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Agile BioFoundry

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

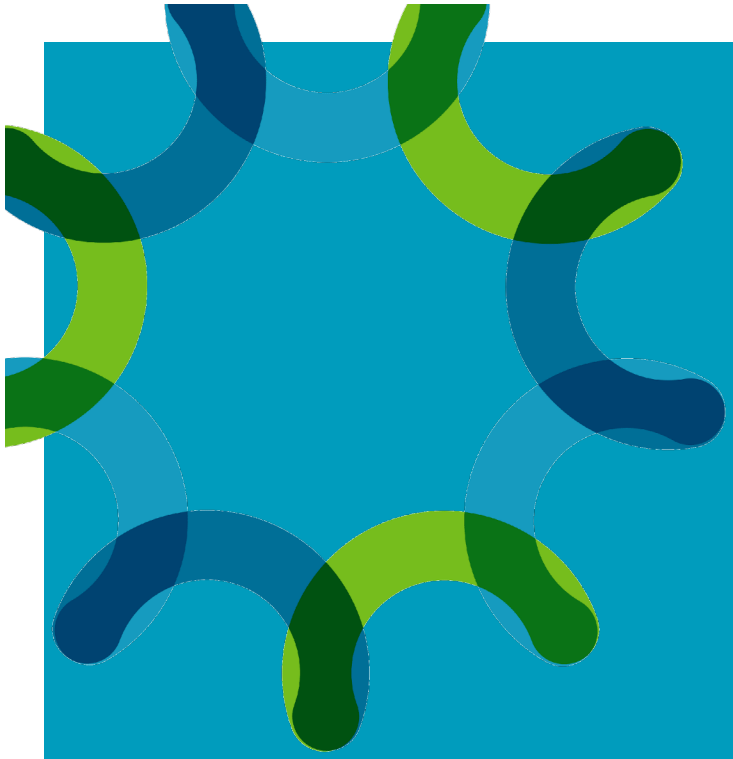
BIOENERGY TECHNOLOGIES OFFICE

ABF DBTL Infrastructure

Nathan J. Hillson

Principal Investigator, DOE Agile BioFoundry

BETO Peer Review 2021
Conversion Technologies
11:45AM-12:15PM EST
March 9, 2021



Project Overview

Goal - Overall ABF

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

- **Outcomes**: Development and deployment of technologies enabling commercially relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts

- **Relevance**: $20M/year public infrastructure investment that increases U.S. industrial competitiveness and enables opportunities for private sector growth and jobs

- **Risks**: Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from

Goal - DBTL Infrastructure

- **Goal**: Design, implement, operationalize, and maintain Design-Build-Test-Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF Tasks and enables the overall ABF goal

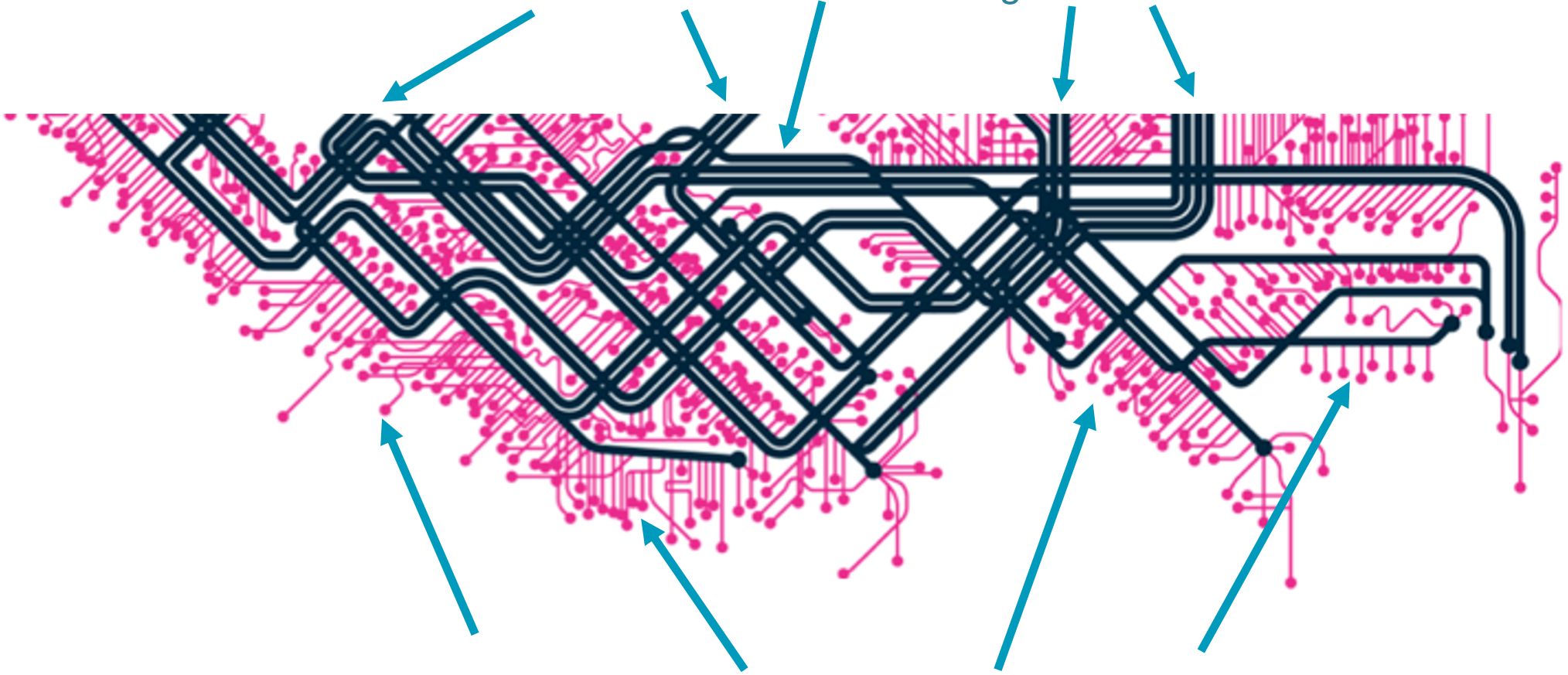
- **Outcomes**: 10X improvement in Design-Build-Test-Learn cycle efficiency, new IP and manufacturing technologies demonstrated and ready for translation to U.S. industry

- **Relevance**: Public infrastructure investment that supports the ABF and other BETO projects, and that can be leveraged by U.S. industry

- **Risks**: Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from

Public Infrastructure Investment Enables Private Industry

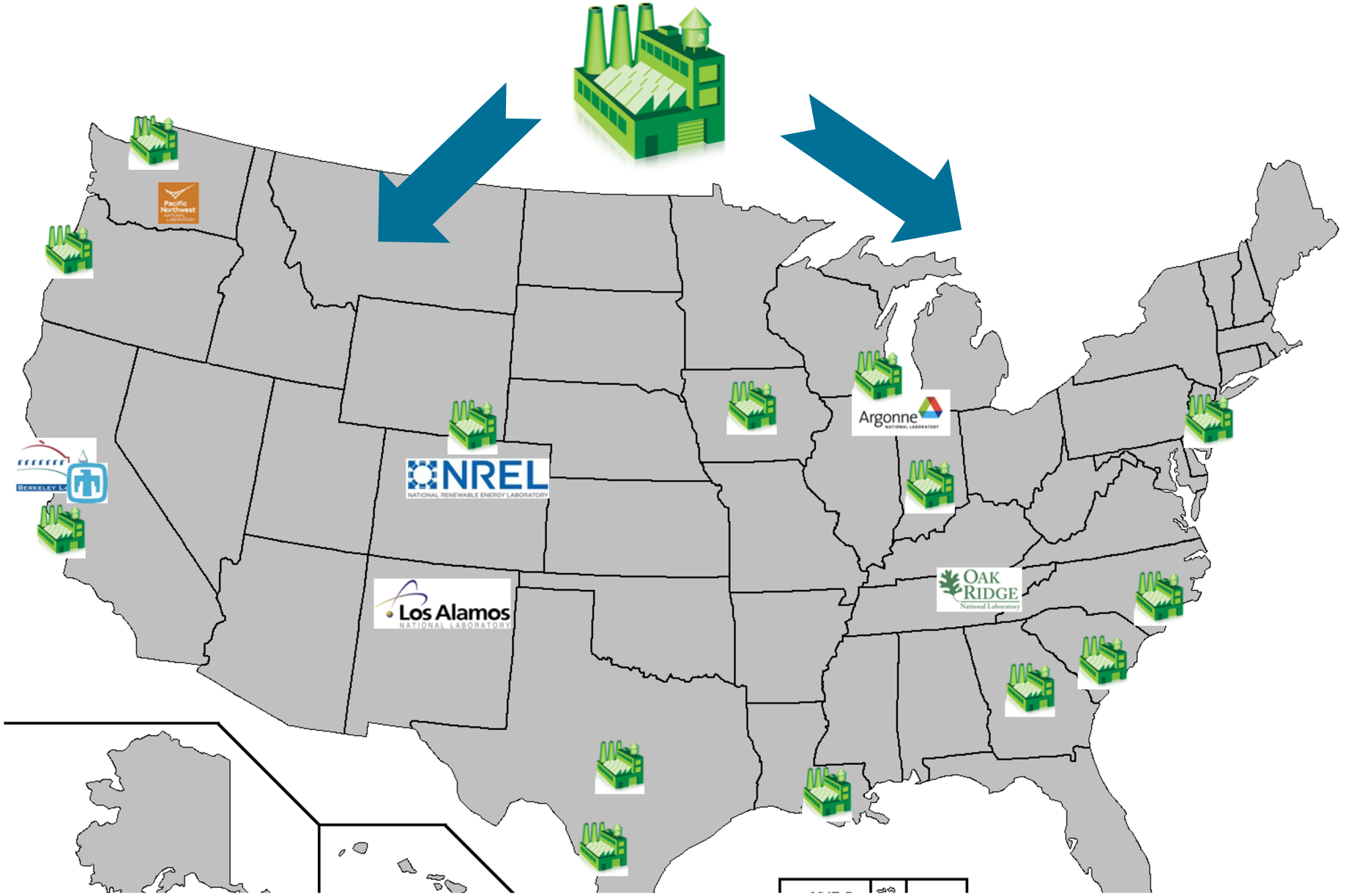
Public investment in biomanufacturing infrastructure



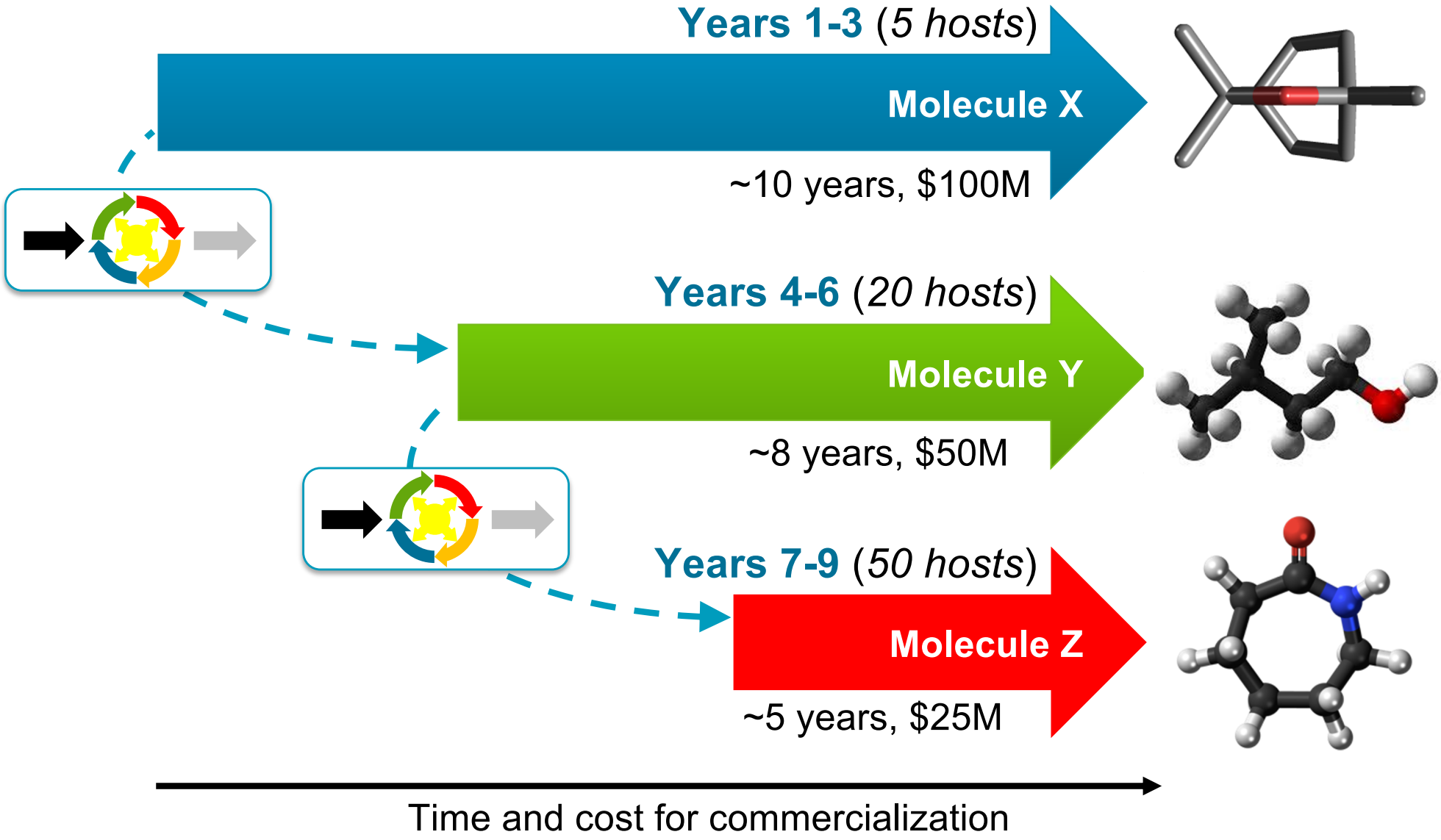
Private investment in product development, scaling, and tailoring to unique pathways and products

