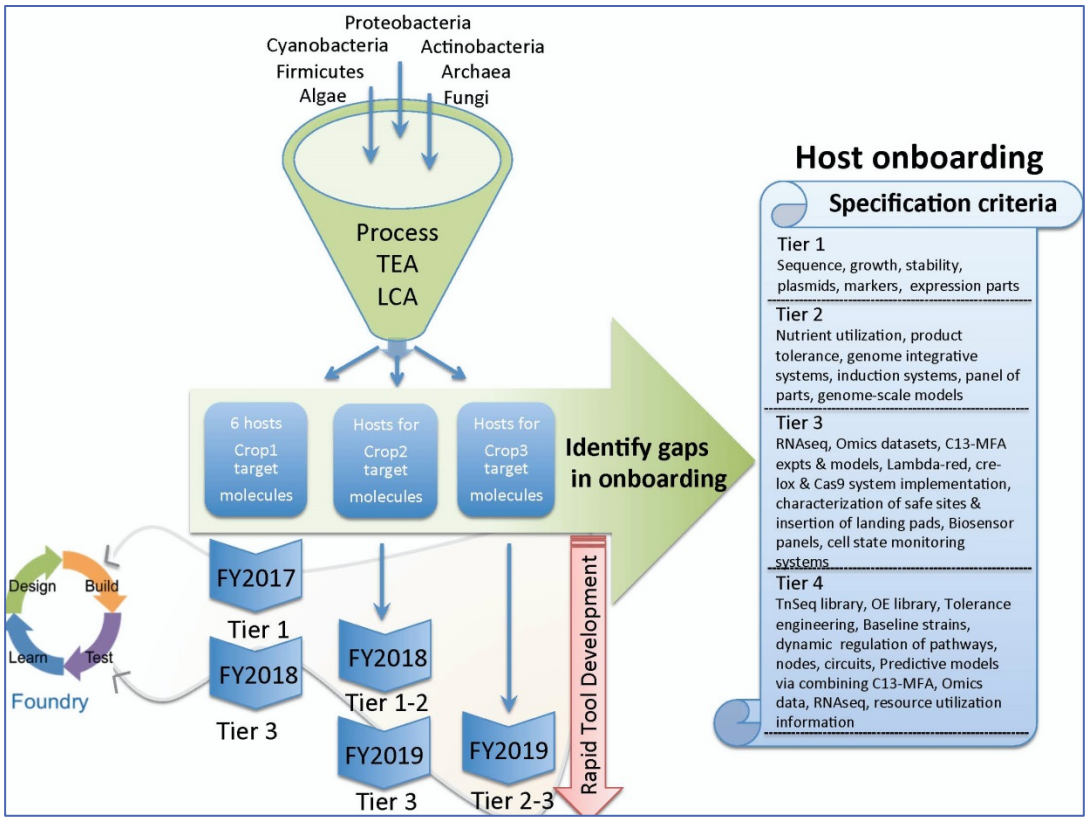

Figure 2. *The host onboarding process.*

operating procedures, and data collected, will be stored in the SMRC and related accessible databases that are tightly integrated with other ABF software tools and with other tasks.

PROCESS INTEGRATION AND SCALING

A primary output of the DBTL cycle, as well as a major component of Design and Test components of DBTL, involves process integration and scaling for the selected target molecules in the proposed ABF effort. This includes a focus on several key aspects including:

- The standardization, production, shipping, and storage of hydrolysates to be tested in DBTL
- The comparison of clean sugar processes with hydrolysates
- Fermentation testing and scaling (coupled to Test) to improve titer, rate, and yield
- Process integration (coupled to Design and Integrated Analysis) to provide integrated, bench-scale data for TEA and LCA
- Scaling of fermentation where necessary to produce data for the Learn component of the DBTL cycle

For a feedstock, the ABF will use corn stover. Currently funded efforts in feedstock handling and pre-processing will be leveraged to provide uniform corn stover compositions for reliable, repeatable biomass deconstruction. Advances from other DOE-funded efforts will be leveraged for improved feedstock properties as developed. Hydrolysate can be produced and shipped to ABF partners in 1-100 L quantities as needed for the Test component of DBTL. In addition, a major component of the Host

Onboarding process will rely on initial screening of candidate hosts with this biomass-derived hydrolysate.

