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Agile BioFoundry

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

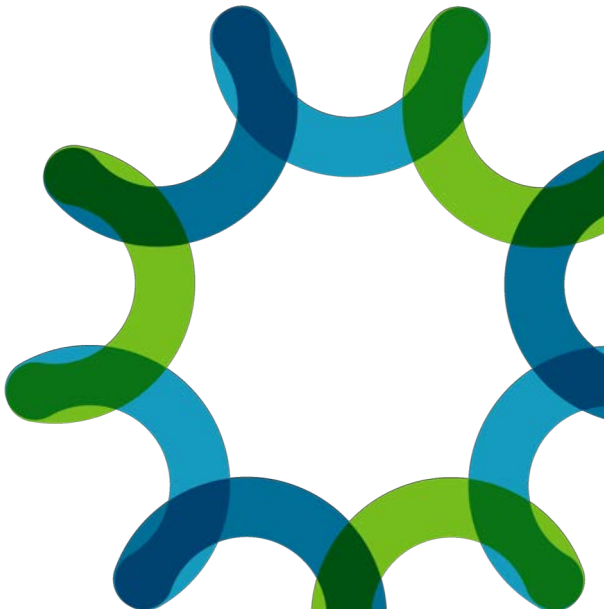
BIOENERGY TECHNOLOGIES OFFICE

DBTL Infrastructure

Nathan J. Hillson

Task Lead - DBTL Infrastructure

BETO Peer Review 2019
Conversion Technologies
1:00-1:50PM
March 7, 2019
Denver, CO

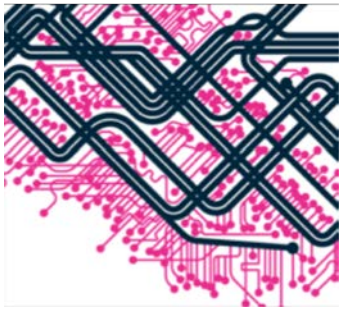


Goal Statement – Overall ABF

- **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 that will productionize synthetic biology.

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

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

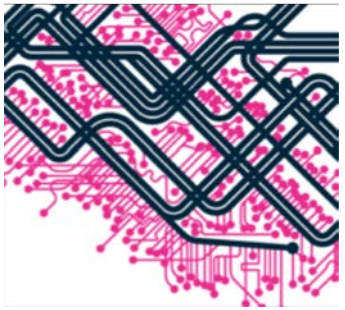


Goal Statement – DBTL Infrastructure

- **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.



Quad Chart Overview

Timeline

- Start: October 1, 2016
- End: September 30, 2019
- 83% complete

Barriers

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

Budget

	Total Costs Pre FY17	FY17 Costs	FY18 Costs	Total Planned Funding (FY19-Project End Date)
DOE Funded	$823k	$2.3M	$3.2M	$3.9M
Partners: LBNL (48%); SNL (21%); NREL (15%); ANL (7%); PNNL (6%); LANL (3%); INL (0.2%); ORNL (0%)				

Objective

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.

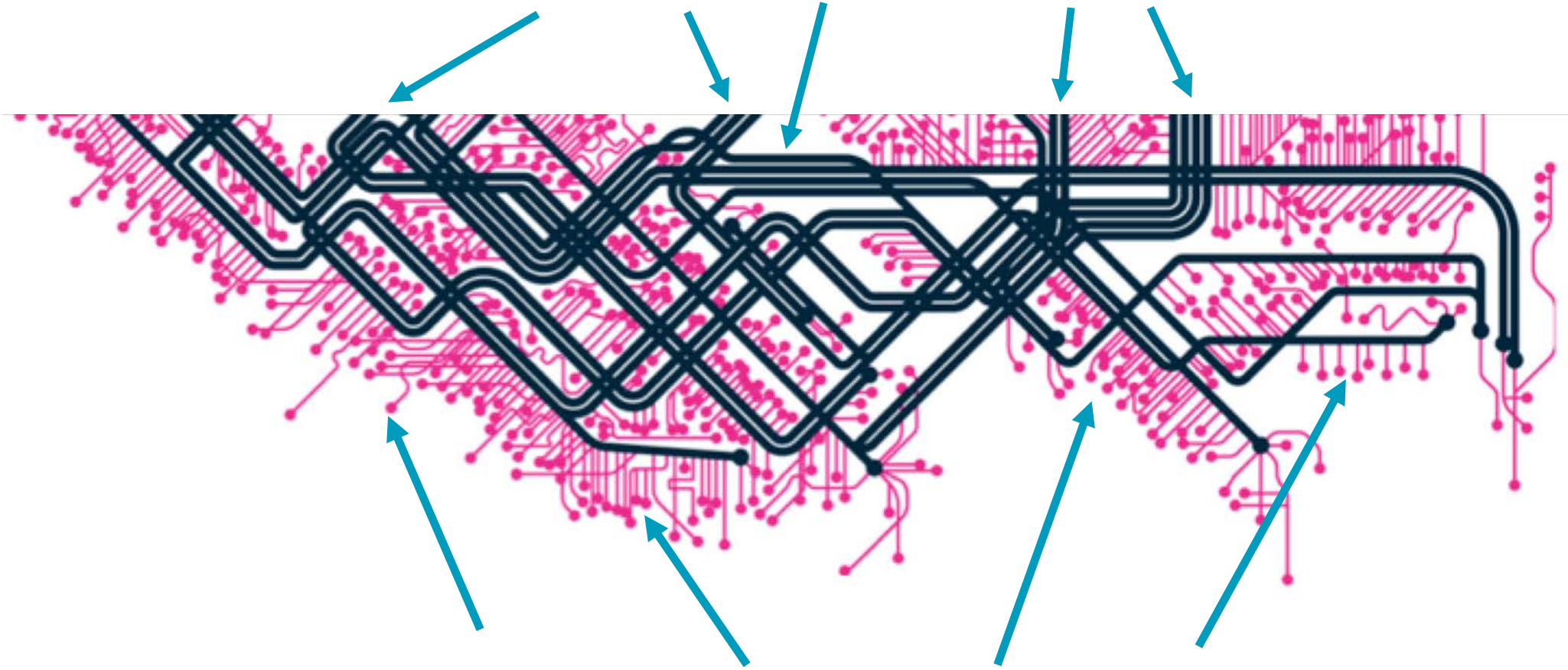
End of Project Goals

Demonstrate target/host pair production of at least 3 molecules at 10 g/L, 100 mg/L/hr, at 40% of theoretical yield from DMR-EH at 10 L. Demonstrate value of non-intuitive Learn predictions.

1 - Project Overview

DBTL Infrastructure Investment Enables Private Industry

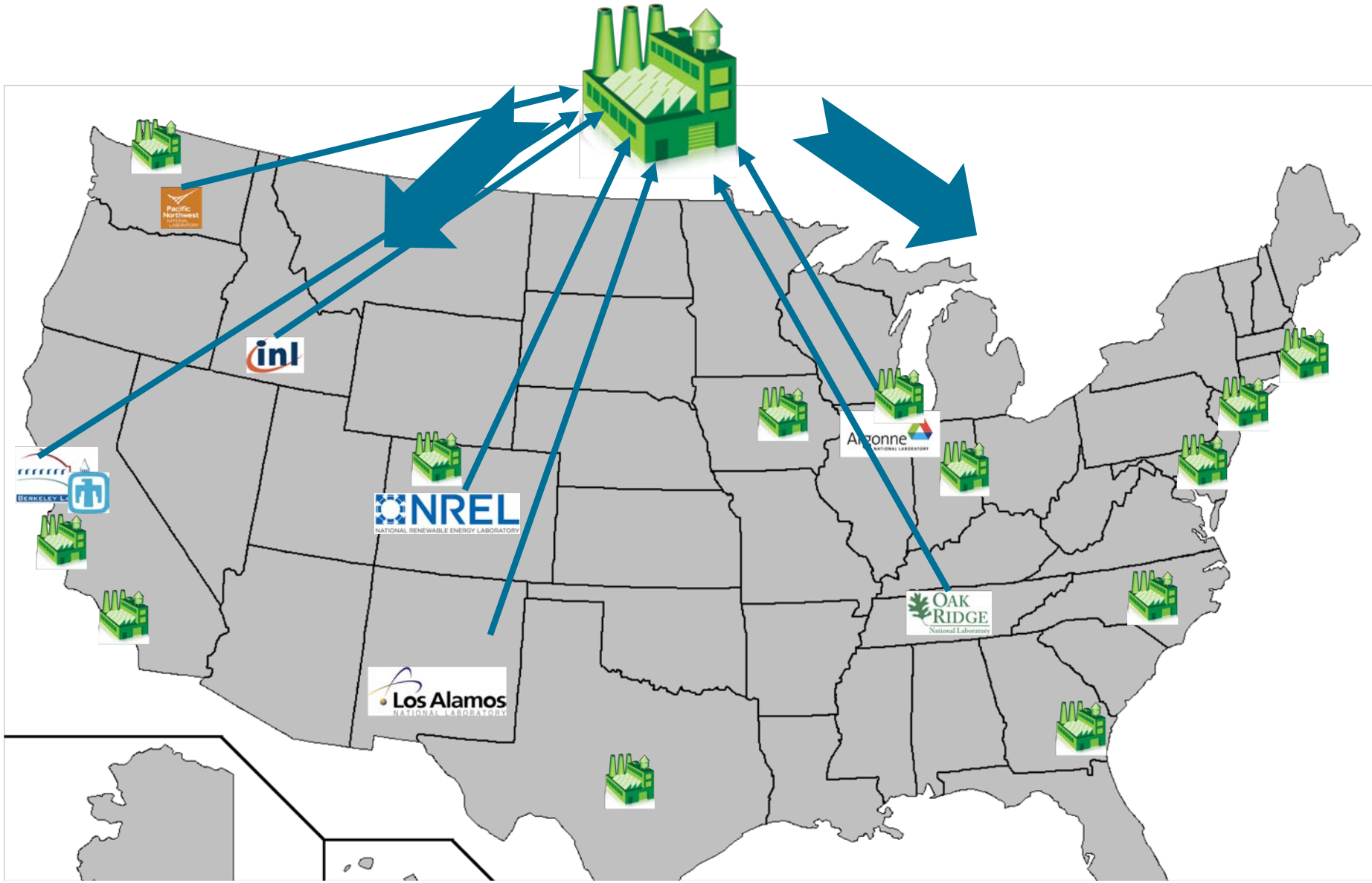
DBTL infrastructure investment



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

Adapted from Lyft

DBTL Infrastructure is Commercially Available or can be Out-Licensed from the ABF



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DBTL Infrastructure Will Reduce Time-to-Scale up

Years 1-3 (*5 hosts*)