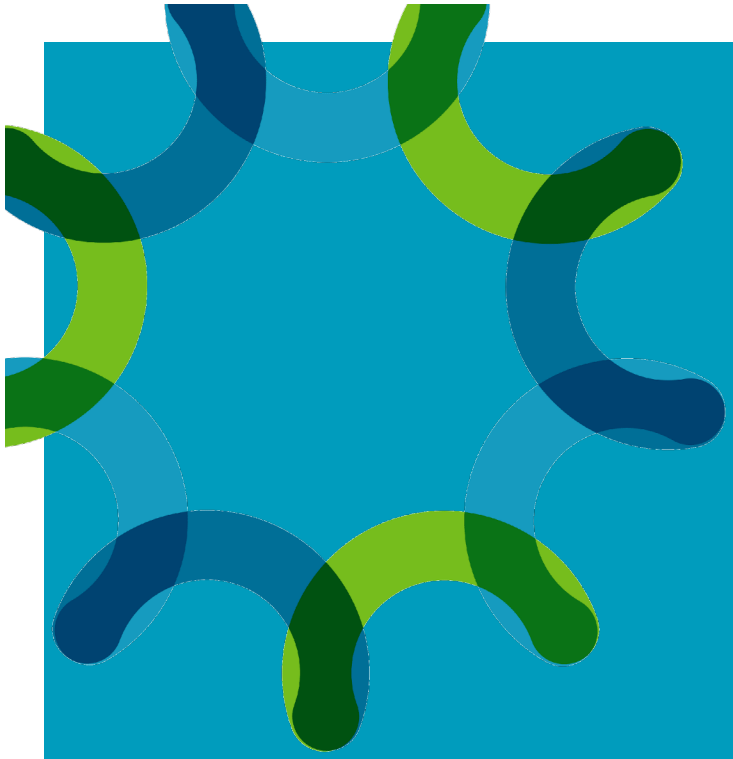
Adapted from Lyft

A Distributed Agile BioFoundry



Agile BioFoundry Will Reduce Time-to-Scale up



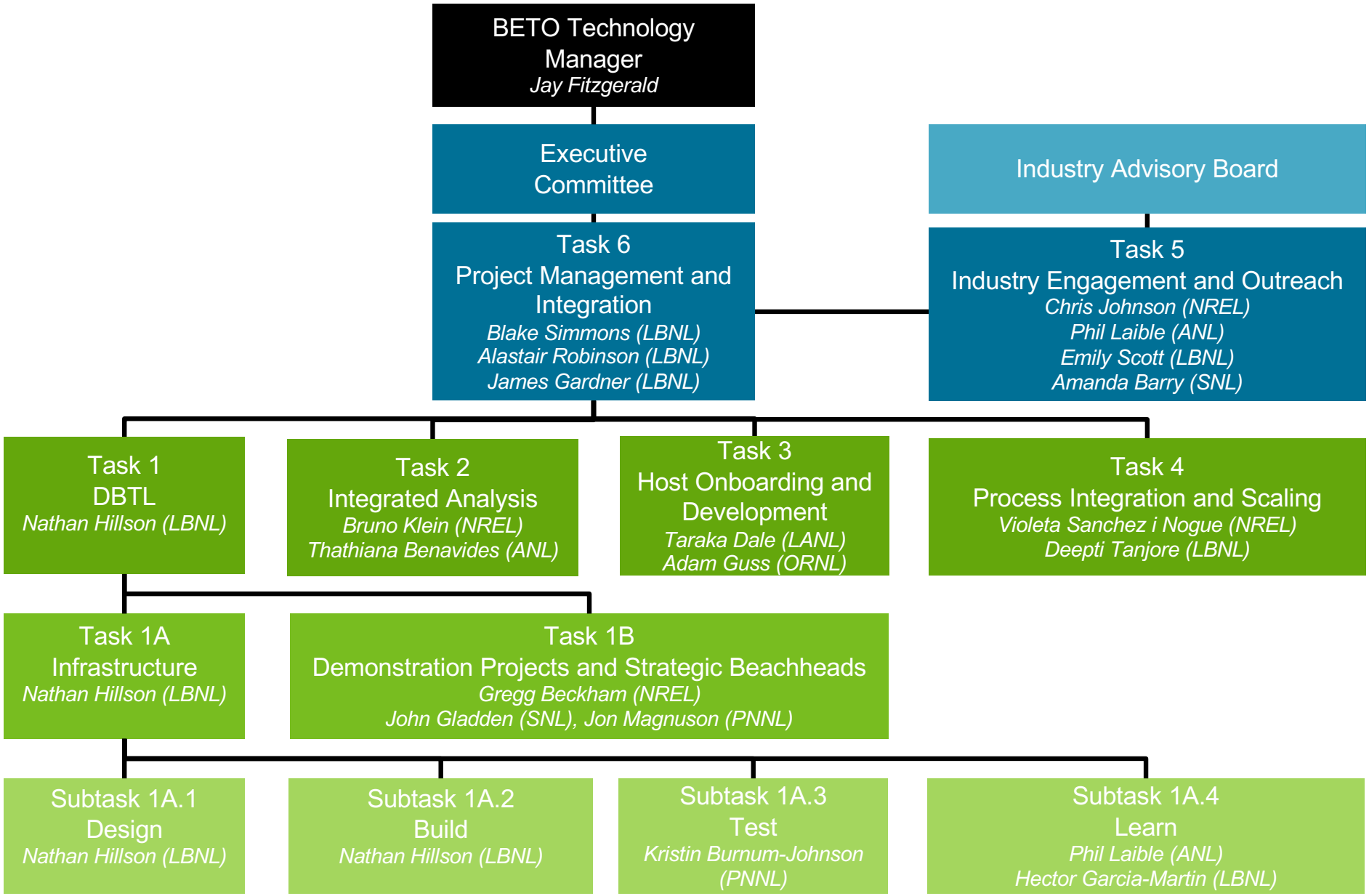


1 - Management

Six Tasks

- **Task 1: Design-Build-Test-Learn** (*Nathan Hillson* - lead)
  - **Infrastructure**: Integrate design-build-test-learn cycle with process automation
  - Demonstration Projects and Strategic Beachheads: Demonstrate uses of DBTL infrastructure and establish and improve routes in microbial hosts to beachhead molecules of high strategic interest
- **Task 2: Integrated Analysis** (*Bruno Klein / Thathiana Benavides* – co-leads)
  - Analyze proposed target and beachhead molecules with TEA and LCA methodologies
- **Task 3: Host Onboarding & Development** (*Taraka Dale* / *Adam Guss* – co-leads)
  - Onboard additional microbial host organisms and further develop them to higher capability tiers through tool development and data collection
- **Task 4: Process Integration & Scale-up** (*Violeta Sanchez i Nogue / Deepti Tanjore* – co-leads)
  - Provide DMR-EH hydrolysates, and test and scale fermentation to improve titer, rate, and yield
- **Task 5: Industry Engagement & Outreach**
  (*Chris Johnson / Phil Laible / Emily Scott / Amanda Barry* – co-leads)
  - Identify barriers to industry adoption of ABF technologies, expand number and diversity of industry partnerships, and establish a set of metrics for determining impact of ABF technologies on industry
- **Task 6: Management** (*Blake Simmons* - lead)
  - Manage project management, develop internal and external communications, provide deliverables to BETO, and make capital equipment purchases

Org Chart


BETO Technology Manager
Jay Fitzgerald
Executive Committee
Industry Advisory Board
Task 6
Project Management and Integration
Blake Simmons (LBNL)
Alastair Robinson (LBNL)
James Gardner (LBNL)
Task 5
Industry Engagement and Outreach
Chris Johnson (NREL)
Phil Laible (ANL)
Emily Scott (LBNL)
Amanda Barry (SNL)
Task 1
DBTL
Nathan Hillson (LBNL)
Task 2
Integrated Analysis
Bruno Klein (NREL)
Thathiana Benavides (ANL)
Task 3
Host Onboarding and Development
Taraka Dale (LANL)
Adam Guss (ORNL)
Task 4
Process Integration and Scaling
Violeta Sanchez i Nogue (NREL)
Deepti Tanjore (LBNL)
Task 1A
Infrastructure
Nathan Hillson (LBNL)
Task 1B
Demonstration Projects and Strategic Beachheads
Gregg Beckham (NREL)
John Gladden (SNL), Jon Magnuson (PNNL)
Subtask 1A.1
Design
Nathan Hillson (LBNL)
Subtask 1A.2
Build
Nathan Hillson (LBNL)
Subtask 1A.3
Test
Kristin Burnum-Johnson (PNNL)
Subtask 1A.4
Learn
Phil Laible (ANL)
Hector Garcia-Martin (LBNL)

Communications

- **ABF is an integrated, geographically distributed multi-Lab team**
  - Effective communications are essential

- **Internal**
  - Bi-weekly Executive Committee meetings
  - Bi-weekly ABF Task Lead meetings
  - Weekly to monthly demonstration project/beachhead meetings
  - Weekly software and automation infrastructure user meetings / webinars
  - Monthly activity summary including DBTL cycle reports to BETO
  - Monthly Host Onboarding and Development Task team meetings
  - Monthly Learn team meetings - activities and milestone planning
  - Monthly Industry Outreach and Engagement Task team meetings
  - Quarterly progress / milestone completion reports to BETO
  - Software infrastructure (e.g. ICE, DIVA, EDD, LabKey, AgileBioCyc, Jupyter, github/bitbucket, etc.)
  - Google Platforms – file storage and sharing
  - Annual Learn Summit
  - Annual ABF All-Hands Meeting

- **External**
  - ABF website (agilebiofoundry.org) and social media (@agilebiofoundry)
  - Presentations, posters, booths at domestic and international scientific / technical conferences
  - Publications
  - Quarterly Industry Advisory Board meetings and Industry Listening Days
  - Annual Global BioFoundry Alliance meeting, and monthly webinar series

Technical Risks and Mitigation Plans

Risk	Severity	Description	Mitigation Plan
Distributed model inefficiencies	Low	Important to consider the effects a distributed model has on the ABF's goals	Monitor and minimize DBTL cycle delays or other inefficiencies due to distributed operations
Insufficient data to fully leverage Learn	Medium	Multi-omics datasets may not be of the quality, quantity, or consistency needed for statistical analysis to identify engineering targets that lead to gains in titers, rates, and yields	Explicitly include the Learn team during the Design process to ensure suitability of generated data
Infrastructure operating costs and value	Low	Costs of infrastructure (both hardware and software) maintenance and asset depreciation becomes unsustainable	Offload maintenance to more cost-effective and sustainable off-the-shelf vendor-supported solutions where possible
Lack of target/host transferability	Medium	Not able to leverage learnings from one demonstration project/ beachhead in work for another	Further Learn the extents/likelihood of transferability
Designs do not work in selected host	Medium	Promoters/enzymes/pathways do not function as intended in the selected host	Further Test and Learn from lack of function, and suggest Design changes to restore function

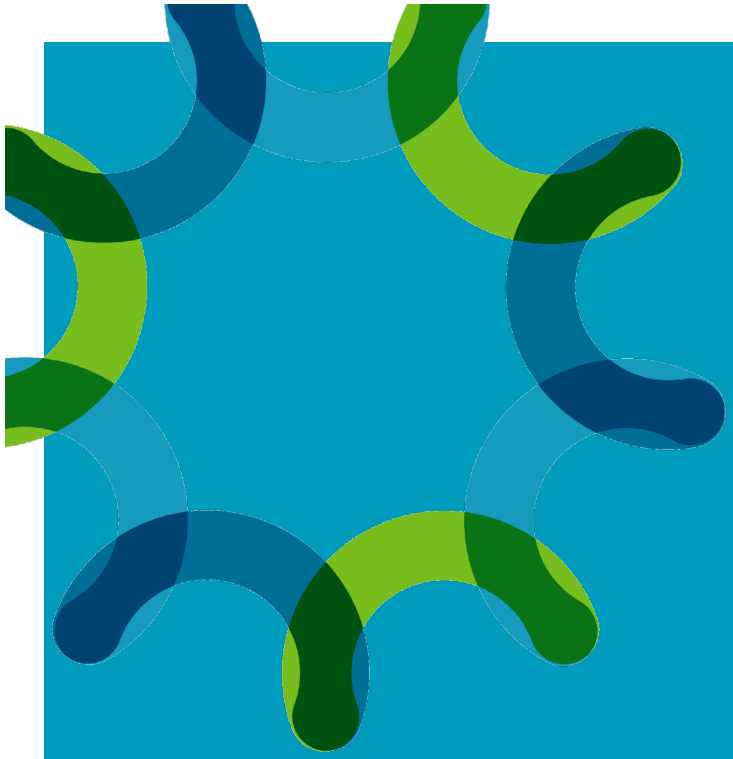
Collaboration with Related Projects and Advisory Boards