Fermentation testing will be conducted as needed in the ABF when titer, rate, and yield measurements need to move beyond results obtainable in shake flask testing experiments. Most fermentation testing will be done at either small scale (e.g., in µ-scale multiplexed bioreactors) up to the 0.5 L scale (e.g., at LBNL's Advanced Biofuels Process Demonstration Unit or NREL's Integrated Biorefinery Research Facility). Fermentation optimization will be conducted on promising strains identified in shake flask trials, and when titer, rate, and yield improvements can be gained by moving to controlled bioreactors. These fermentation results will be crucial for both the Test and Learn components of the DBTL cycle, and 'omics measurements will be employed for bioreactor tests as well to identify metabolic bottlenecks and to inform Learn. Similar to typical efforts in bioprocess development, titer, rate, and yield in fermentation testing will be the primary objective. Scaling will occur beyond 0.5 L (up to 300 L) where needed for harvesting larger biomass samples (e.g., for transcriptomics experiments) and in cases where the Learn component of the DBTL cycle would benefit from scaling up (or down) for predictive scaling purposes, when titer, rate, and yield targets are reached at smaller scale, or when larger-scale production of a target molecule is needed for demonstration purposes.

LABS

The ABF platform will continuously incorporate new technologies that are ready to be onboarded. In order to do this, there will be a staging ground known as the Labs. This is where new technologies will be tested in the context of the platform before being integrated into workflows. This will allow the ABF to ensure that the technologies are properly vetted without diverting resources from normal operations.

STANDING UP THE ABF

To unite the capabilities of the national labs into the ABF, the U.S. Department of Energy's Bioenergy Technologies Office will fund an effort to unite the national labs' capabilities in this space beginning in fiscal year 2017. The goal of these activities is to assemble and demonstrate a functional DBTL cycle for multiple targets and organisms. In the first year, we propose to work with no more than 5 unique organisms and related pathways for the production of at least 5 unique molecular targets. We have identified two organisms, *Streptomyces venzuelae* and *Pseudomonas putida,* to serve as the foundational ABF platforms. Up to three other organisms will be identified by the ABF team, based on genomic maturity and tools, in the first three months of the project. These other hosts may include model organisms, such as *Escherichia coli* or *Saccharomyces cerevisiae* if they prove to be the best hosts for specific target molecules. For this effort, molecules will be grouped for staging through the Foundry, by risk, metabolic pathways, and market analysis. Initial groups will focus on low-risk targets that can be used to build the integrated ABF infrastructure, while later groups will focus on high-impact targets to enable industry. The later groups may include "targets of opportunity," beachhead molecules that allow for many further transformations via biological and chemical upgrading to maximize the potential market opportunities for the targets to enable industry. After the initial 18 months of the project, the ABF platform will be fully integrated and will be able to engage in industry collaborations to develop targets of interest. Industry collaborations before this period will be focused on instrumentation or on development of host organisms to develop technologies needed for an integrated DBTL platform.

MANAGEMENT OF THE ABF

The ABF will be a distributed consortium of national labs. While some elements may be co-located for convenience (e.g., Build technologies that work most efficiently when sited together), others may be geographically dispersed to leverage existing capabilities, facilities, and capital investments.

The ABF aims to have a significant industry-facing component that will perform collaborative research with interested partners. The ABF will operate in a similar fashion to other EERE-industry collaborative efforts, such as the Advanced Biofuels Process Demonstration Unit, the Integrated Biorefinery Research Facility, and others. The ABF will not be a formal User Facility as defined by DOE.

MANAGEMENT STRUCTURE

The ABF will have an Executive Committee composed of members representing each of the partner national labs. The Executive Committee will be responsible for the overall strategy and setting the policies of the ABF, work with the Project Management and Integration team to ensure progress, and the Industry Partnerships team, and work with the Industry Advisory Board to ensure that the ABF is continually addressing challenges faced by industry.

Day-to-day operations of the ABF will be the responsibility of the Project Management and Integration team. This team will be responsible for managing the activities of the various technical teams, as well as ensuring that technical teams are working together. This team will also be responsible for developing the integration of individual technologies. Finally, this team will manage the scheduling of projects through the ABF to ensure that resources and time are allocated appropriately, new technologies are continually evaluated and onboarded, and potential workflow impedances are understood, mitigated, and managed.