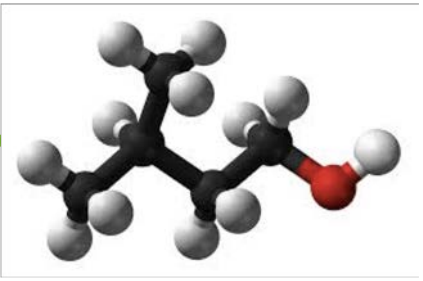
Molecule X

~10 years, $100M

Years 4-6 (*20 hosts*)

Molecule Y

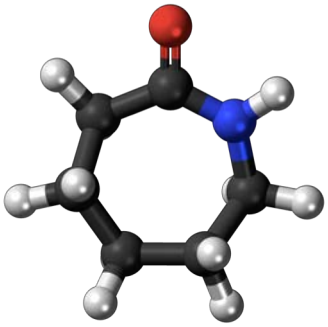
~8 years, $50M



Years 7-9 (*100 hosts*)

Molecule Z

~5 years, $25M



Time and cost for commercialization

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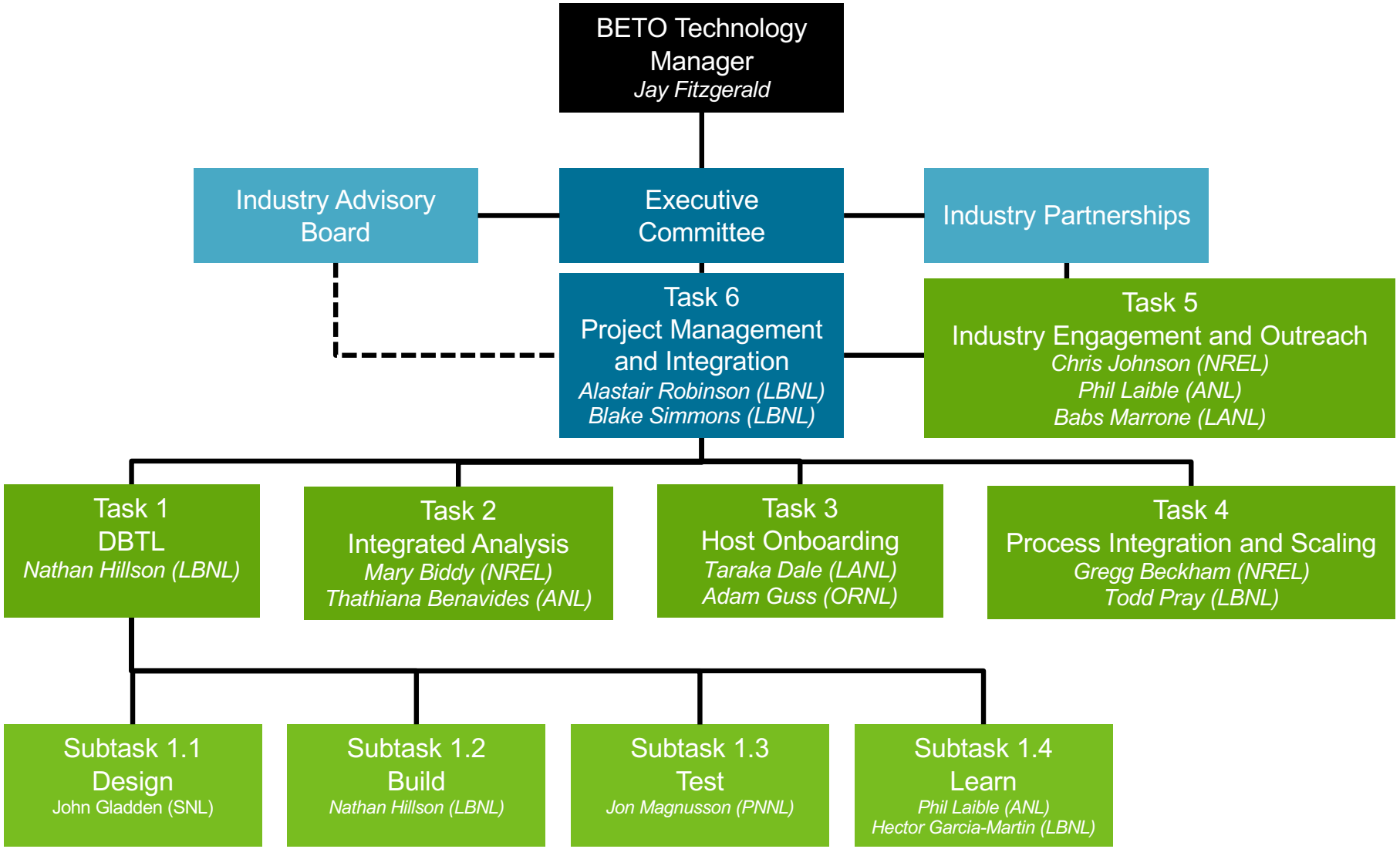
2 – Approach (Management)

Six Tasks for Overall Project

- **Task 1: Design-Build-Test-Learn (*Nathan Hillson* - lead)**
  - **Integrate design-build-test-learn cycle with process automation and sample tracking.**
- Task 2: Integrated Analysis (*Mary Biddy/Thathiana Benavides* – co-leads)
  - Evaluate proposed molecules; develop, update, and improve existing process designs and LCA.
- Task 3: Host Onboarding (*Taraka Dale*/*Adam Guss* – co-leads)
  - Evaluate possible host organisms to determine which on-boarding criteria are not yet met, and fill these gaps through tool development and data collection.
- Task 4: Process Integration and Scale-up (*Gregg Beckham*/*Todd Pray* – co-leads)
  - Standardize, produce, ship, and store hydrolysates; compare clean sugar processes with hydrolysates; test and scale fermentation to improve titer, rate, and yield; provide integrated, bench-scale data for TEA and LCA; scale fermentation to produce data for Learn.
- Task 5: Industry Engagement (*Babs Marrone/Chris Johnson/Phil Laible* – co-leads)
  - Identify barriers to industry adoption of synthetic biology technologies, expand number and diversity of industry partnerships, and establish a set of metrics for determining impact of project technologies on industry.
- Task 6: Management (*Blake Simmons* - lead)
  - Manage project management, develop internal and external communications, provide deliverables to BETO, and make some capital equipment purchases.

Project Management – Org Chart



Project Management – Communications

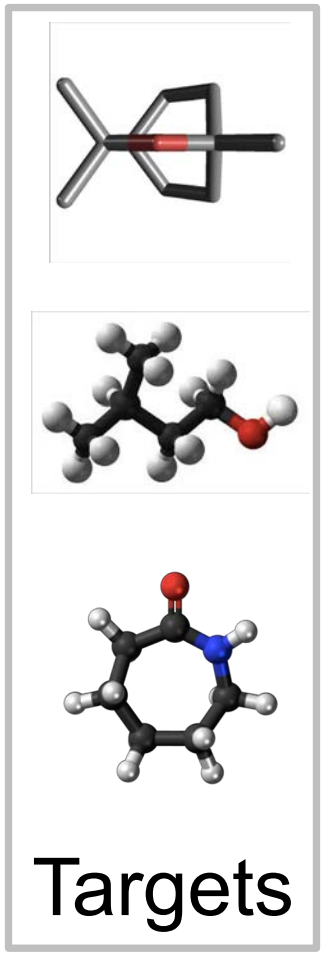
- **ABF is an integrated, geographically distributed multi-Lab team**
  - Effective communications are essential

- **Regular Internal Communications**
  - Bi-weekly Executive Committee meetings
  - Bi-weekly ABF Task Lead meetings
  - Weekly to monthly target/host pair meetings
  - **Weekly software infrastructure user meetings / webinars**
  - Monthly activity reports to BETO Technology Manager
  - Monthly activity summary including DBTL cycle reports to BETO
  - 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.)**
  - Sharepoint – file storage and sharing
  - Annual Learn Summit
  - Annual ABF Meeting

- **External Communications**
  - ABF website (agilebiofoundry.org)
  - Social media (@agilebiofoundry)
  - Presentations, posters, booths at domestic and international scientific / technical conferences
  - Publications
  - Quarterly Industry Advisory Board meetings and Industry Listening Days
  - Semi-annual Global BioFoundry Alliance meetings (pending)

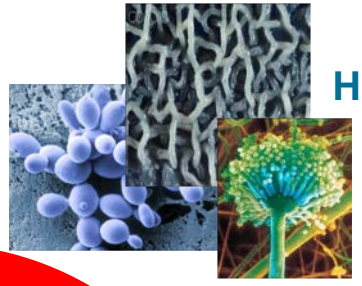
2 – Approach (Technical)

DBTL Infrastructure Enables the ABF Approach



Putative Targets

TEA/LCA



Host On-boarding

Design

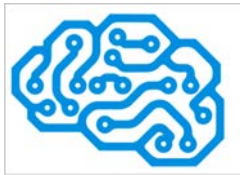
Build

Integration

Scheduler/LIMS

Test

Learn



Target Metrics Achieved

Scale-up:1-1000 L
Predictive toolkits

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

- **The ABF has a variety of teams that work together in a highly collaborative fashion to:**
  - Move target / host pairs 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)

- **Learn component**

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

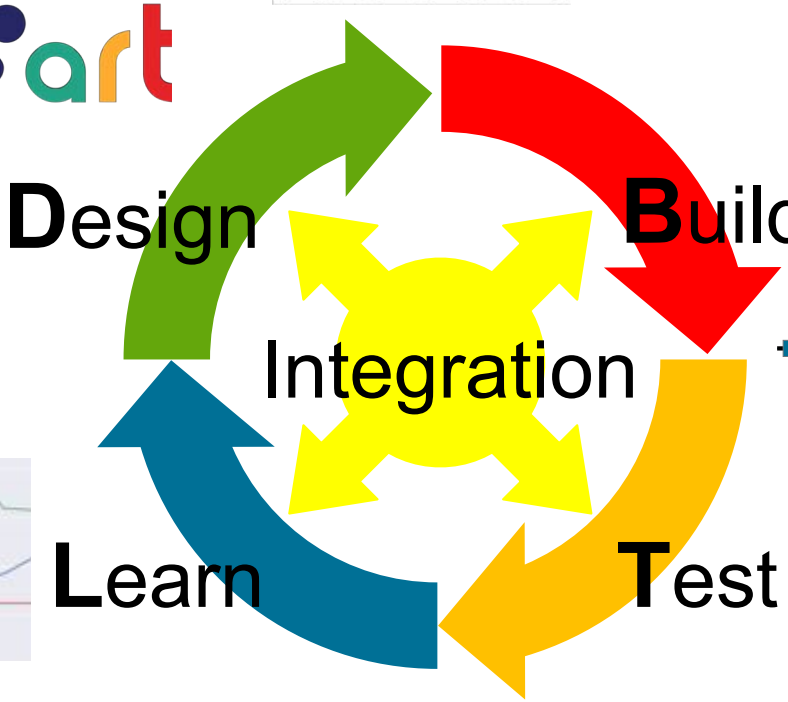
- **Integrated whole that might be separated in other projects**
  - Including Integrated Analysis (TEA/LCA), Host Onboarding, Scale-up

What are our Technical Risks and Mitigation Plans?