- **Other BETO consortia / projects and BETO State Of Technology (SOT)**
  - Other BETO projects could leverage Agile BioFoundry capabilities:
    - Methods, workflows, instrumentation, software, expertise
    - Accumulated enzyme/pathway/host/process Learnings and data
  - BEEPS FOA: DNAda software (supporting DNA construction) collaborations
  - CCPC (BPMS): Bayesian inference of metabolic kinetics collaborations
  - Improve genetic tools for SOT organisms to accelerate & increase DBTL cycle efficiency

- **Other DOE programs**
  - Energy I-Corp: real-time data for in-line process control and predictive scale-up studies
  - BRCs and EFRCs:
    - Target/host suggestions for ABF; technology off-ramping into ABF
    - Shared technical challenges collaboratively addressed (e.g. DNAda/EDD)
    - Provide compelling examples of DOE teams working together
    - Enhance technology transfer and commercialization efforts

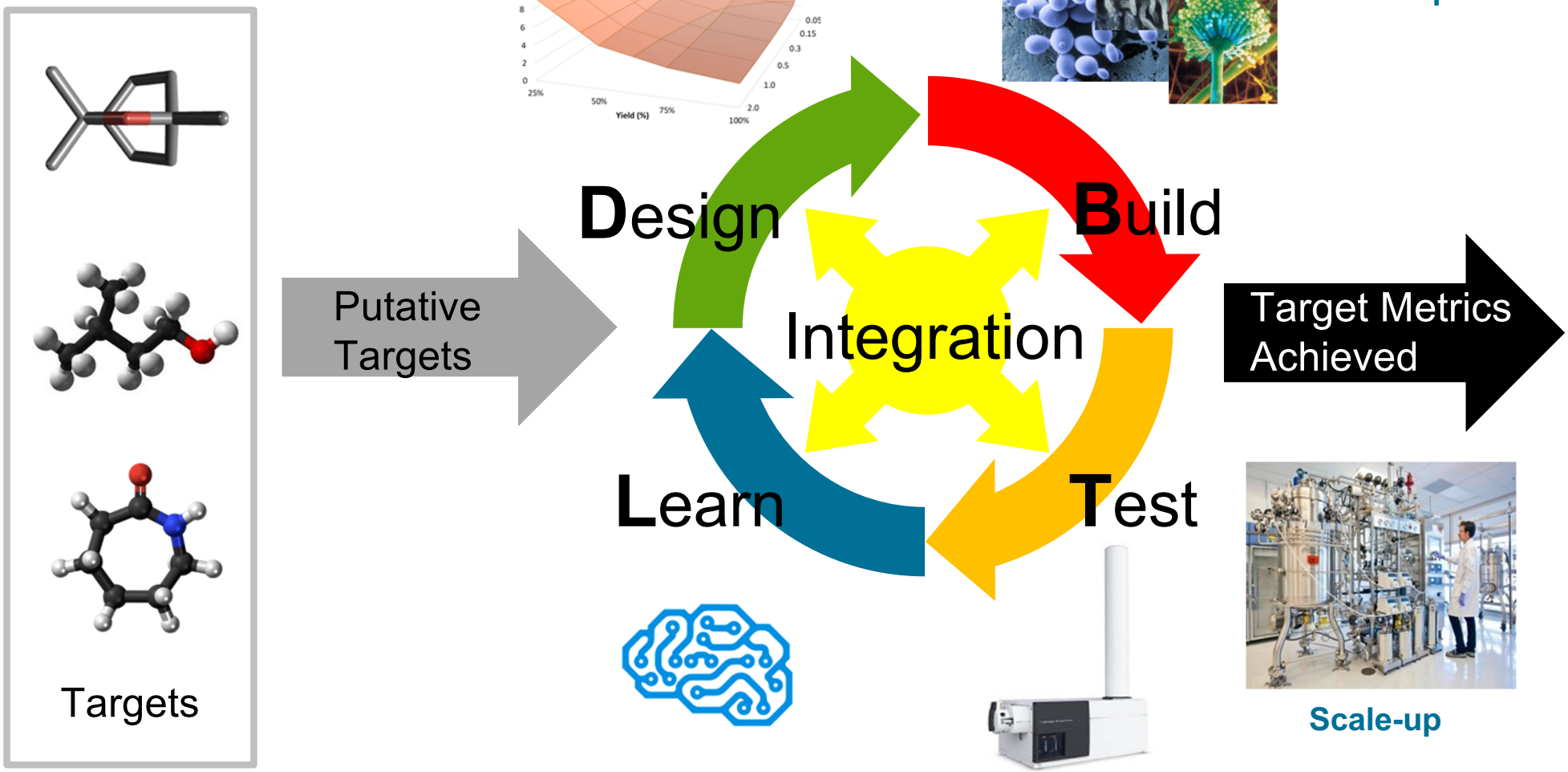
- **Global Biofoundries Alliance:** Software and Metrology/Standards working groups

- **ABF Industry Advisory Board:** Provides guidance relevant to DBTL infrastructure



2 - Approach

The Agile BioFoundry Approach



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What makes ABF different than other BETO-funded metabolic engineering projects?

- The ABF has highly collaborative teams that work together to:
  - Move target / host pairs and strategic beachheads through the pipeline
  - Build the tools and infrastructure to do so
  - More closely mirror industry in terms of breaking effort into domains (e.g. Test team)

- Strategic focus on analysis of and routes through beachhead molecules
  - Requires innovation in TEA/LCA analysis (e.g. via exemplar molecules)
  - Goal is to maximize flux through, as opposed to accumulation of, the beachhead molecule

- **Learn component**

- **Infrastructure to support scale / throughput / depth of analysis / Learn**

- Integrated whole that might be separated in other projects
  - Including Integrated Analysis (TEA/LCA), Host Onboarding and Development, Scale-up

Changes made in light of 2019 Peer Review

- **DBTL cycle specification and efficiency metrics**:
  - Specification: a concrete DBTL cycle specification is now in place
  - Metrics capture: we are increasingly automatically capturing efficiency metrics in our workflow-supporting software infrastructure
  - Software usability: we are engaging a software firm to improve the user interface and user experience of our software to reduce friction and improve efficiency metrics reporting accuracy and completeness
  - Quantitative evaluation: through the Platonic approach, we will be quantitatively estimating efficiency improvements made and identifying the most opportune unit operations(s) to further improve

Top 2 potential challenges

- **Leverage past collaboration learnings with future collaborators**
  - Only portions of past collaborative data or learning methods that do not reveal the underlying primary data may be available

- **Predictive scale-up, and method transferability/reproducibility**
  - Our lack of ability to predict how a process will scale, or how well a method can be transferred across facilities, may limit the impact of our research and development efforts

Go/no-go decision points

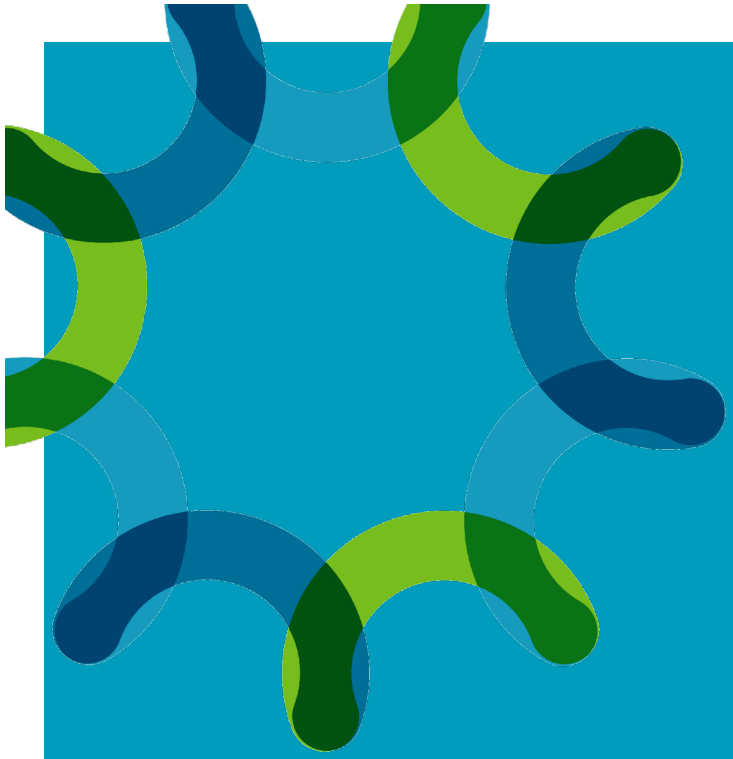
- **Date**: 3/31/2021

- **Description**: 5 target molecules or tools transferred between host organisms that are able to at least achieve 1 g/L or higher in the first host. Successful target molecule transfers will have product titers greater than 1 g/L. For 3 of 5 of these, 2X biological engineering cycle efficiency gains demonstrated over attempts made in prior host organisms

Target Molecule/Tool	Original Host	Transfer Host (s)	Efficiency gain
3HP	*A. pseudoterreus*	*A. niger; R. toruloides*	9X; 6X
Muconate	*P. putida*	*C. glutamicum*	In progress
Beta ketoadipate	*P. putida*	*C. glutamicum*	In progress
Muconate biosensor	*P. putida*	*C. glutamicum*	6X
Microfluidic screening	*P. putida*	*C. glutamicum; Rhodobacter*	2X; 2X
Integration tools	*P. putida*	*C. necator*	10X
Fungal transporters	*A. pseudoterreus*	*R. toruloides*	9X

Economic/technical metrics

- **DBTL and tool/target transfer efficiency**:
  - Efficiency: unit operations or objectives achieved, per time (wall/clock), per resource (human/instrument)
  - Platonic DBTL cycle: efficiency estimated from underlying unit operations



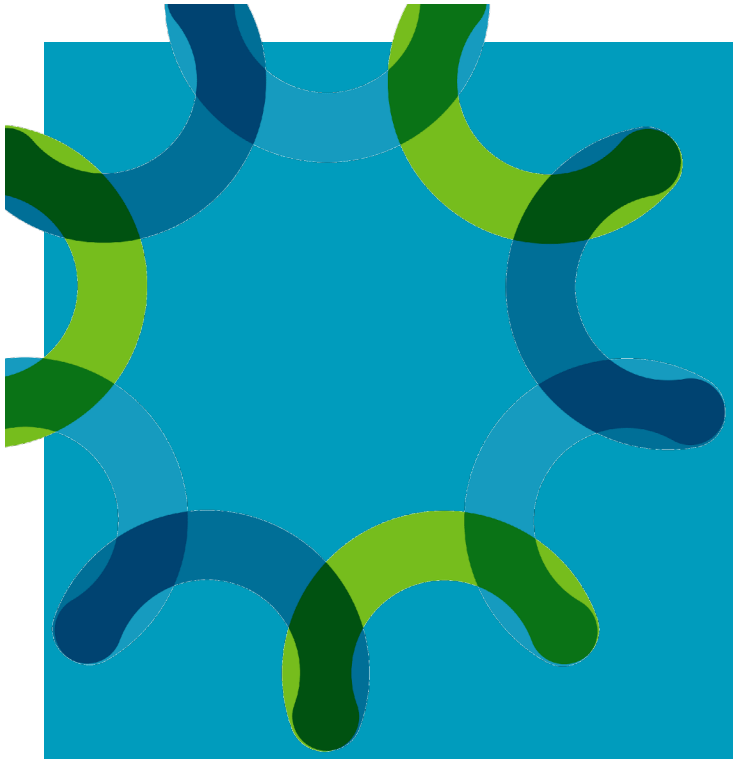
3 - Impact

Impact Highlights

- **Impact on state of technology/industry if successful**:
  - Accelerated biomanufacturing commercialization
  - No need to re-establish metabolic routes and hosts
  - Likelihood assessments / demonstrations of process transfer
  - Increased access to broadly enabling DBTL infrastructure

- **How disseminating results**:
  - 250+ citations across 50 publications to date (since FY17)
  - 67 citations across 17 publications since FY20
  - 5.91 impact factor
  - 6 records of invention, 7 software disclosures, 16 patent applications, 5 licenses
  - Reducing barriers to commercialization

- **Memoranda of Understanding (pending)**:
  - NSF and DOE
  - Global Biofoundries Alliance



4 - Progress and Outcomes

Progress made towards goals

- **Acceleration of biomanufacturing commercialization**:
  - Collaboration: Industrial and academic collaboration projects will be presented the over the next two days. As leading indicators, progress therein is very promising (e.g., Lygos - 20X increase in isobutyrate titers)! Through the outcomes of these collaborations over time, the ABF endeavors to definitively establish end-to-end impacts on time from bioprocess conception to scale-up and commercialization
  - Internal: The ABF, as assessed through target/tool transfer and DBTL efficiency metrics, along with established beachheads and hosts, is itself making good progress towards this goal