The technical teams (Targets, Hosts, Design, Build, Test, Learn, and Labs) will be performing the work of the ABF. The Targets team will evaluate exemplar molecules for the ABF and provide TEA, LCA, and process considerations for design. The Hosts team will evaluate host organisms for use at the ABF, define the needed requirements for onboarding, and fill in gaps in those requirements. The Design, Build, Test, and Learn teams will be the technical experts in their associated technologies and executing the R&D for ABF projects. The Labs team will be continually testing new and emerging technologies to ensure that they can be onboarded into the platform without impeding the normal operations of the ABF.

The Industry Partnerships team will be responsible for developing relationships with industry partners, developing scopes of work with the industry partners and the Project Management and Integration team, and handling the intellectual property agreements between the ABF and its industry partners. This team will report to the Executive Committee and will work closely with the Project Management and Integration team to ensure effective partnerships with companies. To facilitate interactions with industry, the ABF will have a single point of contact, or concierge, to manage interactions between the labs' intellectual property offices. The ABF will also maintain a public website that contains information about working with the ABF, along with recent publications, patents, and other disclosures of work, to aid in dissemination of ABF R&D products to interested stakeholders. In order to disseminate its progress to the wider research community, the ABF will host workshops and webinars regularly.

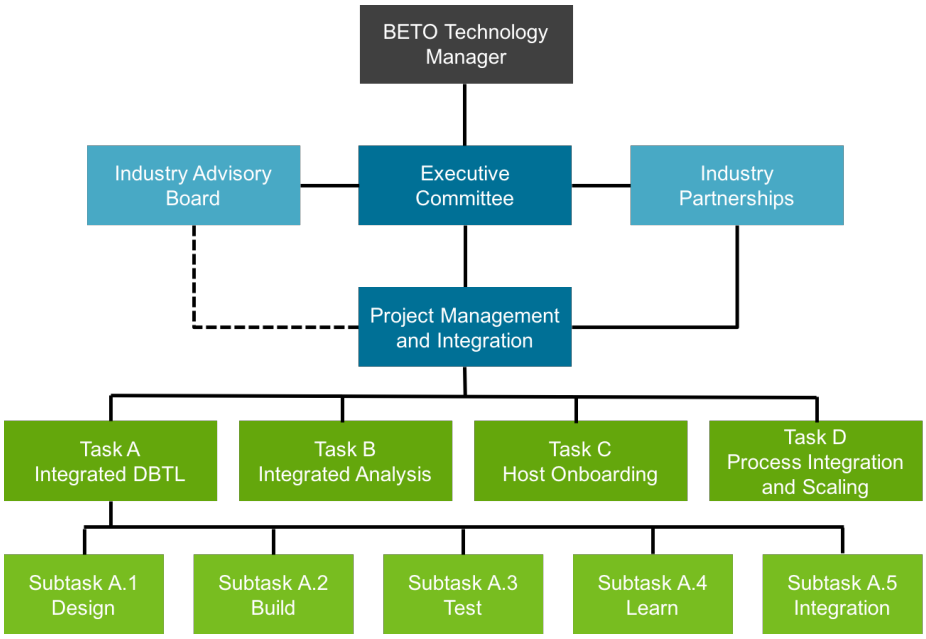

Figure 3. *Proposed management structure for the ABF.*

INDUSTRY ADVISORY BOARD

To ensure that the ABF addresses the needs of companies in the bio-based fuels and chemicals industry, an Industry Advisory Board (IAB) will be established. The IAB will work closely with the Executive Committee to establish strategic goals for the ABF. The IAB will also ensure that the ABF is addressing the critical barriers to rapid, cost-effective, and efficient biological engineering for industrial processes to remain relevant to its industry stakeholders. The IAB could also provide strategic direction for potential opportunities offered through the ABF, such as voucher programs, that allow for companies to compete for sponsored projects through the ABF.