Risk	Severity	Description	Mitigation Plan
Designs do not work in selected host	Medium	Promoters / enzymes / pathways / etc. do not function as intended in the selected host.	Further test and learn from lack of function, and suggest design changes that could restore function
Lack of transferability of between target/hosts	Medium	Not able to leverage past efforts and learnings in one target-host pair for subsequent work in another	Further learn extents / likelihood of transferability
Infrastructure operating costs and value	Low	Costs of infrastructure (both hardware and software) maintenance and asset depreciation becomes unsustainable	Where possible, offload maintenance to more cost-effective and sustainable off-the-shelf vendor-supported solutions
Insufficient data to fully leverage Learn capabilities	Medium	Multi-omics datasets are not 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 Learn team Test data consumers during Design process to ensure Learn suitability of generated data

3 – Technical Accomplishments/ Progress/Results

Highlights – DBTL infrastructure



DiVA

art

Sequence Validation

EASy

Biosensors

Transcriptomics, Metabolomics, Proteomics, & Lipidomics

Data Quality Assessment

EDD

LabKey

Bayesian Inference: Metabolic Kinetics

Kinetic Learning

Metabolic Modeling

Deep Learning

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Design Highlight – ART Software

- **Automatic Recommendation Tool**
  - A machine learning tool for improving the effectiveness of strain engineering using probabilistic predictive modeling

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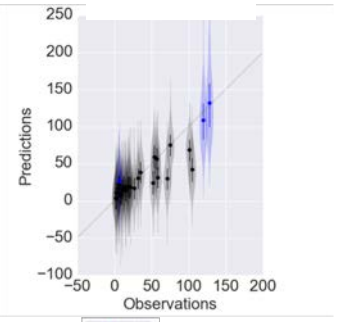
An Objective

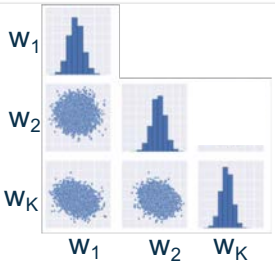
Recommended Designs

Machine learning

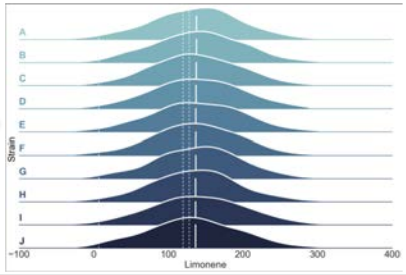


Model 1
Model 2
Model K
w_1
w_2
w_K
Ensemble Model

Bayesian ensemble modeling



Global optimization through Parallel Tempering



Probabilistic characterization of predictions

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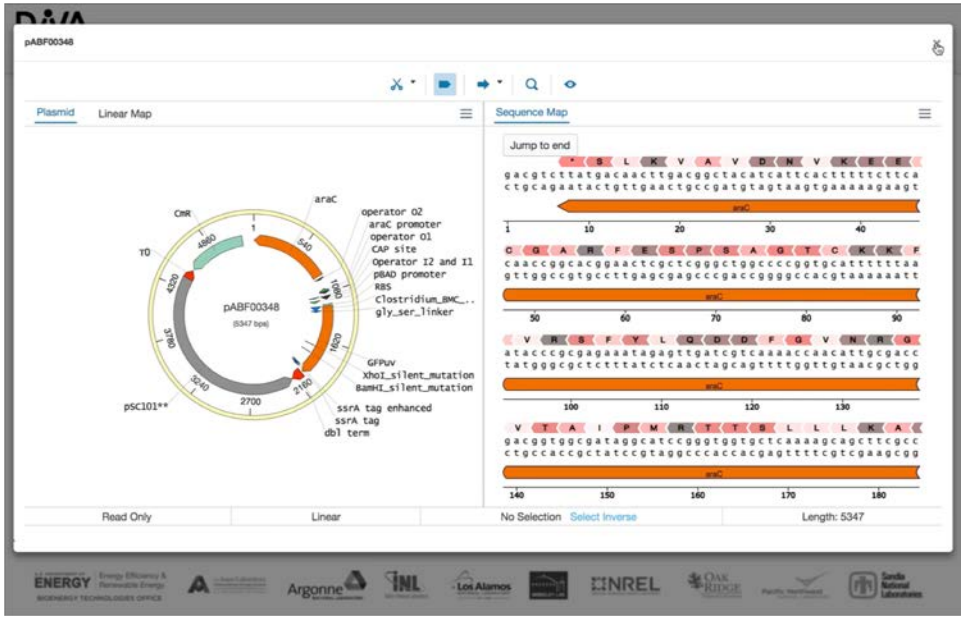
Design/Build Highlight – DIVA Software

- **Design Implementation Validation Automation**
  - Software platform that integrates tools for designing and building DNA constructs

- **Recent improvements**
  - Open VectorEditor integration
  - IP and BioSecurity questions
  - Users can now stop j5 runs in progress
  - Users can create their own custom DIVA teams



To the best of my knowledge, the sequences in this design:

- ☑ ***Will not infringe existing intellectual property***
- ☑ ***Have not been misappropriated***
- ☑ ***Will only be used for research purposes***

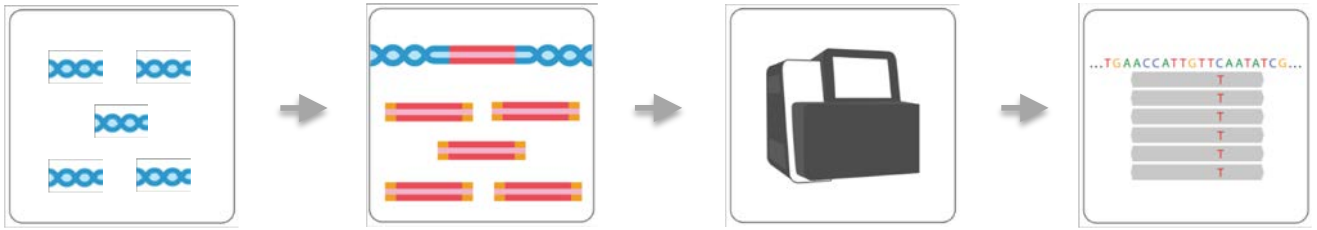
To the best of my knowledge, the sequences in this design:

- ☑ *Are not export controlled*
- ☑ *Are not a bio-security risk*
- ☑ *Do not require registration*

Jennifer Chiniquy	Sequences passed screening by BLiSS. Cost estimate is $757.65 (cloning, sequencing). The project ID should be provided by the PI. Note that the cost may be adjusted for troubleshooting. Pls provide necessary templates. Ex: False, Sec: False, Reg: False
Samuel Coradetti	Dropped one problematic sequence IP: True, Mis: True, Res: True

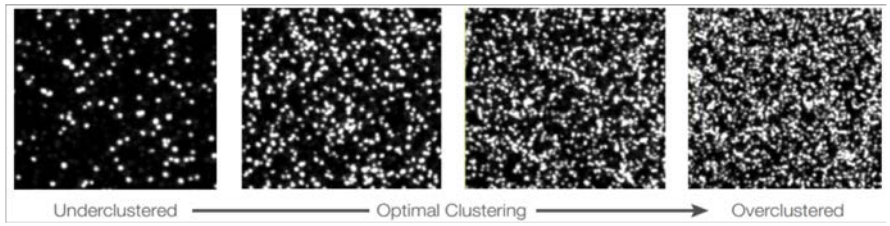
Build Highlight – DNA sequence validation

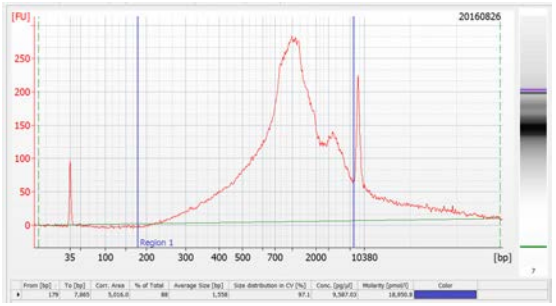
- **Overview**
  - $8 per sample (full amplicon/plasmid coverage, no oligos required)
  - Sample types: boiled cell culture, mini-prepped plasmid, PCR amplicon
  - 384 samples per week (1536 samples per week by end of March 2019)

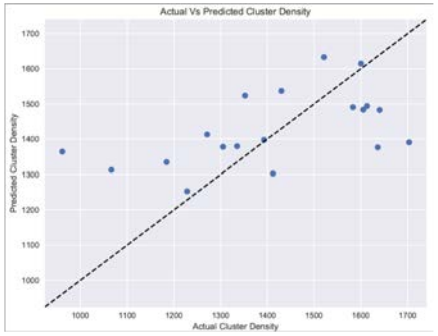


DNA input | Nextera preparation | MiSeq sequencing | Alignment analysis

- **Machine learning-enabled sample loading software tool**
  - Predict sample loading amount that will maximize sequencing reads (i.e., optimize MiSeq cluster density)