- **Broadly enabling DBTL infrastructure**
  - The following slides will offer concise highlights thereof

- **Assessments and demonstrations of bioprocess transferability**
  - Target and Host Engineering, and the Host Onboarding and Development ABF presentations are later this afternoon
  - These subsequent presentations will further detail our progress

DBTL infrastructure

DiVA

DNAda

DNA Construction

Design

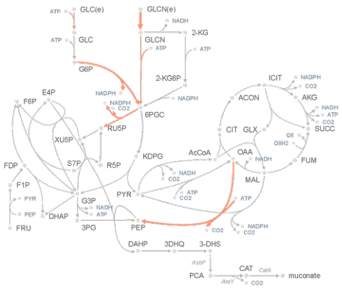
Build

Integration

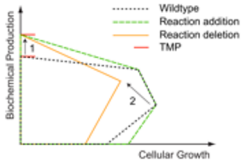
Learn

Test

art



Bayesian Inference: Metabolic Kinetics



Metabolic Network Modeling

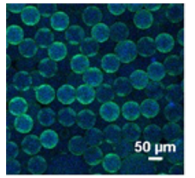
Deep Learning

EDD

LabKey

Automation

Sequence Validation



Biosensors + Cytometry/ Microfluidics

Scale-up

Transcriptomics, Metabolomics, Proteomics, Lipidomics

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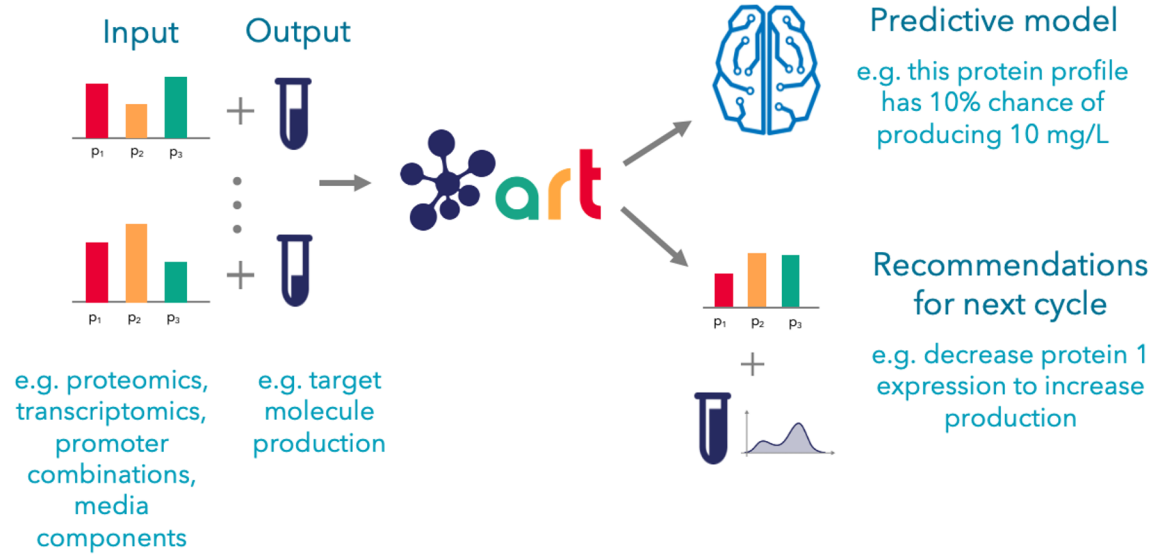
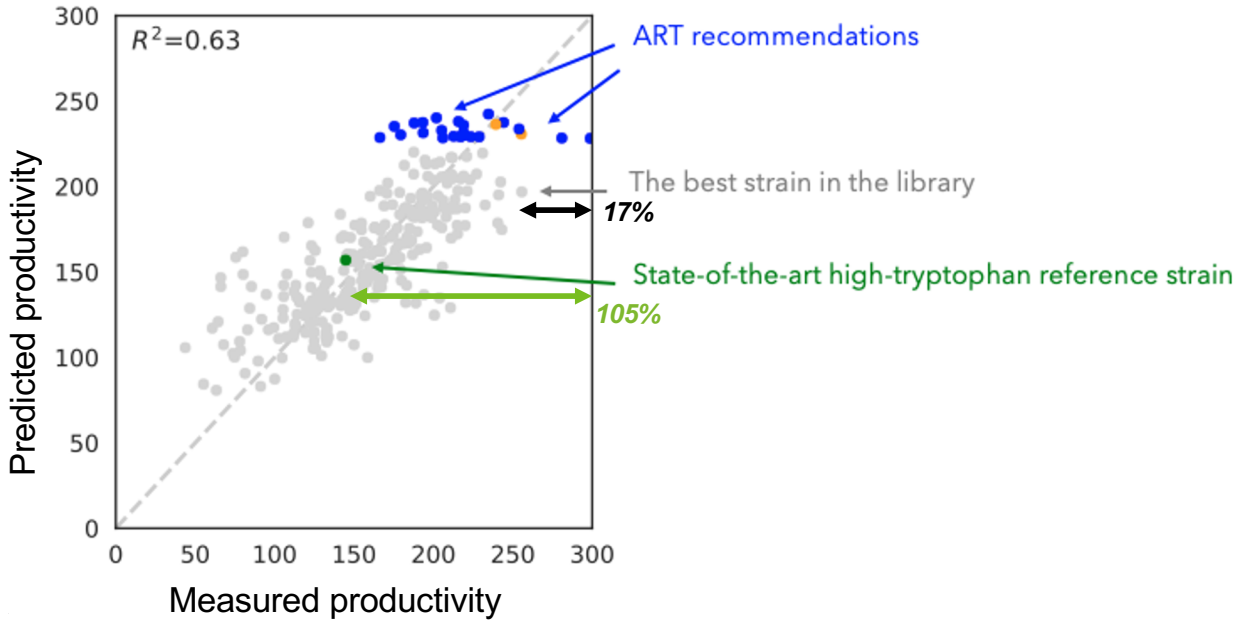
Learn/Design Highlight – ART Software

- **Automated Recommendation Tool**
  - Machine learning tool **specifically adapted to synthetic biology's needs**: small data sets, uncertainty quantification.
  - Builds a **predictive model from data** and uses that model to **recommend new designs** for next cycle.
  - Can be used via Web-UI@art.agilebiofoundry.org or Jupyter notebooks



  - ART has been used to successfully **guide the bioengineering process.**



Radivojević, et al.
Nature communications 11.1 (2020): 1-14.

Zhang, et al.
Nature communications 11.1 (2020): 1-13.

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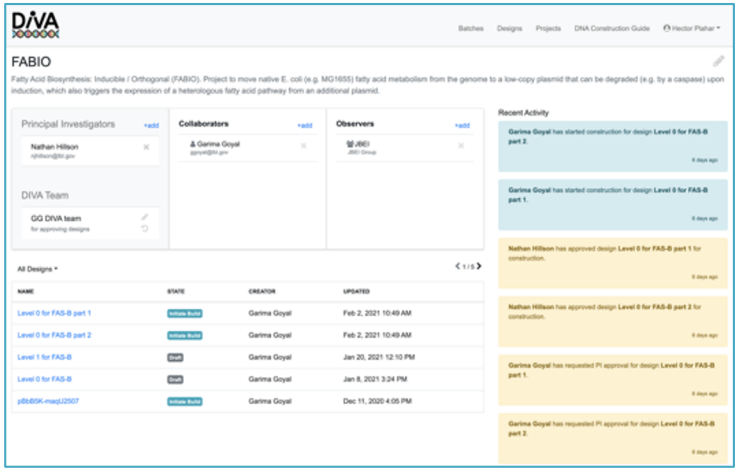
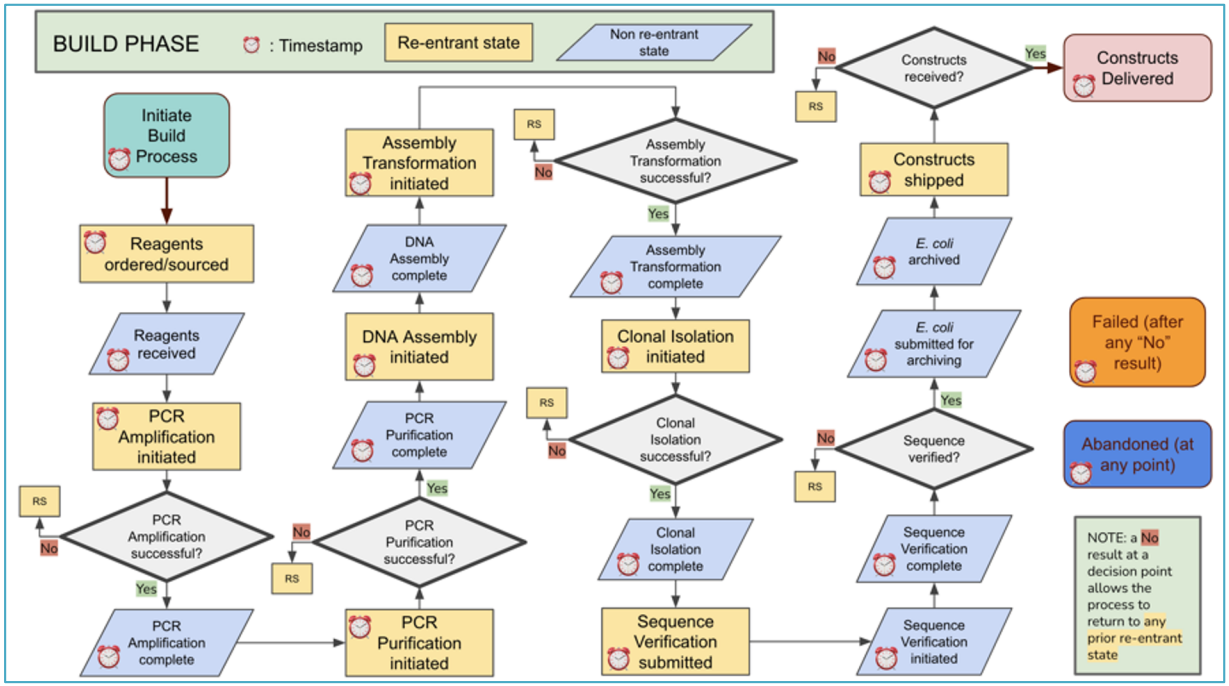
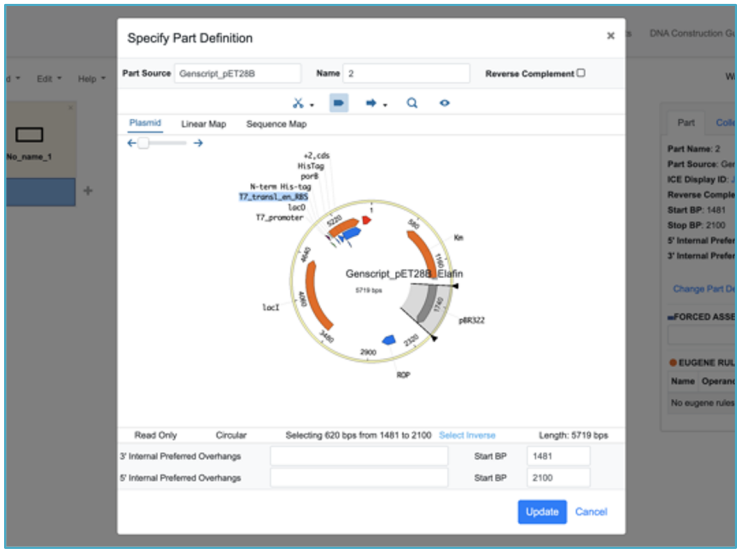
Design/Build Highlight – DIVA Software

- **Design Implementation Validation Automation**
  - Software platform integrating tools for Designing and Building DNA constructs

- **Recent improvements**
  - Design batching for increased Build efficiency (FY20Q3_DBTL_R4)
  - Design and Build cycle time metric capture (FY20Q3_DBTL_R4)
  - User interface redesign and client web framework modernization
  - Support for "empty" parts in a design
  - Support for custom DIVA Teams
  - Improved OpenVectorEditor integration for part and construct visualization