INTELLECTUAL PROPERTY AND WORKING WITH INDUSTRY

INTELLECTUAL PROPERTY PRINCIPLES

Whenever possible, the ABF intends for intellectual property (IP) generated by the national lab partners or with public funds to be accessible to the larger community. The national labs will work together to establish a portfolio of background IP that can be brought to bear in standing up the ABF. The labs will establish a streamlined process for managing IP developed under the ABF. The ABF Industry Partnerships team will investigate models for accessible IP and develop methods of licensing ABF IP to ensure that it is minimally encumbered for use by industry. In general, the ABF will protect IP it generates through non-exclusive licenses to maintain accessibility for the broadest spectrum of stakeholders. For specific interactions with companies, the IP will follow the process of inventorship outlined in the specific agreement.

The preferred IP management model for industry is a single point of contact and a streamlined set of agreements for partnering with the ABF. The Industry Partnerships team will lead up the IP management activities. To facilitate single point-of-contact IP management, the ABF may reserve some

funds for IP management. The ABF will use these funds for patent prosecution and IP licensing. In this model, the Executive Committee, in consultation with the Industry Partnerships team, will decide which IP will be patented and licensed. If the ABF declines to prosecute a patent, the national lab(s) that developed the IP will be able to prosecute if desired.

The ABF will have streamlined agreements for industry projects. Companies that wish to pursue Cooperative Research and Development Agreements (CRADAs) or Work for Others agreements (WFOs) will work with the Industry Partnerships team to develop statements of work for ABF projects that apply to all labs in the consortium. Additionally, Materials Transfer Agreements and Non-Disclosure Agreements will be standardized and apply to all labs in the ABF. It is expected that the first industry partners for the ABF will be focused on individual elements of technology development, including instrumentation, with an integrated platform available for specific target development ready for industry collaborations within two years of standing up the ABF. By the end of year three, the ABF will have the capacity pursue multiple industry-led targets.

At the Industry Listening Workshop, companies expressed interest in creative mechanisms for funding CRADA or WFO projects with the ABF. Besides standard cost-recovery models, the ABF will investigate a voucher program, similar to the Small Business Voucher program, with its EERE sponsors. A voucher program would allow companies to compete for EERE-sponsored projects with the ABF with minimal cost share. Industry members also expressed an interest in cooperative technology development activities with the ABF to develop new DBTL technologies. In this case, the Executive Committee would consult with the IAB to develop strategic opportunities in this space that could be funded by a voucher program or a competitive solicitation with the ABF's EERE sponsors.

Companies may also be interested in testing or learning about the ABF platform prior to licensing and bringing technologies in house. To facilitate that, the ABF could host visiting researchers from private industry. In these cases, the companies would receive a non-exclusive license to the platform technologies after a specified duration of training.

DATA MANAGEMENT

One of the first management tasks for the ABF will be to define a data management plan. While this plan will require negotiation between the labs' intellectual property offices, some general principles can be identified. Data resulting from publicly-funded R&D at the ABF will be made accessible to stakeholders as much as possible through standardized databases and software. The data will be quality controlled to ensure accuracy. Companies that work with the ABF will have the option to make their data available for improving Learn capabilities. Should a company choose not to share its data, any data and/or IP generated during a project will be firewalled from the public-facing data.

REPORTING

The ABF intends to be an open and accessible resource for the rapid, cost-effective biological engineering. To that end, the ABF will report regularly on its progress in a transparent manner. Regular reports will be made to EERE through the Annual Operating Plan process and its associated reporting structure of quarterly reports. Results from publicly funded projects will be published in scientific and technical journals, as well as highlighted on a public website. Available licensing opportunities will also be published on the website.

The Industrial Advisory Board will be convened on a quarterly basis. The Executive Committee and ABF staff will report out on progress, new strategic foci, and other business to the IAB. The IAB reports will be available to EERE following the meetings.

The ABF will convene an all-hands retreat on an annual basis to facilitate interactions between staff. EERE and IAB stakeholders will also be invited to attend the annual meeting. This will provide an opportunity for ABF leadership to update on progress, future directions, and highlight ABF successes. It will also serve as an opportunity for staff in the distributed consortium to meet and network, facilitating improvements in the integration of activities and identification of new R&D directions.

CONCLUSION

This document outlines a framework of operations for the Agile BioFoundry of national labs, including the R&D foci for the ABF and a proposed management structure. This document is informed by discussions with industry stakeholders, EERE staff, and staff from the national labs. Further engagement with industry, other Federal government stakeholders, academic researchers, and the community will be sought out to improve the framework.