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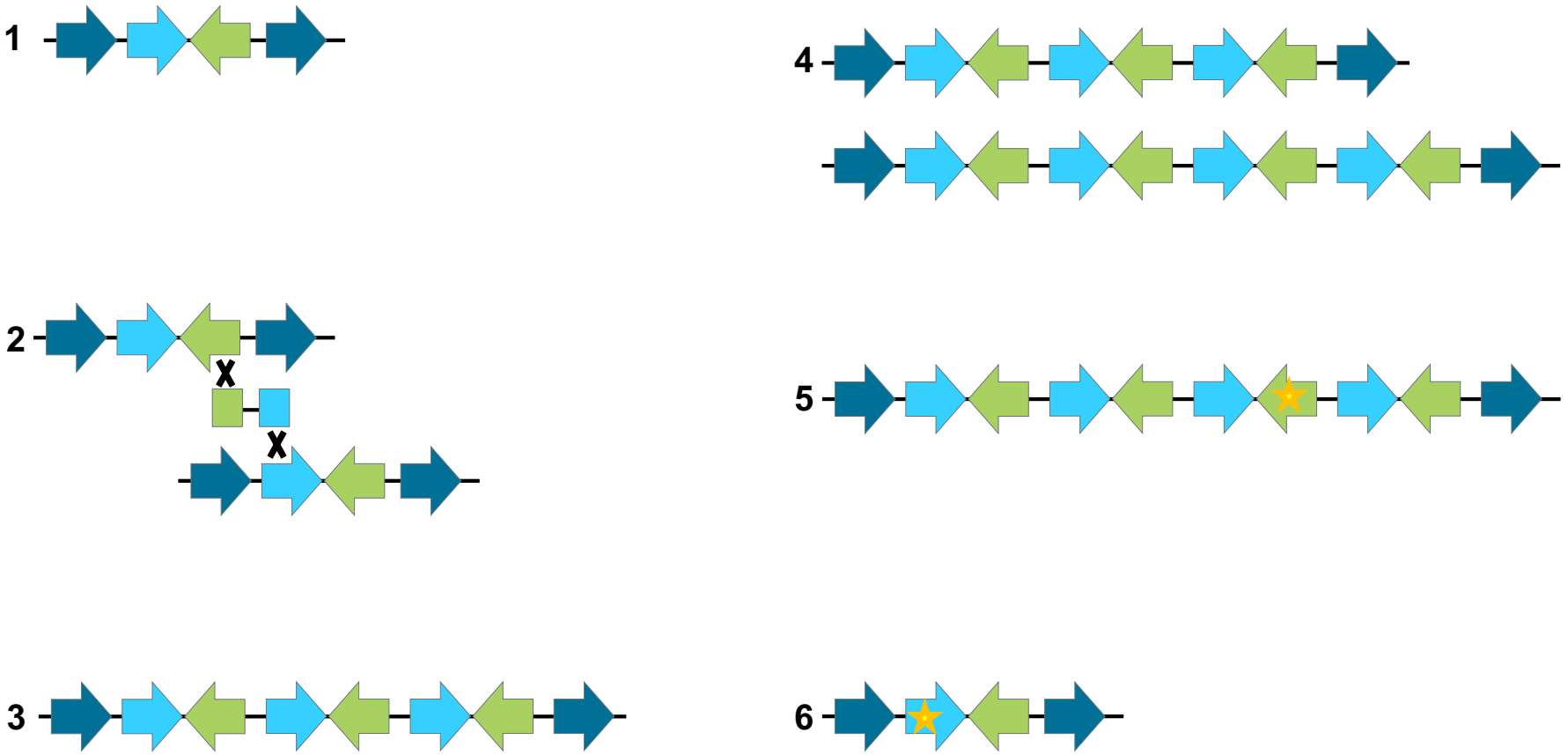
Build/Test Highlight – EASy

- **Evolution by Amplification and Synthetic biology**
  - Method developed by ABF collaborator Ellen Neidle (Tumen-Velasquez et al. *PNAS* 2018)
  - Can be applied to different target/host pairs, in combination with selection or screening
  - ABF is developing strategies to accelerate and improve the process



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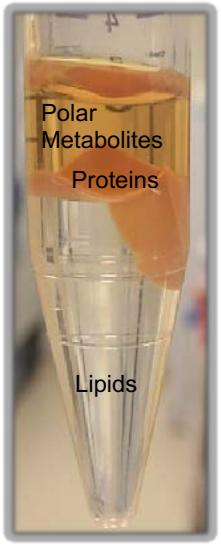
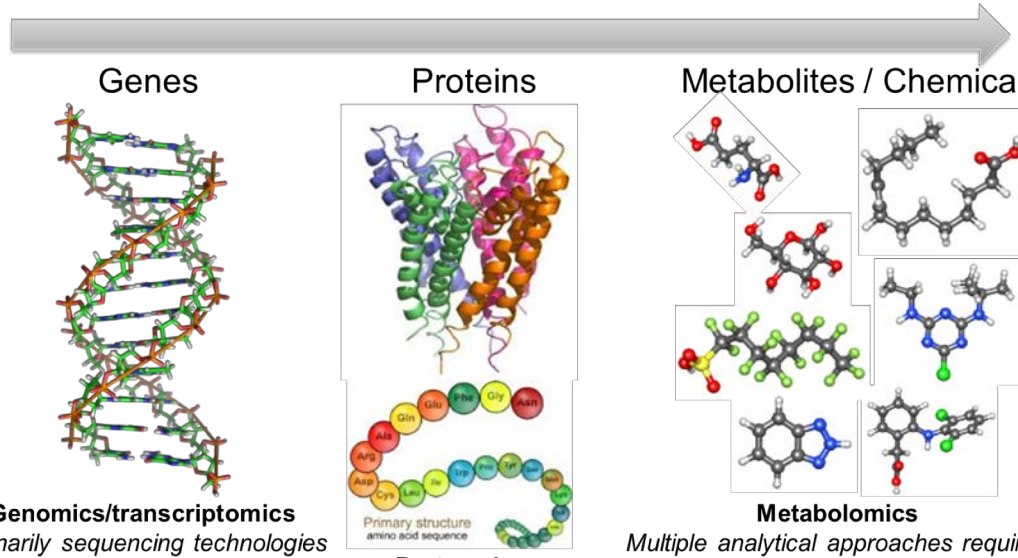
Test Highlight – Multi-omics Analysis

- **Single-sample Metabolite, Protein and Lipid Extraction**
  - Method developed previously: Burnum-Johnson *et al.* Analyst, 2017, 142, 442-448
  - Now used at the ABF

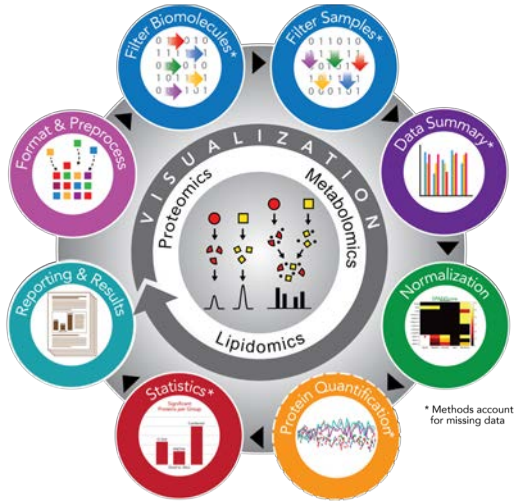




Genomics/transcriptomics
Primarily sequencing technologies

Proteomics
Global LC-MS/MS & targeted proteomics, whereby a complex sample is spiked with a stable isotope-labeled peptide that acts as internal standard

Metabolomics
Multiple analytical approaches required GC-MS, LC-MS, & ^{13}C metabolic flux analysis



Host	Proteomics (global + targeted)	Metabolomics/Lipidomics (Intra/Extracellular)
P. putida	>300 datasets	>500 datasets
A. pseudoterreus	>250 datasets	>450 datasets
R. toruloides	>250 datasets	>300 datasets

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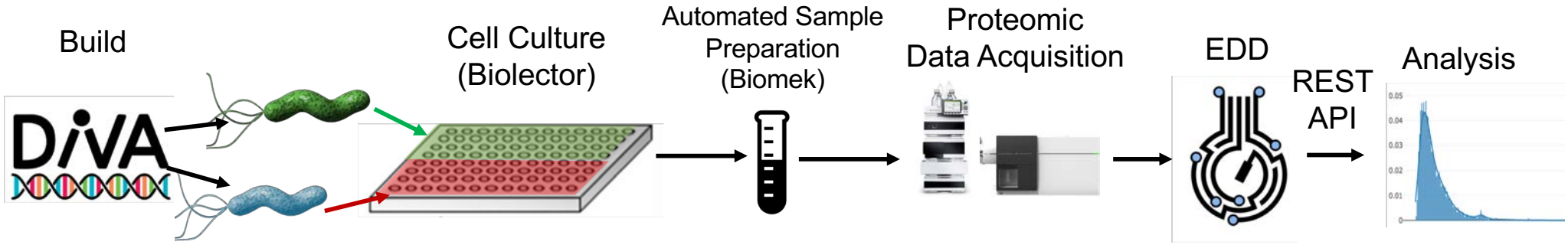
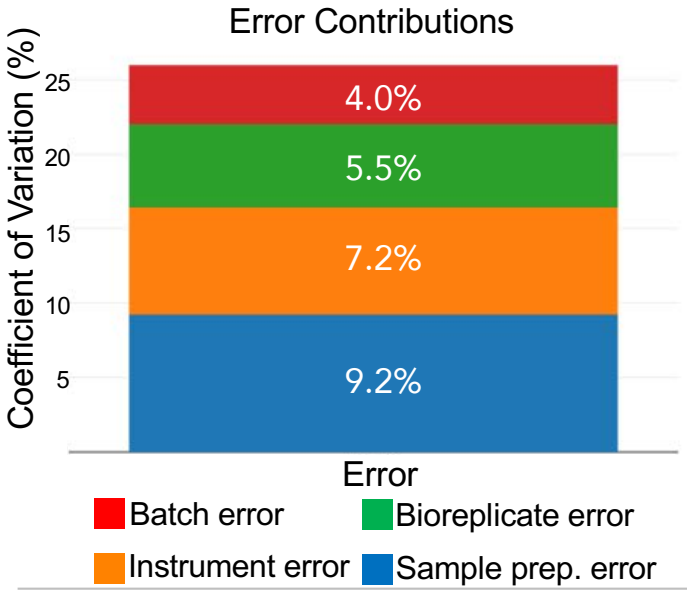
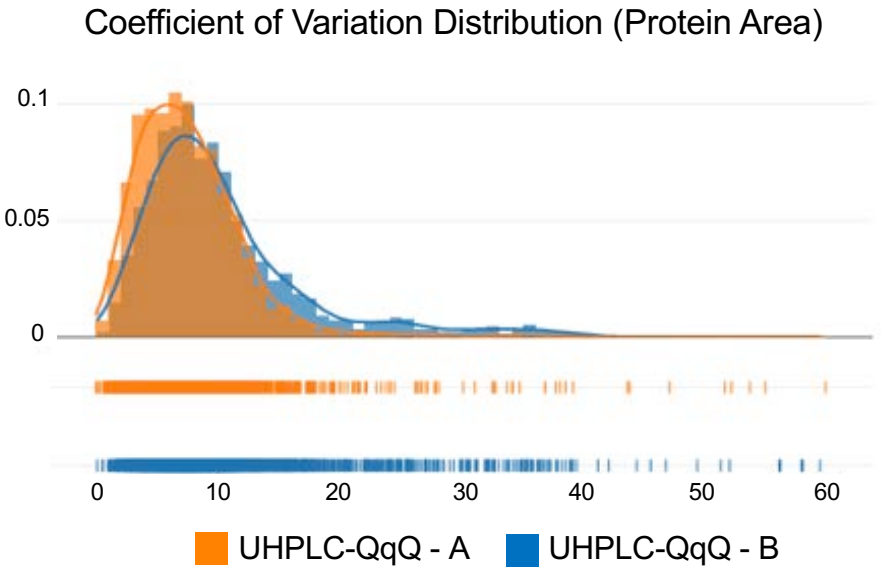
Test Highlight – Preliminary Targeted Proteomics Data Quality Assessment Efforts

57	3	4	2	5	>2000
Engineered strains	Biological replicates	BioLector batches	QQQ instruments	Technical replicates	**Total Samples**