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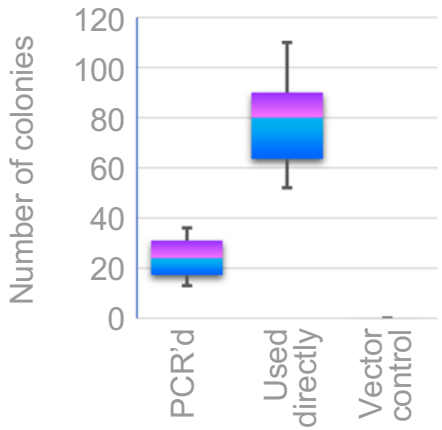
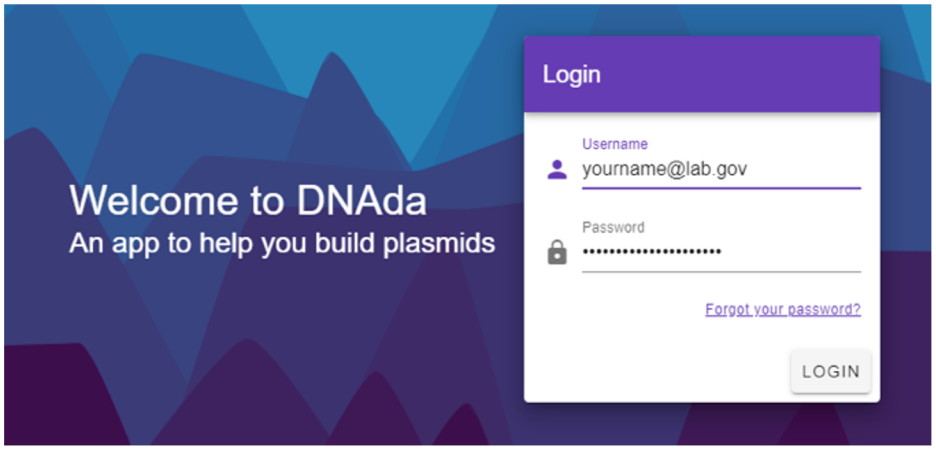
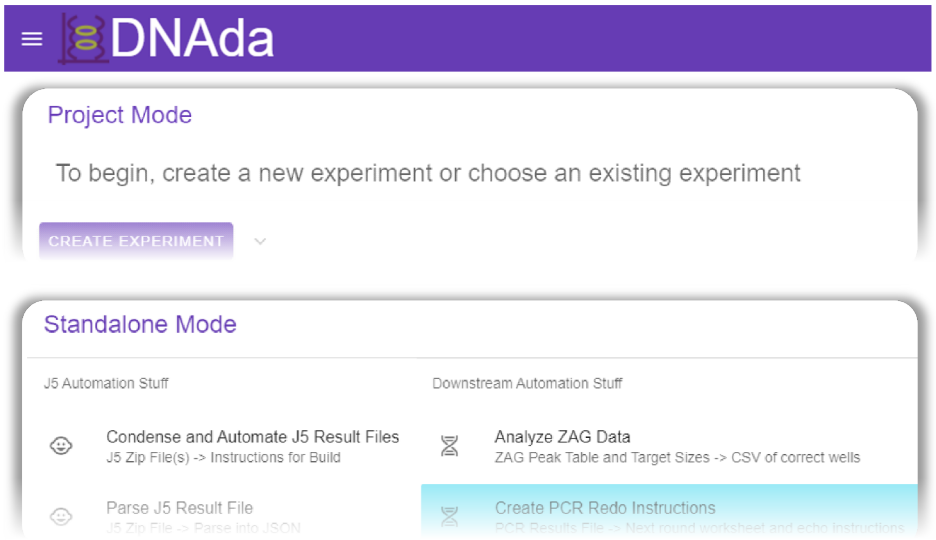
Build Highlight – DNA Construction

- **Informatics**
  - Collaborations with BETO- and BRC- supported projects on software interfacing dry and wet-labs
- **Process improvement**
  - Throughput increase from automated (e.g., 4x for *E. coli* transformation) and non-automated methods (e.g., large scale DNA purification) - 32 days from synthesis to delivery of 100+ constructs
  - Progress on plasmid DNA copy-control strain for accelerating Build of large constructs
  - Higher efficiency and reduced hands-on time for cloning synthetic DNA w/o adapters (skipping PCR/purification)







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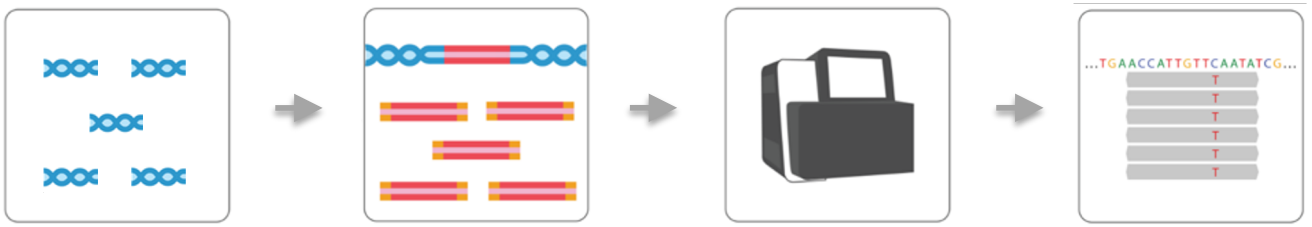
Build Highlight – DNA sequence validation

- **Overview**
  - $8 per sample (full amplicon/plasmid coverage, no Sanger-method oligos required)
  - Sample types: boiled cell culture, purified plasmid, or PCR amplicon
  - Up to 1536 purified DNA samples, or 384 cell cultures per MiSeq run



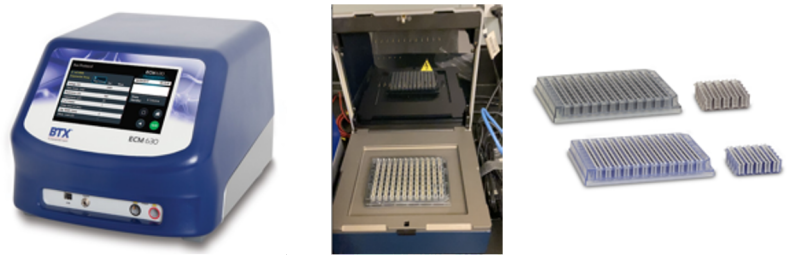
DNA input | Nextera preparation | MiSeq sequencing | Alignment analysis

- **In progress**
  - Automating plasmid minipreps on the Biomek to facilitate higher-throughput MiSeq runs

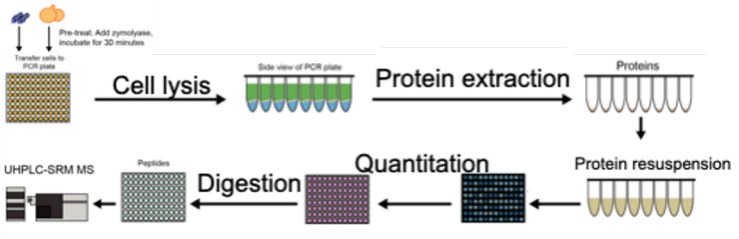
  - Developing sequencing statistics web app (SSGUI) to streamline analysis for users
  - Coordination with microbial strain archivists to buffer demand for more frequent/consistent sequencing cycles
  - Evaluating alternatives to Nextera library kits

Build/Test Highlight – Automation

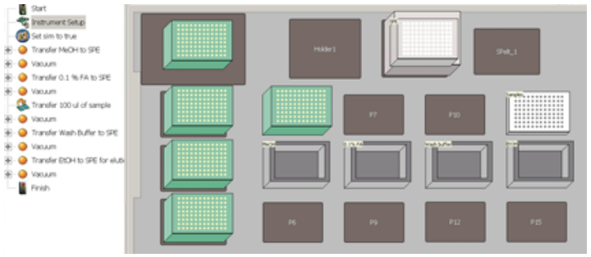
- **Build**: ***Rhodosporidium toruloides & Pseudomonas putida* transformation**
  - New 96-well plate based methods created
  - 400% throughput improvement
  - Created and validated method with live samples (>5 cycles of improvements completed)



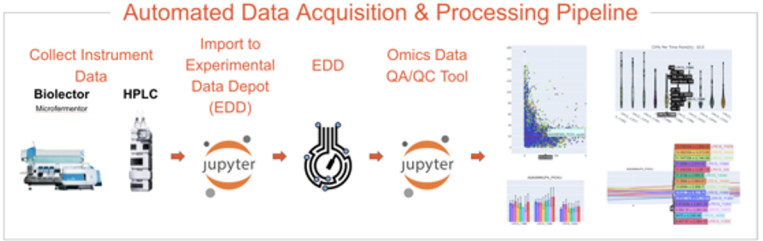
- **Test: Proteomics Sample Preparation**
  - Modularized the sample preparation workflow and established modules on multiple biomek liquid handlers
  - ~50% faster and more flexible



- **Test: Analytical Sample Prep (Solid Phase Extraction)**
  - 96-well plate based method on Biomek FX
  - Facilitates cleaner samples
  - 400% improvement in throughput over manual method in same time



- **Test: Omics Data QA/QC Tool**
  - Analysis tool that calculates the repeatability, precision, and quality of experimental data uploaded to the Experiment Data Depot (EDD)
  - Supports transfer of high-quality data to Learn activities
  - Expanded to HPLC, GC, and bioreactor data types in the EDD



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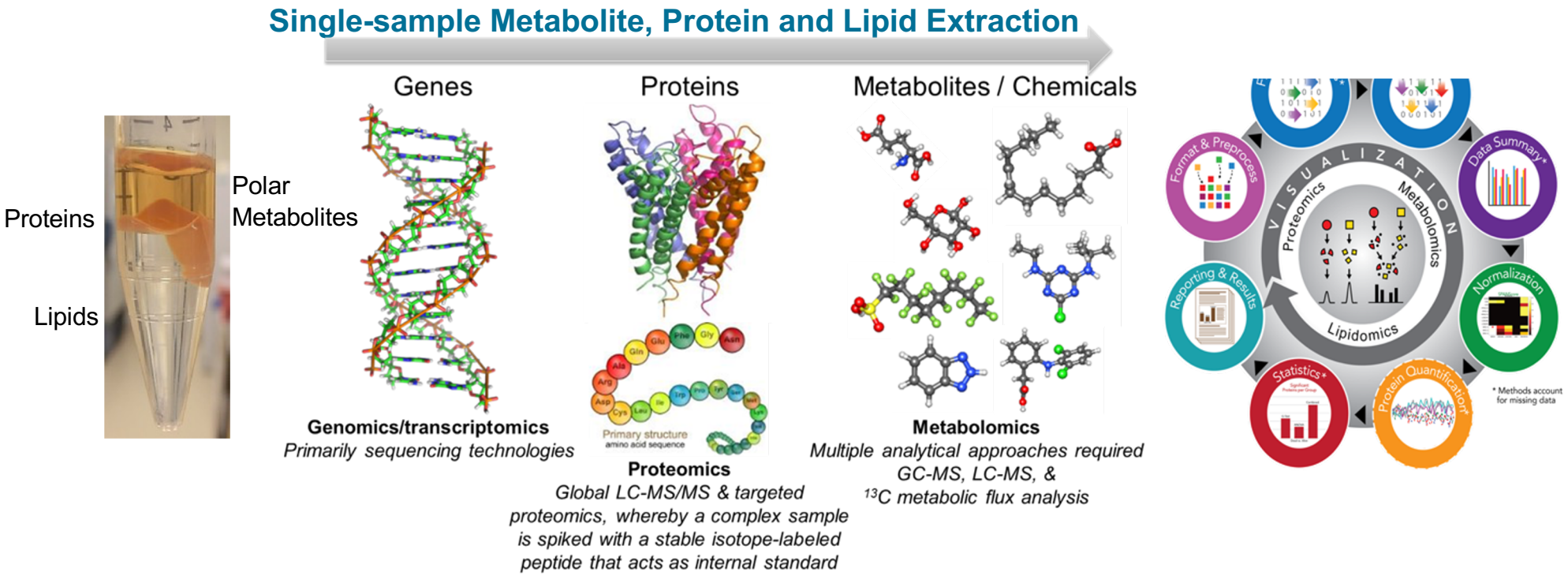
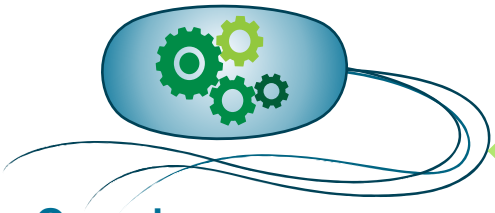
Test Highlight – Multi-omics Analysis

- **New ABF targeted proteomic method increases throughput of protein quantification by 4 times**
- Gao, Yuqian, et al. “High-Throughput Large-Scale Targeted Proteomics Assays for Quantifying Pathway Proteins in Pseudomonas putida KT2440” Front. Bioeng Biotechnol. 2020 Dec 2;8:603488. doi: 10.3389/fbioe.2020.603488.



Host	Proteomics (global + targeted)	Metabolomics/Lipidomics (Intra/Extracellular)
P. putida	> 780 datasets	> 520 datasets
A. pseudoterreus	> 590 datasets	> 550 datasets
A. niger	> 150 datasets	> 150 datasets
R. toruloides	> 660 datasets	> 1000 datasets

Test Highlight – Biosensors and Cytometry



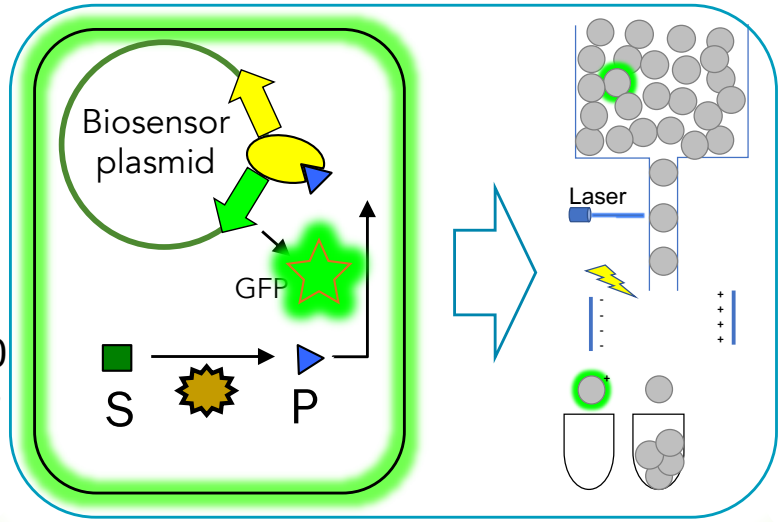
Organisms

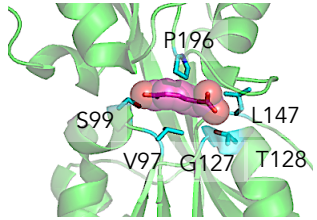
Established in

- *Pseudomonas putida* KT2440
- *Corynebacterium glutamicum*
- *Acinetobacter baylyi* ADP1

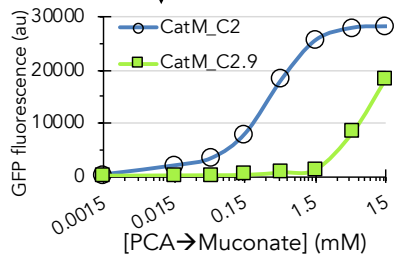
In progress

- Anaerobes



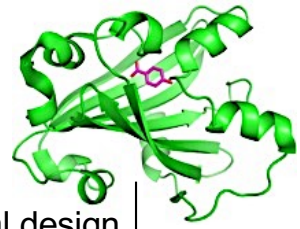


Custom design

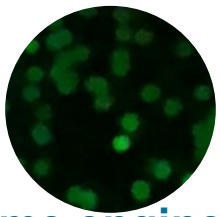


Sensors with variable sensitivities

Established sensors



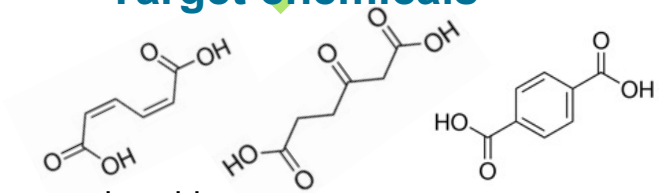
Rational design



Enzyme engineering

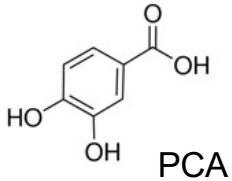
Selection based on fluorescence

Target chemicals

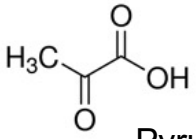


cis,cis-muconic acid β-ketoadipic acid TPA

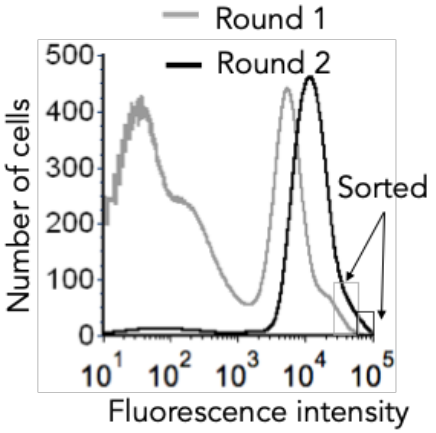
Beachheads



PCA



Pyruvic acid



Strain evolution

Sorting heterogeneous population

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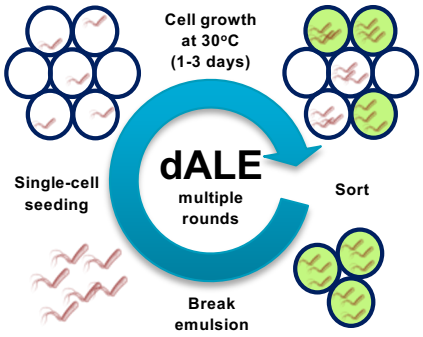
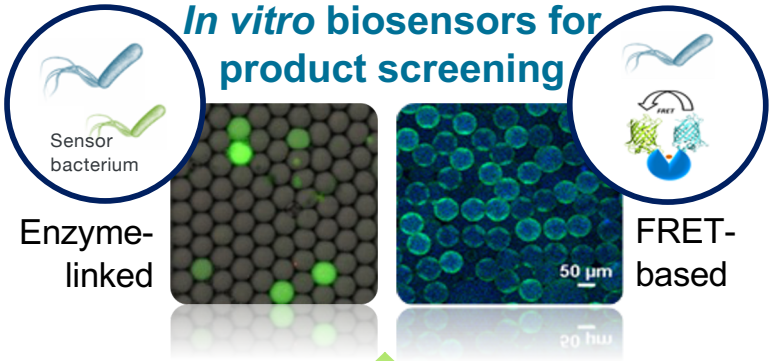
Test Highlight – Biosensors and Microfluidics



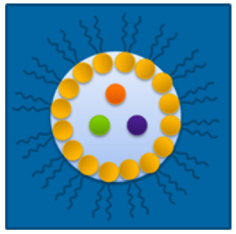
Droplet-based adaptive laboratory evolution (dALE)

- Enriches for fast growers
- Avoids over-growth of non-productive cells
- Maximizes strain diversification



High-speed LC-Mass Spectrometry

- Analysis time: **~1 min/sample**
- Monitor four single ions
- Simultaneous UV/vis

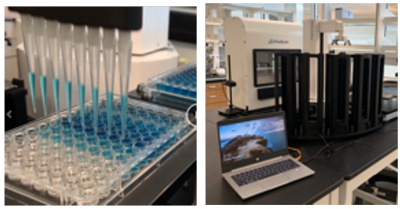


Biologically friendly droplets

- Aqueous droplets in fluorinated oil
- Volume: **1-30 pL**
- Droplets/run: **~10,000,000**

Droplet-based microfluidics

- Mixing and encapsulation
- Strain evolution
- Enzyme evolution



Laboratory automation

- Liquid handlers allow parallel strain testing
- Efficient validation in multi-well plates or shake flasks

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Test Highlight – Scale-up

LBNL

- RoboLector + Biolector
- 12 X 250 mL Sartorius Ambr®
- 4 X 2 L Sartorius Biostat B
- 1 X 50 L ABEC
- 2 X 300 L ABECs
- Thermofisher Gallery for micronutrient analysis

NREL

- RoboLector + BioLector Pro
- 2 X 250 mL Applikon my-control
- 36 X 500 mL Sartorius BioStat-Q
- 6 X 3 L Applikon single-wall
- 5 X 10 L 320 Eppendorf BioFlo
- 1 X 30 L, 2 X 160 L, 2 X 1450 L, 4 X 9000 L

PNNL

- 3 x Sixfors (6 x 0.5 L each)
- 1 x 2-10 L Sartorius Biostat
- 2 x 30 L Sartorius
- 120 L Sartorius

SNL

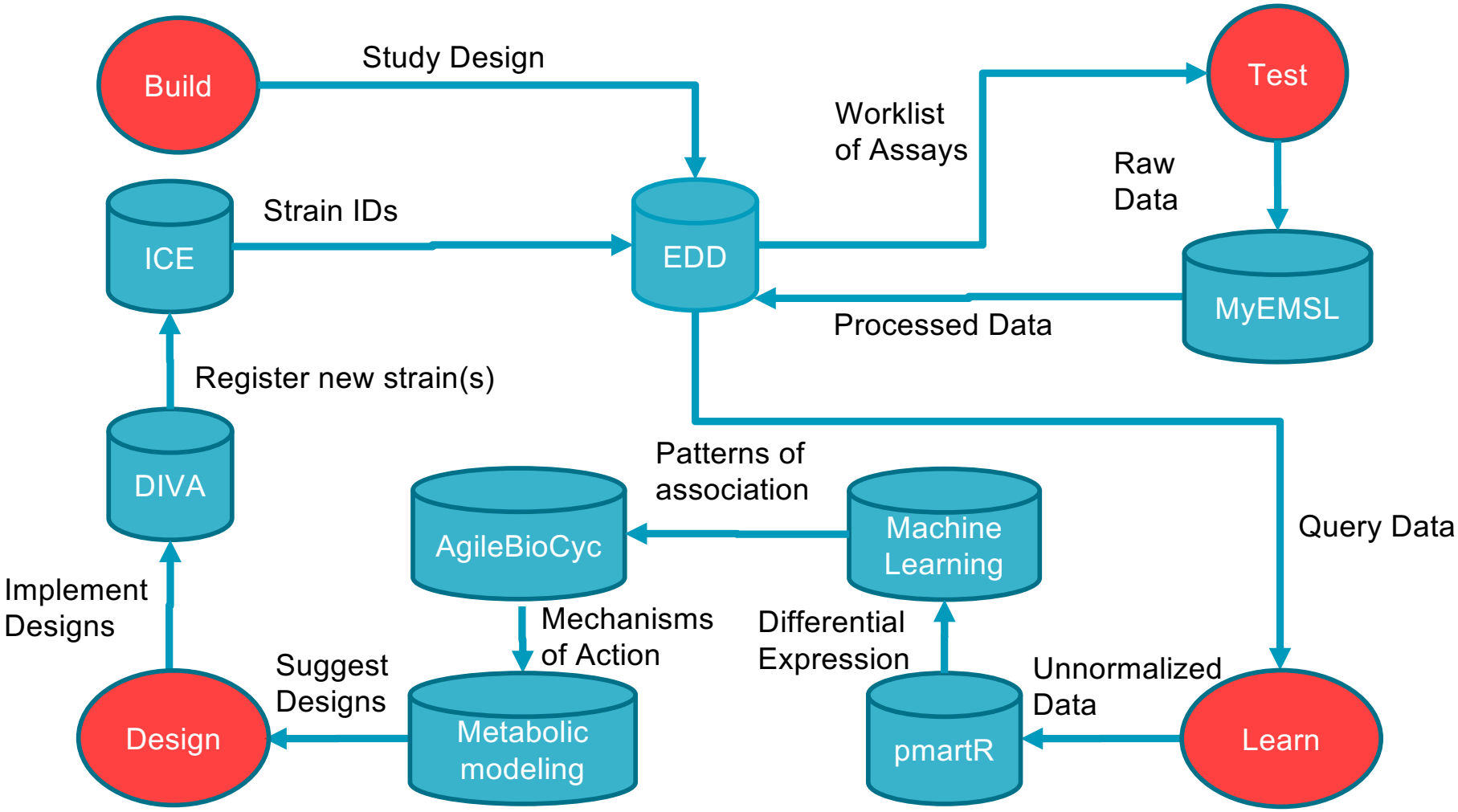
- Biolector Pro

12 X 250 mL bioreactor

4 X 9000 L bioreactors

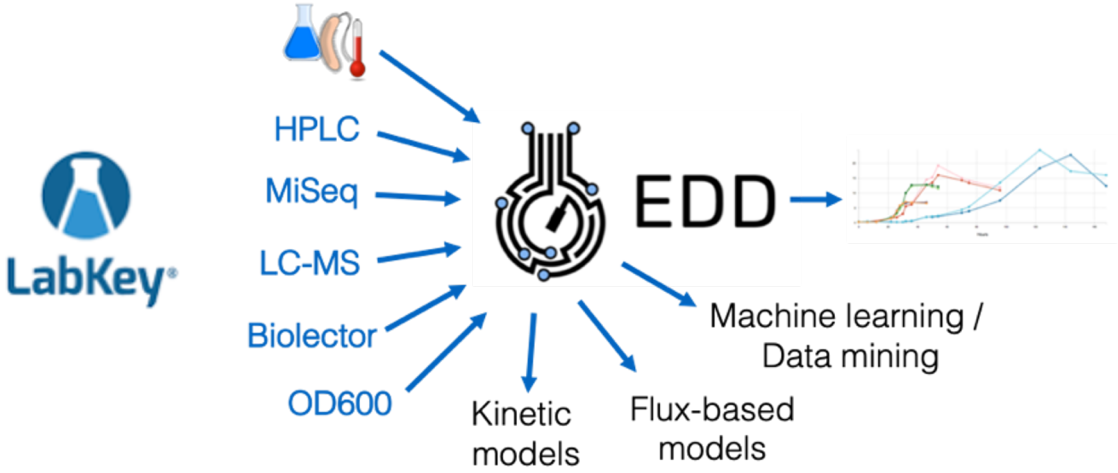
Test/Learn Highlight – ABF Data Flows

- Raw, processed, assay, and sample data and metadata from Test activities
  - May additionally be channeled into the ABF data ecosystem via MyEMSL



Test/Learn Highlight – EDD Software

- **Experiment Data Depot**
  - Software platform repository for actionable biological datasets and metadata



- **Recent improvements**
  - Internal updates
    - Updated script builds to Webpack 4
    - Improved organization of code modules
    - Automated building, testing, and deploying
    - Added scripts to generate deployment configuration
  - Added features
    - Account approval and management tools
    - Improved import workflow
    - Support / requirement for UniProt, PubChem IDs
    - Speed improvements by working in background
    - Include Assay metadata in building worklists
    - JSON Web Token login option in REST APIs
  - In-Progress features
    - Replaced in-house table implementation with library
    - Further improved import workflow
    - Replicate support & improved graphing
    - Support for AWS S3 -- removing upload size limits
    - Campaigns to group related Studies (i.e., DBTL cycles)
    - Link to Protocols from protocols.io
  - New EDD-related paper:
    - Roy *et al.* (2021) Frontiers in Bioeng Biotech (see additional slides for full reference)