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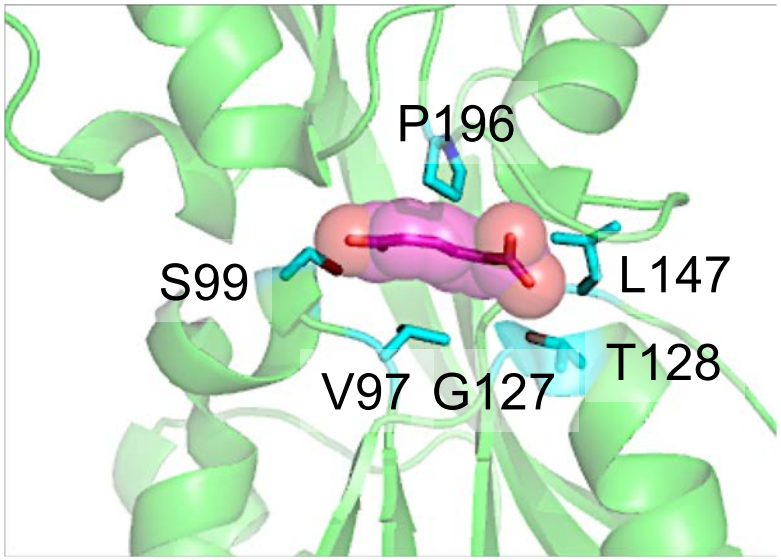
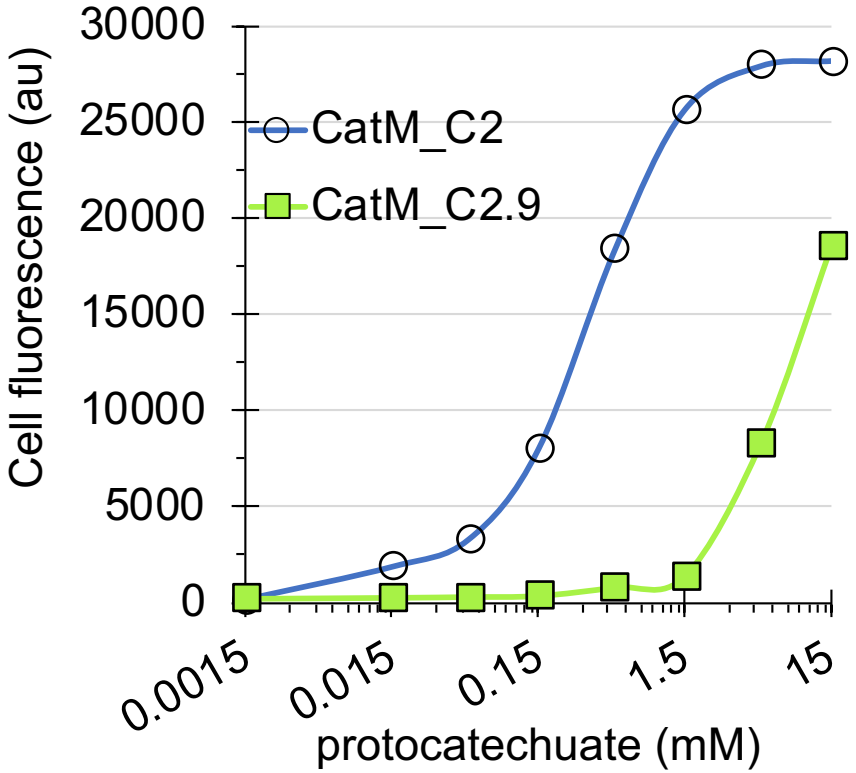
Test Highlight – Biosensors and Cytometry

- **Structure-based design to shift muconate biosensor dynamic range**
  - An engineered *P. putida* strain can import and bioconvert protocatechuate to muconate
  - CatM transcription factor activity is modulated through muconate binding
  - FACS positive/negative selection identified C2 *catM* promoter variant as starting point for *gfp* expression
  - FACS identified CatM mutant that shifts muconate-sensing dynamic range as desired



FACS



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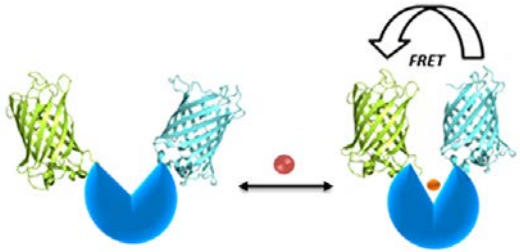
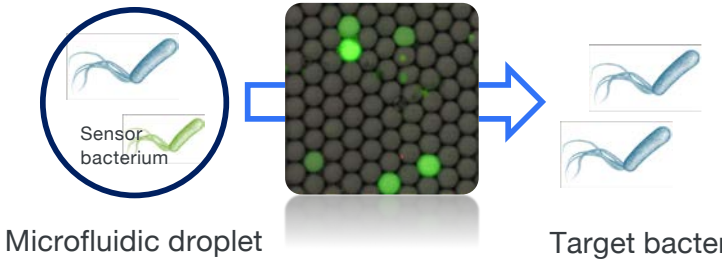
Test Highlight – Biosensors and Microfluidics

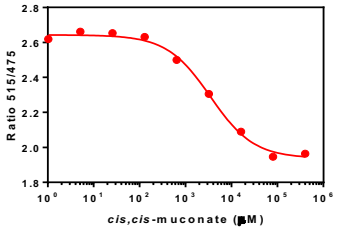
- **FRET Biosensors:**
  - Rapid, real–time signal response
  - Follow metabolic or catabolic (conversion) processes
  - Adaptable to a wide range of targets
  - Ratiometric signal is independent of sensor concentration



- **Enzyme-linked biosensors:**
  - TF sensors with >100-fold increase in signal to noise



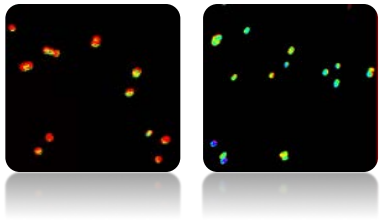
- **Diverse experimental approaches:**
  - In vitro measurements



Titration of biosensor with cis,cis-muconate

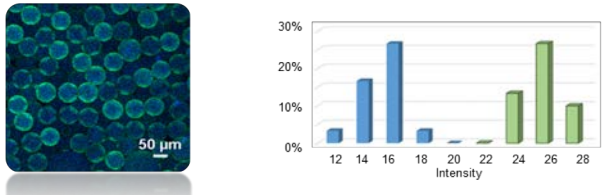
  - In vivo measurements

- muconate + muconate



Images of P. putida expressing biosensors

  - Detection in microfluidic droplets



Biosensor-containing droplets sorted by muconate concentration

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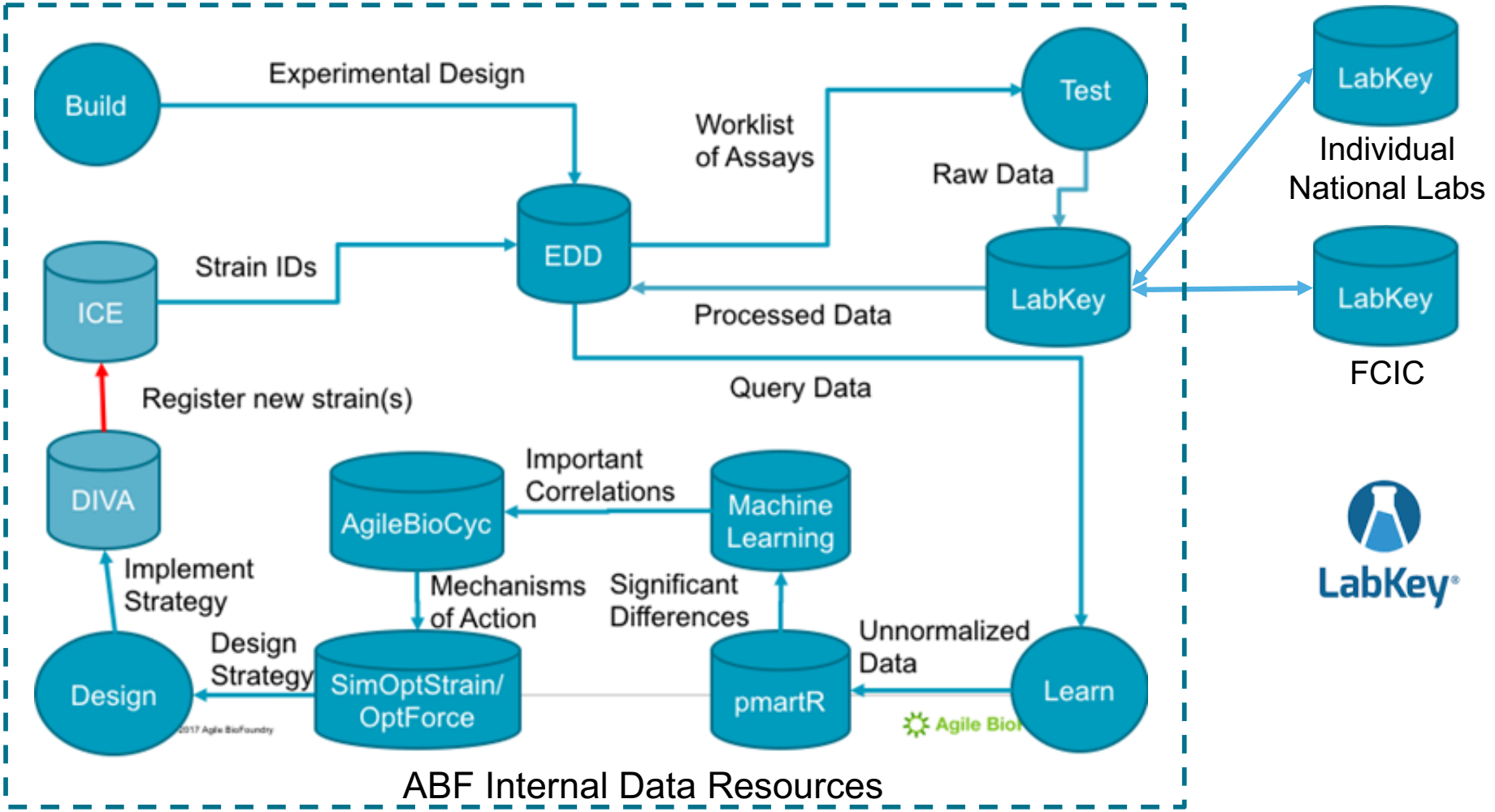
Test Highlight – LabKey Software