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Learn Highlight – Metabolic network modeling

Metabolic network reconstruction, modeling, and validation

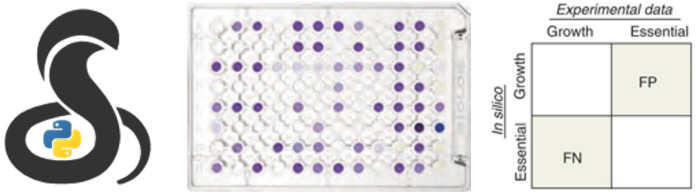
BiGG Models

KEGG

MetaCyc

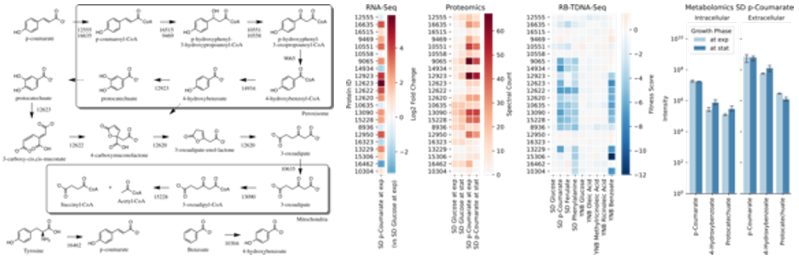
Transporter Classification Database

- Reconstruction of metabolic networks using high-quality metabolic models and databases
- AgileBioCyc at https://cyc.agilebiofoundry.org

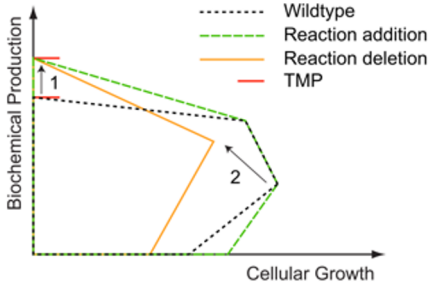


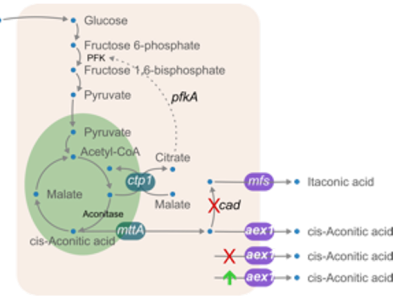
- Curation and refinement of metabolic networks using multi-omics and high-throughput data
- The genome-scale metabolic network model of *R. toruloides* and associated data available at https://github.com/AgileBioFoundry/Rt_IFO0880

Multi-omics data integration and computational strain design



- Analysis and visualization of multi-omics data using metabolic models and maps





- Computational strain design approaches using genome-scale metabolic models at https://github.com/AgileBioFoundry/Strain_Design

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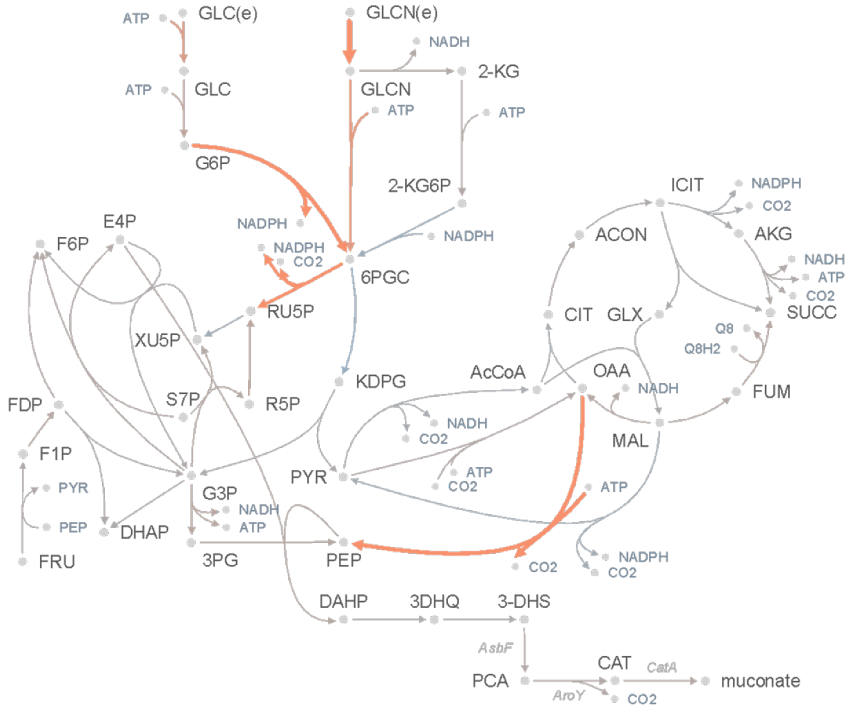
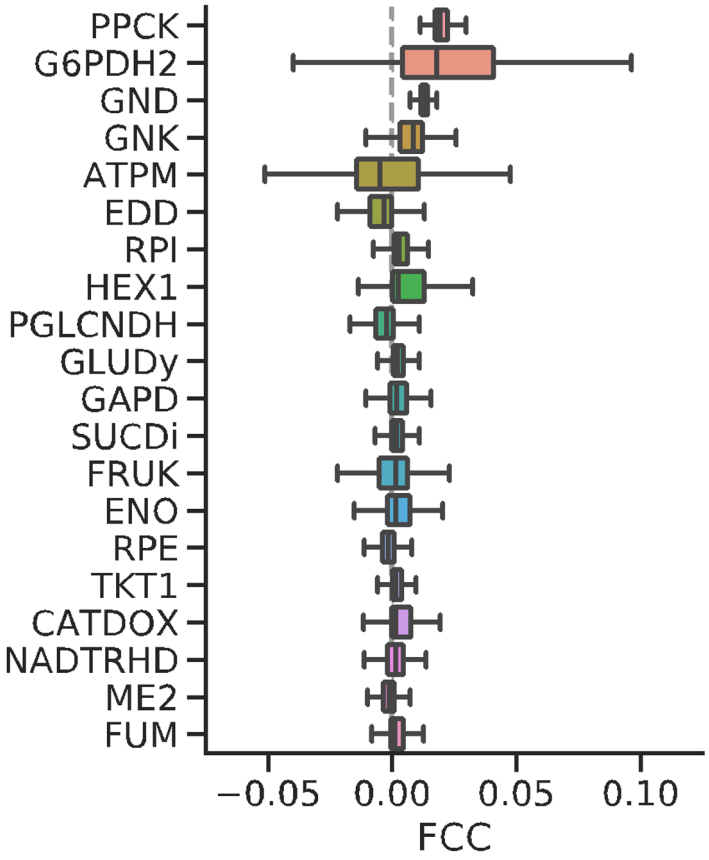
Learn Highlight – Bayesian inference of metabolic kinetics from multi-omics data

- Infer probabilistic relationship between variables we can control (enzyme expression; media composition) and those we cannot (intracellular fluxes and metabolomics)
- Method applied to P. putida media and strain experiment
- Revealed several core-carbon enzymes that might lead to higher muconate flux





Red arrows indicate overexpression is predicted to result in higher muconate flux

- The resulting model offers predictions that are more mechanistic than black-box approaches

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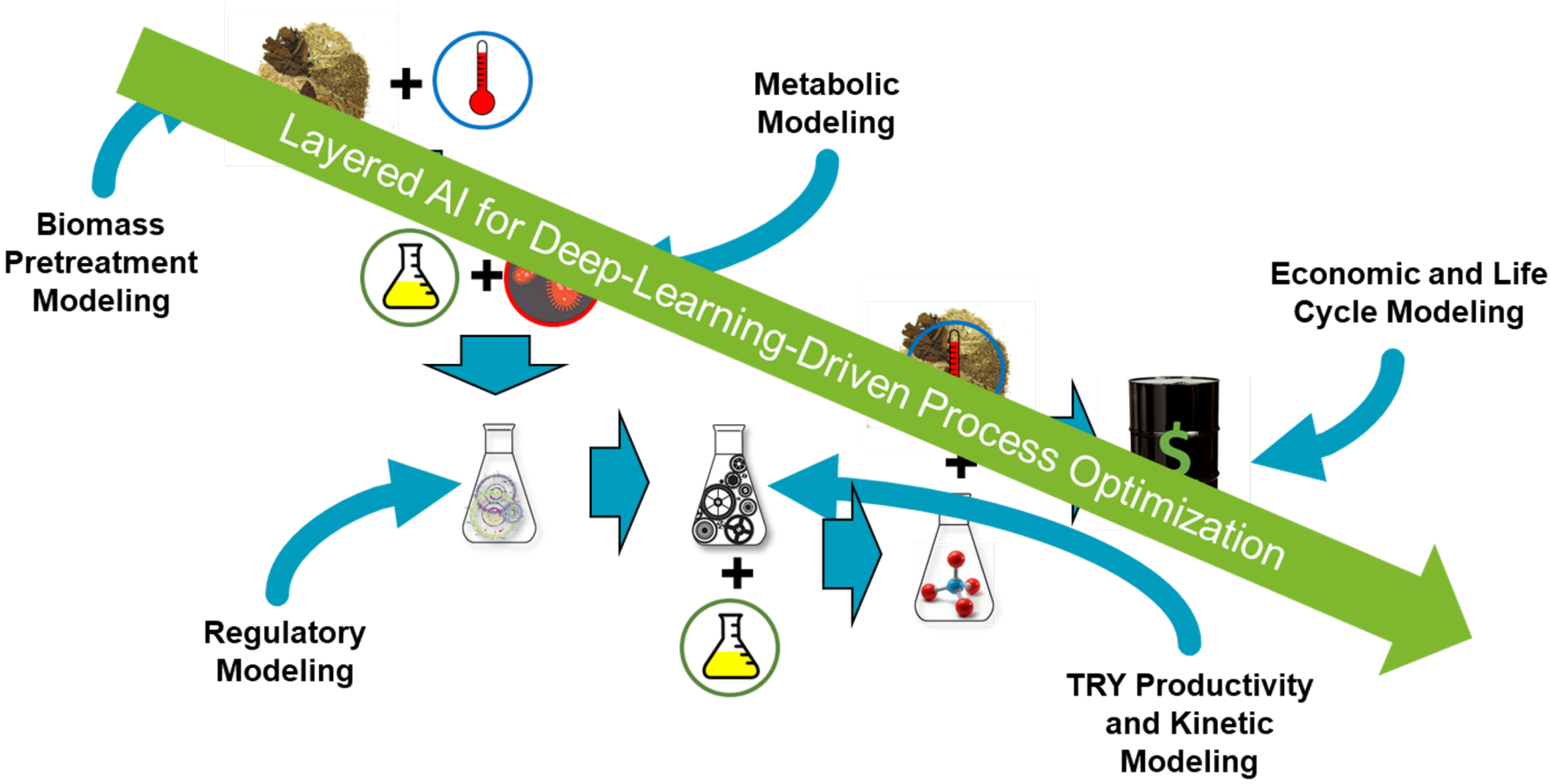
Learn Highlight – Deep Learning

- **Integrated AI subsystems for Deep Learning in Biomanufacturing**
  - An ecosystem of learn models for continuous data collection and integration
  - Outcome: An integrated layering of modules where output of one is input of next
  - Ongoing: Required complexity and inter-lab coordination being established



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Status of key milestones

- **Completed in FY20 (representative)**:
  - Q1: DBTL cycle defined with specific required unit operations comprised
  - Q1: 4 DBTL unit operations formally specified
  - Q3: Opportunities for DBTL efficiency improvements identified, development initiated for these in at least 3 workflows and improvements quantified
  - Q3: Power analysis of -omics datasets for two organisms completed to determine number of replicates needed for Learn
  - Q3: Opportunities for DBTL task automation identified and prioritized, and development initiated of automation workflows for 2 identified priorities
  - Q4: Reproducibility of 3 Test unit operations quantified through comparison of results for on-site vs. off-site sample analysis for 3 or more variables

- **On track for completion in FY21 (representative)**:
  - Q2: (Go/No-Go) 5 metabolic pathways and/or tools transferred between hosts, with 2X improvements in second host, with metrics defined for each case
  - Q3: Efficiency gains in DBTL workflows assessed
  - Q3: Biosensors developed for two beachheads or energy/redox indicators
  - Q4: 1-2 DBTL automation workflows finalized that improve efficiency by => 2X
  - Q4: Cross-validated 20% improvement in predictive power demonstrated for two or more ABF Learn methodologies, for multiple vs. single data modalities

Risk mitigations - all on-track / ongoing

Risk	Severity	Description	Mitigation Plan
Distributed model inefficiencies	Low	Important to consider the effects a distributed model has on the ABF's goals	Monitor and minimize DBTL cycle delays or other inefficiencies due to distributed operations
Insufficient data to fully leverage Learn	Medium	Multi-omics datasets may not be of the quality, quantity, or consistency needed for statistical analysis to identify engineering targets that lead to gains in titers, rates, and yields	Explicitly include the Learn team during the Design process to ensure suitability of generated data
Infrastructure operating costs and value	Low	Costs of infrastructure (both hardware and software) maintenance and asset depreciation becomes unsustainable	Offload maintenance to more cost-effective and sustainable off-the-shelf vendor-supported solutions where possible
Lack of target/host transferability	Medium	Not able to leverage learnings from one demonstration project/ beachhead in work for another	Further Learn the extents/likelihood of transferability
Designs do not work in selected host	Medium	Promoters/enzymes/pathways do not function as intended in the selected host	Further Test and Learn from lack of function, and suggest Design changes to restore function

Summary - DBTL Infrastructure

- **Goal**: Design, implement, operationalize, and maintain Design-Build-Test-Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF Tasks and enables the overall ABF goal

- **Outcomes**: 10X improvement in Design-Build-Test-Learn cycle efficiency, new IP and manufacturing technologies demonstrated and ready for translation to U.S. industry

- **Relevance**: Public infrastructure investment that supports the ABF and other BETO projects, and that can be leveraged by U.S. industry

- **Risks**: Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from

Quad Chart Overview

Timeline

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

	FY20	FY21	FY22	Total Active
DOE Funding	$2.3M	$4.8M	$5.4M	$12.5 M

Project Partners

- LBNL (34%), SNL (21%), PNNL (19%), NREL (11%), ANL (4%), LANL (7%), ORNL (5%)

Barriers addressed

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

Project Goal

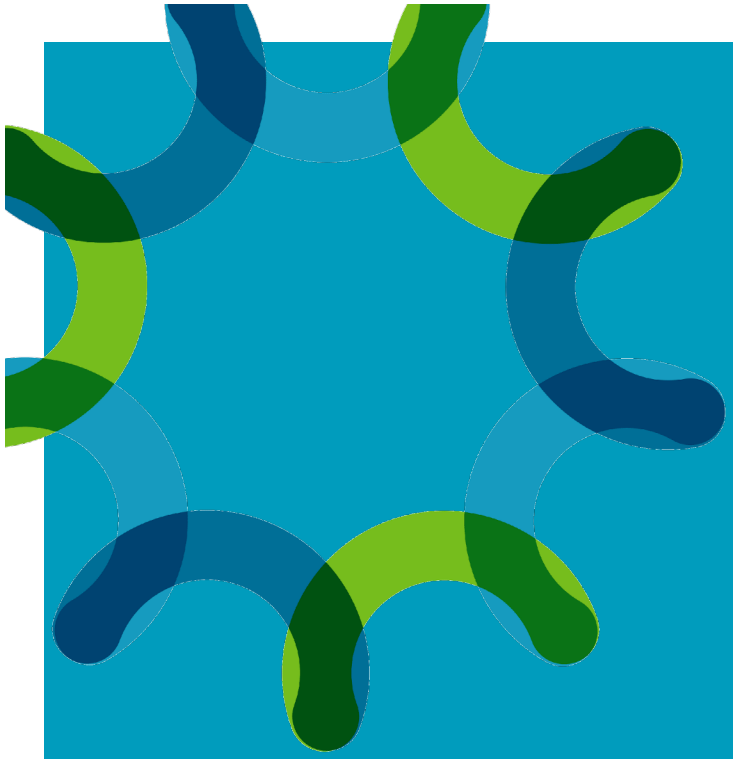
Design, implement, operationalize, and maintain Design-Build-Test-Learn infrastructure as a core component of the Agile BioFoundry that supports other ABF Tasks and enables the overall ABF goal

End of Project Milestone

- 5X efficiency improvement in DBTL engineering cycle

Funding Mechanism

AOP



Additional Slides

Responses to Previous Reviewers' Comments

- **C:** The DBTL infrastructure is the core of the ABF and supports all other tasks. Having this infrastructure 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 acquired a number of tools. These include DNA construct design and assembly, various types of metabolic modeling, deep learning algorithms, and LIMS for data storage and sharing. New Build tools are NextGen 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.

- **R:** We agree that our DBTL cycle time (as of Peer Review 2019) 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.

Responses to Previous Reviewers' Comments (cont.)

- **C:** 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.
- **R:** 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, non-exclusive, 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 non-exclusive (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 non-exclusive licensing options in Collaborative Research and Development Agreements (CRADAs), and we plan to explore these options in the next phase of the ABF.

Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Geographic distribution of the different steps poses logistical challenges, requiring strains and samples to be shipped around different sites. This could slow down the cycle.
- **R:** We are aware of the distributed logistical challenges. While these do slow down DBTL to an extent, it is a rather minor (at least currently) contributor to overall DBTL cycle time.

- **C:** Weakness: It would be helpful if they provided more quantitative benchmark for progress and success. These are a little vague; as a consequence, it is difficult to evaluate whether the team is on track to achieve their final goals.
- **R:** Not clear what the Reviewer is referring to specifically. We did agree elsewhere that more closely connecting sub-tasks with the overall ABF goal is important, and we also mentioned that that we used simplified milestone language in the presentations, which resulted in not sharing all the quantitative benchmark details with the reviewers.

- **C:** Weakness: Specific relevance of the current work toward the 10x efficiency improvement is not quantitatively explained. The goals of the ABF should be focused on the performance period, especially for peer review. Descriptions for individual projects should include explanation of hour their contributions quantitatively feed into the program-level goal(s) for the period.
- **R:** We don't have this quantitative data yet. Until we have a clear definition of DBTL and granular performance metrics captured (next 3-year AOP objectives), this won't be possible. Discussed elsewhere how we will use this data to prioritize and inform subsequence core technology development. It is a fair point that presenting 9-year ABF objectives during a 3-year Peer Review can make assessments less straightforward.

Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: More data/results are required to determine how much non-intuitive learning has been enabled to date. It will be important to keep a scoreboard on such successes to they can be highlighted in future reviews.
- **R:** Agreed. We won't have the results from evaluating the unintuitive predictions until the end of FY19. It should be noted, though, that we did not ask the Reviewers to assess the value of the unintuitive predictions (can't be done until we have the data!), but rather we asked them to assess if the predictions were unintuitive (or not) independent of their future successes or failings.

- **C:** Weakness: Many different tools are being developed, but no clear plan on how they will put together in a workflow. It would be good to have a case study to showcase all the tools, taking a strain through a full cycle.
- **R:** This would have needed to have been done in the Target/Host presentations. There was no time to do this in the DBTL infrastructure presentation. If there had been more time, a case study would have been nice and effective.

- **C:** Weakness: Quantitative benchmarks would be helpful for evaluating success, be it partial or complete. Also, it would help to more clearly define what is meant by non-intuitive predictions. The basic idea is clear; however, the metric for success here is somewhat murky. In particular, non-intuitive for some may be intuitive for others. Clearly, rigorously defining this metric will be challenging (and the team recognizes it). Nonetheless, the team should continue to work towards refining this metric, given its central role in justifying the Agile Biofoundry.
- **R:** Quantitative benchmarks addressed elsewhere. We did define what we meant by unintuitive in the overview presentation - namely a prediction or design choice that a skilled metabolic engineer would not have come up with without access to the deep/wide Test data and Learning methods employed at the ABF. This comment/response is very similar to that addressed above regarding how to quantitatively assess the value offered by any particular Test dataset or Learn methodology.

Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Management of individual projects appears to follow typical collaboration dynamics (regular meetings, etc…). Although they are working fine now, they may not scale as ABF's portfolio grows (especially with the CRADAs). It may become important to institute additional project management tools and/or layers of project management. The overall approach to the organization of the core technology development efforts was not presented. By presenting only highlights, the team did not communicate a clear, comprehensive picture of the approach as a whole, so it is hard to assess the planned capability. The team should articulate the entire tech-dev effort, explain how it is broken down into individual components, provide rationales/justifications for the areas where it is placing its biggest bets, and explain how they all quantitatively add up to achieve each of the high-level objectives for the ABF. In this way, it will be possible to regularly, holistically review the portfolio to ensure that all component efforts are still relevant going forward. Similarly, the team did not communicate a clear, comprehensive picture of the current state of technology development as a whole. As a result, it is not possible to evaluate the current state (nor development progress) of the ABF capability as a whole, and review is limited to highlights. This is due, in part, to omissions from the presentations of important activities that the team is certainly perusing. For example, regarding "Build," Target-Host-pair presentations mention strain construction as a bottleneck, yet there was no coverage of this critical component of ABF, nor its plans going forward. Strategically, in order to attain the 10x efficiency goal, there must exist a sub-goal/sub-plan for strain construction efficiency. (I am assuming that this was simply not communicated.) Similarly, there must be an assessment of what ABF's current status is toward these sub-goals and intermediate milestones. The same kinds of omissions are also assumed for the other aspects of "DBTL."
- **R:** This is an important point - how will the ABF scale its CRADA project management along with operations? We will need to discuss this internally with DOE BETO. Our current approach appears to be working well (lead lab PIs doing project management of CRADA projects, whereas Alastair is doing it for core ABF activities), but this may or may not scale. In the first three year AOP cycle for the ABF, we have identified many places around the DBTL cycle that present as opportunities for further efficiency gains. To date, we have largely been pursuing an all-the-above approach to core technology development. With increasingly precise and granular DBTL performance metric data capture, we can more holistically and strategically approach core technology development prioritization and staging. Since the presentations were limited by time, we were not able to present all of the work that we have been doing. This includes "Build", which other than the sequence validation component, was not discussed or presented.

Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: A lot of great software tools have been developed, but there are no examples showing how the software tools have facilitated the Learn function. Some were covered in the host presentations, but it is still too soon to tell how much the modeling helped identify targets. Much of the Test function is performed in small-scale plates. It is not clear how well this translates to actual fermentation conditions. Cycle time is still too long. It is understandable that the Build function will take a long time for novel hosts. However, in many cases Test is very long too. Since they have all culturing, analytical capability, and omics within the ABF, Test should only take a few weeks.
- **R:** The structure of the talks, which split the presentations of the tools themselves from their applications / predictions did not make it easy for the Reviewers to connect the tools with their unintutive predictions. Agree that most Test activities are at small scale, with a minority taking place in bioreactors; in the next 3-year AOP for the ABF, we will be working more on scale-up and scale-down, and the addition of Ambr250 and BioLector instrumentation will help us very much in this regard. Cycle time is addressed elsewhere. A given Test unit operation clock time may be fast, but the wall time of an overall Test phase depends on the different types and numbers of unit operations, along with how resources - personnel and instrumentation - are allocated and prioritized. For multi-omics analysis, several weeks is probably not a realistic expectation.

Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: A comprehensive picture of the entire DBTL capability is not presented, so it is hard to review. (See comment under "Approach.") It would help to better understand impact to explain who/which projects are using these tools, and how much. For example, Diva was only mentioned by one other project, which gives the (false?) impression that there is only one user. Similar questions can be applied to EASy, EDD, or microfluidics, etc.. It is not clear how are all of these tools combined into a DBTL cycle. Ad-hoc is ok, but it should be stated explicitly if so. Regarding newer AI/Learn efforts, many were described superficially, so the approaches are hard to evaluate, especially since there are no validated predictions yet. For the purpose of review, it would help to present these innovations at a deeper level, perhaps by the ABF's ML experts. An overarching concern is that these methods can often require large numbers of experiments, even if the amount of data from each individual experiment is large (e.g. metabolomics). It will be important to show that findings from any new ML approach are substantially better than what would be generated via "old" ML (regression/clustering). The layered AI approach has even greater technical risk, as it would require substantial data for each step.
- **R:** It is a good point that we do not have not collected comprehensive data about which target/host projects are using which tools / infrastructure. During the Peer Review presentation, there was not time to go into depth on any one tool or capability. We are assessing how much data is required for each Learn approach. For example, for kinetic learning, we have used simulated data to help us know how fast the methods will begin to converge.

Responses to Previous Reviewers' Comments (cont.)

- **C:** Weakness: Motivations/justifications for particular directions (e.g. specific software features, new analytical capabilities) is not provided, so it is hard to evaluate whether the collective set of directions make sense as whole. As a hypothetical example, maybe more emphasis should be placed on "Build" or on collaboration software, and less on sequence verification-- not enough contextualization is provided to judge. Another example would be to assess why "Test" times are currently so long, and to address this in future plans. The dynamic that the leadership should aim to avoid is one in which the shape of the portfolio starts be be determined by inertia rather than by careful, strategic assessment and re-assessment. For some technical directions (e.g. biosensors), it will be important to quantify performance requirements (dynamic range, S/N) that are needed in order to have relevance, and then to evaluate progress against these requirements. One alternative might be to institute a scientific advisory board (domain experts; could include some members of IAB), to perform deep-dive portfolio review.
- **R:** The Reviewers were presented with highlights, and not a comprehensive and detailed survey of all of the work we are doing. Just because something wasn't presented (at all or in sufficient detail) doesn't imply that it is not being done. "Build" scope and "Test" times are addressed elsewhere. DBTL core technology development prioritization mentioned elsewhere. We wil be discussing with DOE BETO the addition of a under-NDA Scientific Advisory Board that would complement the IAB.

Publications, Patents, Presentations, Awards, and Commercialization

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

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

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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

Publications, Patents, Presentations, Awards, and Commercialization (cont.)

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