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



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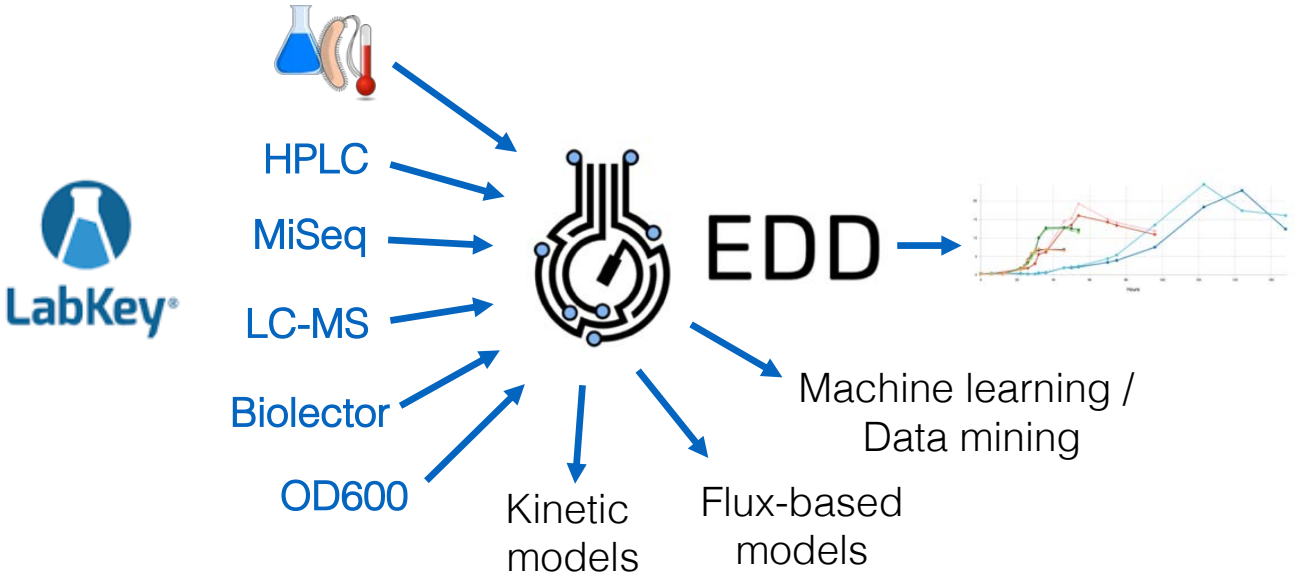
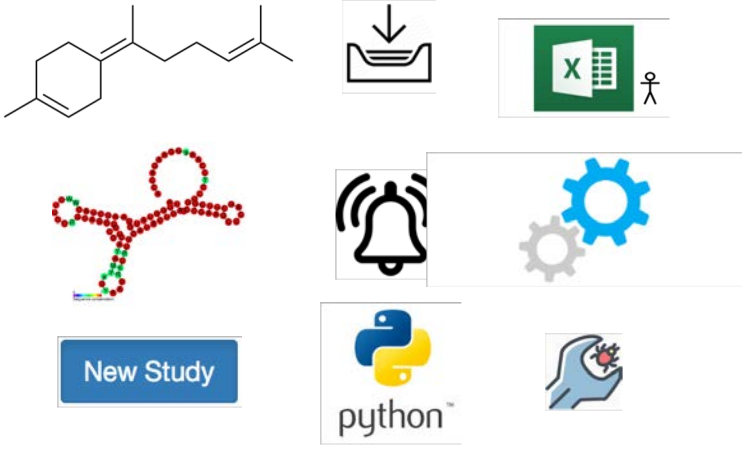
Test/Learn Highlight – EDD Software

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

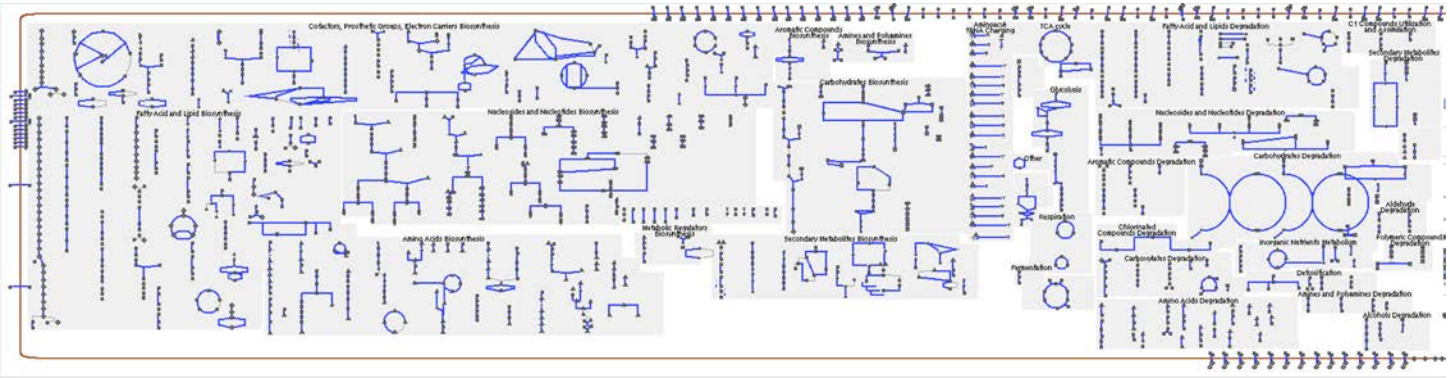


- **Recent improvements**
  - Designed replacement import process, targeted proteomics beta test
  - PubChem-based Metabolomics pipeline implemented and tested
  - Initial transcriptomics pipeline (beta)
  - Streamlined study creation
  - Support for larger file imports
  - Browser & email notifications for long-running tasks (e.g. import)
  - Added metadata types for use by PNNL, NREL, ABPDU
  - REST API for data export / search (e.g. from Jupyter)
  - Python 3 migration – long-term maintainability
  - Many minor improvements & bug fixes



Learn Highlight – Metabolic network reconstruction and modeling

- **AgileBioCyc Pathway/Genome Database (cyc.agilebiofoundry.org)**

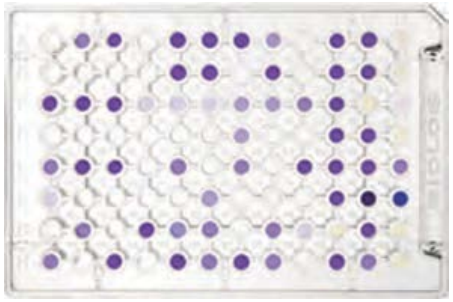


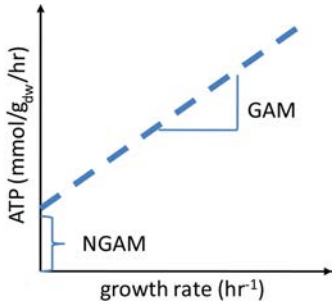
BioCyc Database Collection

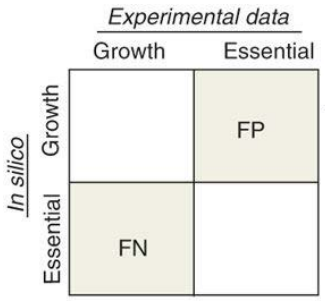
Pathway Tools

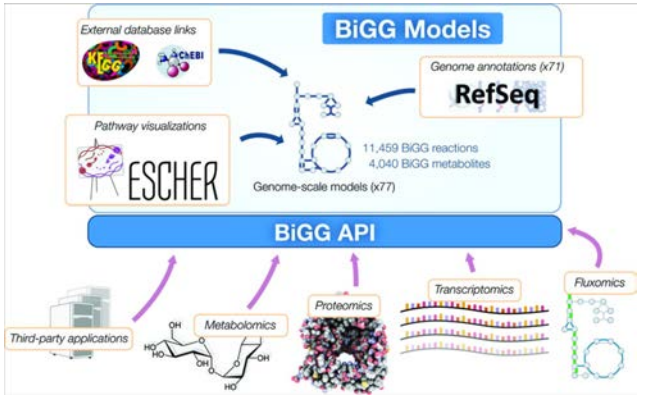
- **Metabolic model building using BiGG Models and openCOBRA**
  - High quality metabolic models with gene association
  - Orthologous gene mapping from OrthoMCL
  - Metabolic map building and omics data visualization

- **Experimental validation and refinement**
  - Biomass composition and ATP-maintenance
  - Growth phenotyping and gene essentiality analysis









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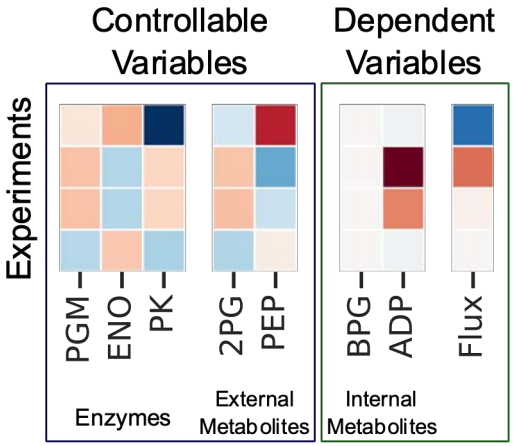
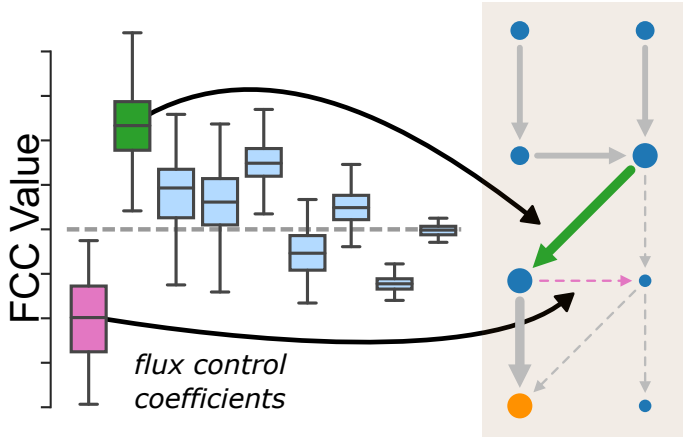
Learn Highlight – Bayesian inference of metabolic kinetics from multi-omics data

- Infer probabilistic relationship between variables we can control (enzyme expression; media composition) and those we cannot (intracellular fluxes and metabolomics)

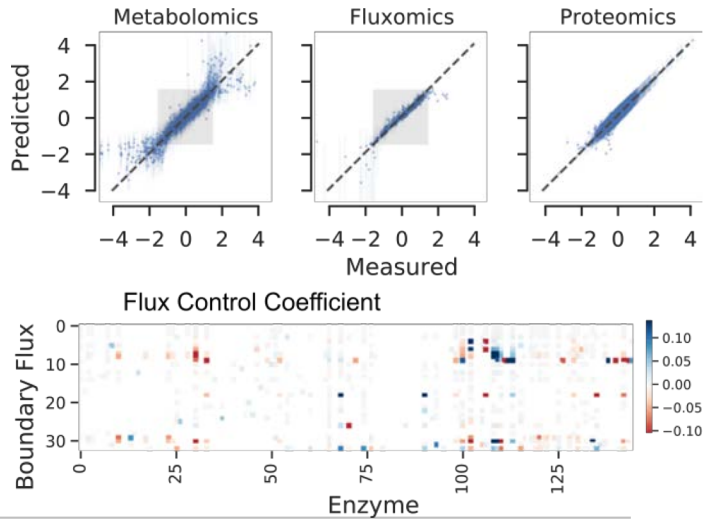


- Finds parameters for kinetic model that reproduces observed steady-state data
- Distributions in ***Flux control coefficients*** *(FCCs)* can be found to determine which enzymes to over/under-express to achieve a desired metabolic phenotype



- Method can be scaled to genome-scale models and multi-omics datasets (*right*)

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



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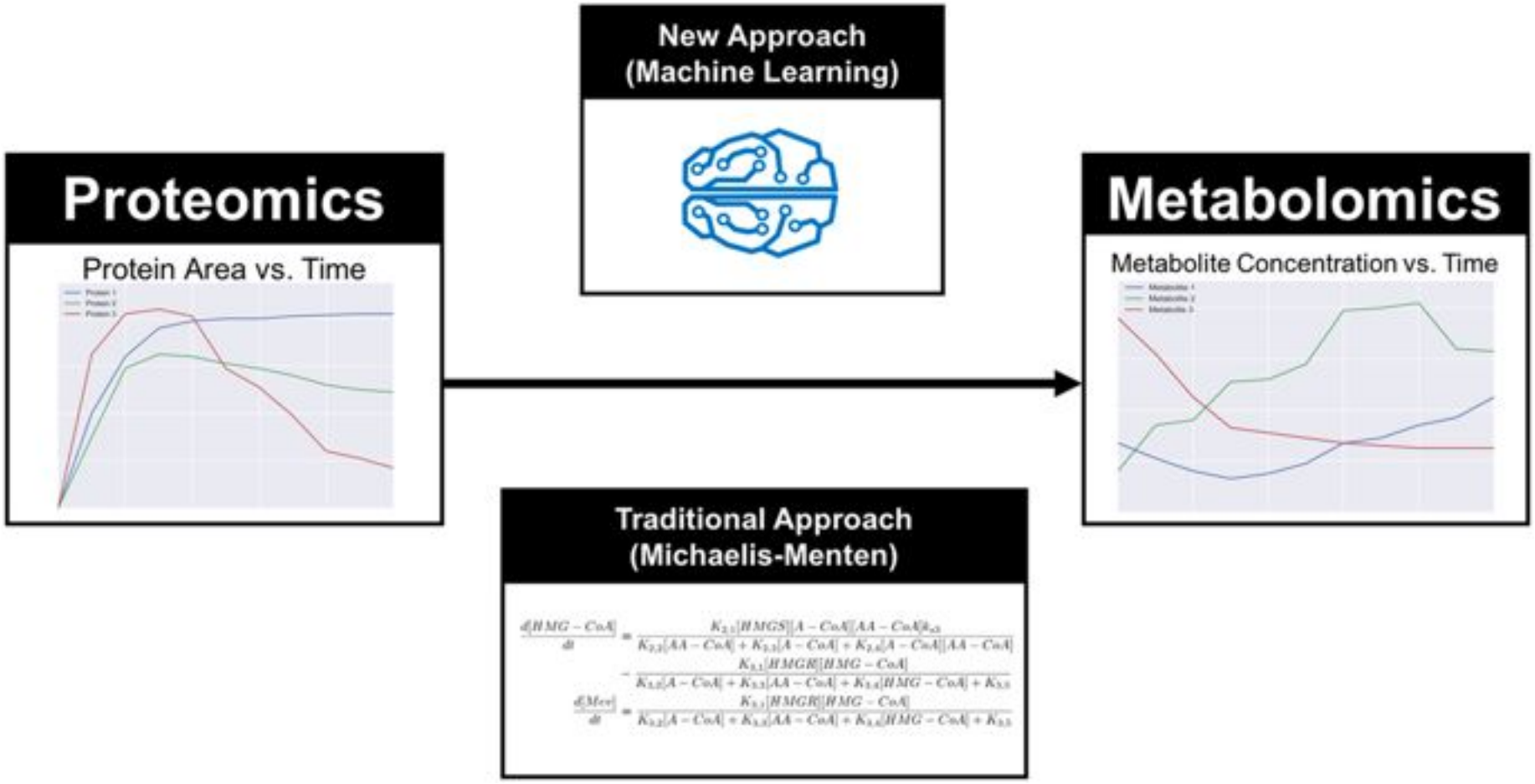
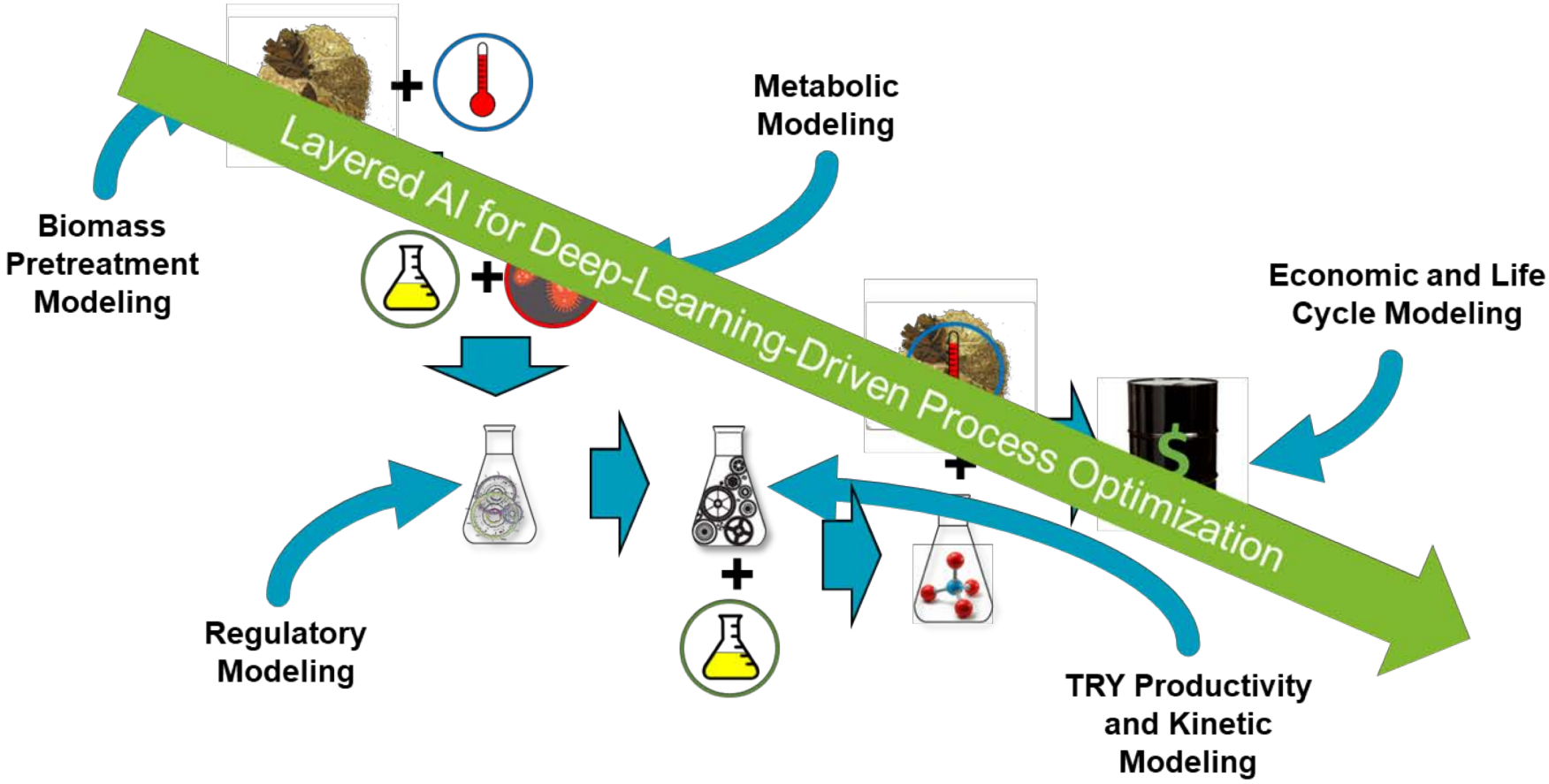
Learn Highlight – Kinetic Learning

- **Additional New Machine Learning Technique**
  - A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data



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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FY17/18 Project Milestones Completed

Highlighted FY17/18 ABF Milestones relied upon DBTL Infrastructure

- **FY17 Annual SMART milestone**
  - Demonstrate the Agile BioFoundry process by **successfully completing one or more Design, Build, Test, Learn cycles** for 5 molecules in their designated onboarded hosts, hitting baseline titers of 100 mg/L in mock or DMR-EH hydrolysate for at least 2 molecules.

- **Go/No-Go Decision, Q2 FY18**
  - Demonstrate process integration and scaling in 2 L bioreactors in DMR-EH hydrolysate using a target molecule introduced into the BioFoundry in FY17 with a target titer of at least 1 g/L.

- **FY18 Annual SMART milestone**
  - From a set of 10 target molecules, demonstrate successful production of 40% with titers for FY18 target molecules of at least 100 mg/L in mock or DMR-EH hydrolysate, and titers for FY17 target molecules of at least 500 mg/L in DMR-EH hydrolysate.

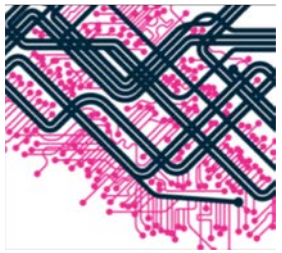
FY19 Milestones Completed

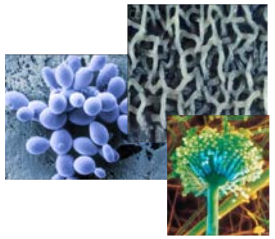
Milestone (synopsis)	Task	FY19 Quarter	Type
Selections of new target molecule & existing molecule in different host	Target/Host	Q1	Quarterly (Regular)
4X Build sequence validation capacity increase from FY18 to FY19	DBTL Infrastructure	Q2	Quarterly (Regular)
TEA and LCA on new FY19 target molecule	Integrated Analysis	Q2	Quarterly (Regular)
Deep Learning non-intuitive predictions	DBTL Infrastructure	Q2	Quarterly (Regular)
Titer goals in range of 1 to 10 g/L	Target/Host	Q3	Quarterly (Regular)
Transformation in new organism(s)	Host Onboarding	Q3	Quarterly (Regular)
5X Test capacity increase from FY17 to FY19	DBTL Infrastructure	Q3	Quarterly (Regular)
Promoters in new SOT organisms	Host Onboarding	Q4	Annual (Regular)
10L scale using DMR-EH hydrolysate, with 10 g/L, 100 mg/L/h, 40% yield	Process Integration & Scaling	Q4	Annual (Regular)
SWOT Analysis	Industry Engagement & Outreach	Q4	Annual (Regular)
DBTL Activity, Quarterly/Milestone, and final AOP reports sent to BETO. Updates to ABF website	Management	Q4	Annual (Regular)
Value of non-intuitive Learn predictions demonstrated	Target/Host	Q4	Go/No-Go

4 – Relevance

Relevant Outcomes

- 50% reduction in time-to-scale up compared to the average of ~10 years
- 10X improvement in Design-Build-Test-Learn cycle efficiency
- Public infrastructure investment that increases U.S. industrial competitiveness and enables new opportunities for private sector growth and jobs
- New IP and manufacturing technologies effectively translated to U.S. industry ensuring market transformation
- New industrially relevant, optimized chassis organisms for fuel and chemical production





The Agile BioFoundry is complementary to BETO's other projects

- BETO's projects frequently target specific molecules/hosts

- In contrast, the **Agile BioFoundry** is a **broadly enabling platform**
  - **Applicable across biorefinery** fuel or chemical production **processes**
  - **Other BETO projects could leverage** Agile BioFoundry capabilities
    - Methods, workflows, instrumentation, software, expertise
    - Accumulated enzyme/pathway/host/process learnings and data

- Agile BioFoundry development/assessment through several use cases
  - Sufficient number/diversity of molecules/hosts to demonstrate broad utility

Connections to other BETO projects

- **Other BETO consortia**
  - Continue to integrate TEA/LCA support across consortia
  - Closer collaborations to further inform the DBTL cycle
  - ChemCatBio: catalytically convert ABF molecules into value-added compounds
  - SepCon: secreted hydrophobic, acid, and intracellular products recovery
  - FCIC: understanding the effect of feedstock variability on strain robustness
  - Performance-Advantaged BioProducts: ABF molecules could be used

- **BETO State Of Technology (SOT)**
  - Improve genetic tools for SOT organisms to accelerate & increase DBTL cycle efficiency

- **Application of Energy I-Corp Learnings**:
  - Better Utilization of Real-time Data for in-line process Control
  - Predictive Scale-Up studies in lab-scale bioreactors

ABF and other DOE programs

- **Complementary ABF domain expertise, infrastructure, and operational TRL range offer opportunities for synergy with other DOE programs**
- **The ABF is open to working with other DOE funded projects and centers, such as the BRCs and EFRCs**
  - Target/host suggestions for ABF
    - Scientists can propose biofuel and bioproduct targets for the ABF to work on and further optimize
  - Technology off-ramping into ABF
    - Early stage DBTL infrastructure (e.g. software, devices, methods) and microbial hosts can be brought into the ABF and further developed and operationalized
  - Shared technical challenges collaboratively addressed
    - Example: experiment data storage and dissemination – EDD co-development
    - Resulting resources made accessible across projects – *P. putida* mutant libraries
  - Provide compelling examples of DOE teams working together
    - Across TRLs and bridging the gap between fundamental and applied science and technology
  - Enhance technology transfer and commercialization efforts

Working with Industry: FY17 Direct-Funded Opportunities and FY18 BEEPS FOAs

- **Poster Session (Tuesday March 5)**
  - Will include presentations on the ABF DFO projects (be sure not to miss them!)
- **Process and Management**
  - Will be discussed in ABF Directed Funding Opportunities and Partnerships presentation
- **Why these projects and BETO investments are so important**
  - Expand the range of ABF targets and hosts
  - Stress-test ABF capabilities and identify weaknesses and opportunities
  - Bring new technologies in to the ABF and opportunities to license ABF technology out
  - Early stage investments that will be crucial to the ABF accomplishing its overall goal and its desired outcomes (many relate directly to industry impact and technology transfer)
  - Ensure that ABF development is responsive to industry
  - Increase industry exposure (beyond funded companies) to the ABF and its capabilities
  - Quantitatively demonstrate industry interest in leveraging the ABF
- **New for FY19: template ABF CRADA**
  - Publicly accessible from the ABF website: https://agilebiofoundry.org/work-with-us/
  - Non-negotiable for projects receiving DOE funding
  - Includes new "Extended Non-Exclusive Option" IP model

Relevance to Industry (in their own words)

CORRECTING and REPLACING – Lygos Partners with Agile BioFoundry and U.S. Department of Energy to Accelerate BioProduct R&D and Commercialization

Two-year, $1.43 million pilot collaboration to automate microbial engineering research technology

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October 20, 2017 16:00 ET | **Source:** Lygos

In a release issued under the same headline on October 17th by Lygos, Inc., please note that the subheadline contained an incorrect value for the pilot collaboration and that a quote from John Gladden of Sandia National Laboratories was not included. These have been corrected below, and a new description of Agile BioFoundry has also been included. The corrected release follows:

BERKELEY, Calif., Oct. 20, 2017 (GLOBE NEWSWIRE) -- Lygos, a leading innovator in the development of sustainable high-value specialty chemicals, announced today that the U.S. Department of Energy is providing multi-year funding for Lygos' collaboration with the Agile BioFoundry (ABF) to automate research technology. Lygos' pilot collaboration is part of a two-year, $5 million, multi-company effort coordinated by the ABF.

Profile
Lygos

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Berkeley, UNITED STATES
http://www.lygos.com

"This DOE funding underscores the importance of our work with the Agile BioFoundry," said Jeffrey Dietrich, Lygos' Chief Technology Officer. "Harnessing the power of microbes to produce important chemicals requires a less expensive, faster engineering cycle as well as new technologies to more effectively interrogate microbe performance. By pairing Lygos' expertise designing, building, and optimizing pathways with the ABF's capabilities in advanced automation we'll be able to dramatically decrease the time required to commercialize new microbial products."

total of $1.43M in funding over two years, $1M of which the DOE will provide for work on the project at the ABF and the remainder to be provided by Lygos in support of its in-house R&D.

"A primary goal of the ABF is to accelerate bioproduct development and deployment into the market to enable rapid growth of the bioeconomy," said John Gladden of ABF consortium member Sandia National Laboratories, "and this opportunity to work closely with industry partners like Lygos helps demonstrate the real-world impact of the ABF while providing the ABF invaluable feedback, laying the groundwork for future ABF partnerships with industry and other interested parties, especially as companies like Lygos bring products to market more rapidly because of collaborations like this."

"We're impressed with the capabilities, expertise, and elite staff at the Agile BioFoundry, and we're excited about increasing the power of our research to create more products and serve more customers," said Eric Steen, CEO, Lygos. "The past five years have seen revolutionary reductions in the time and cost of engineering biology. Working with the ABF, I think we can do even more over the next five years."

5 – Future Work

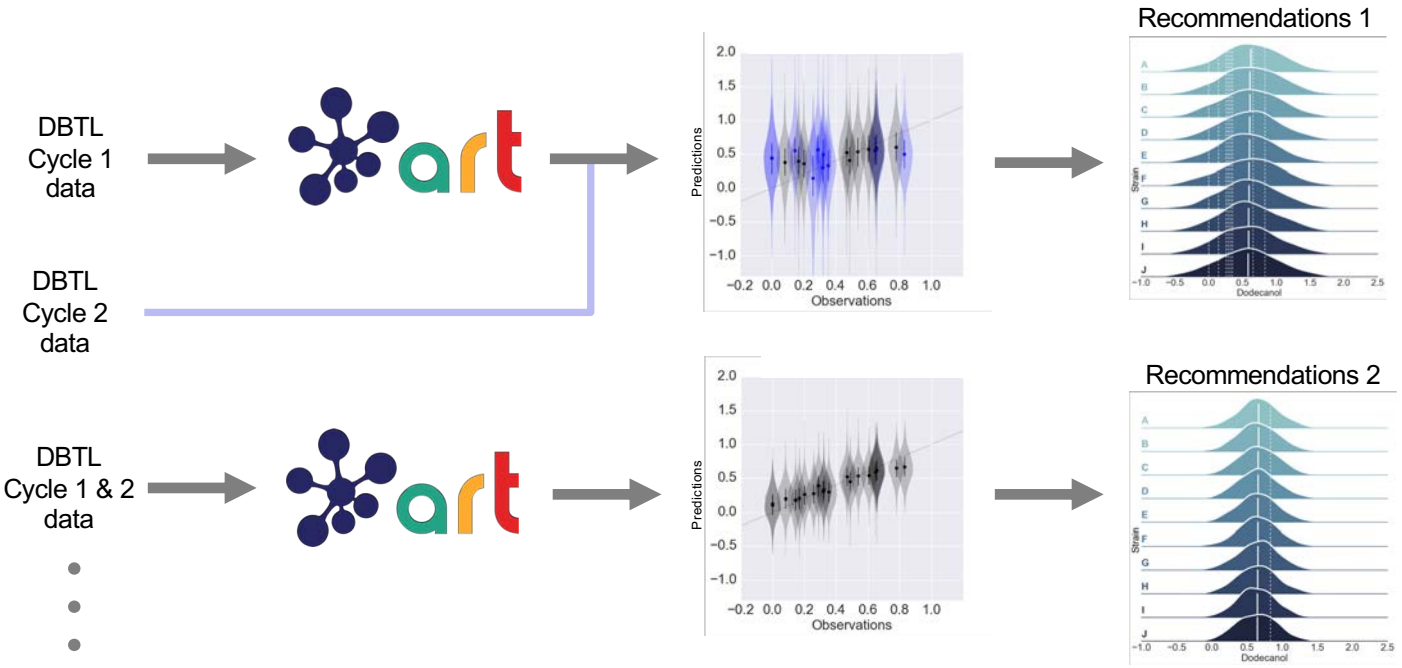
How we are thinking about our future work

- **We have a long term strategic vision for the ABF DBTL Infrastructure**
- **Our future work will focus on the technical and operational barriers to achieving the overall ABF goal and its desired outcomes**
- **Some challenges facing the ABF:**
  - Show Learn can add value through non-intuitive predictions
  - Demonstrate industry-relevant ABF competencies across targets and hosts
  - Onboard new hosts and develop tools for them
  - Increase DBTL cycle capacities and efficiencies; reduce cycle time
  - Keep current strength / weakness / opportunities / threat (SWOT) assessments
  - Demonstrate reproducible geographically distributed unit operations
  - Find sustainable ABF IP / licensing / contracting model(s)
  - Demonstrate that past work and Learnings increase the efficiency of new work
- **Next slides show our current planned FY19 activities**
  - DBTL Infrastructure contributions to FY19 and pending FY20-22 milestones then follow

Design Highlight – ART Software

- **Automatic Recommendation Tool**
  - A machine learning tool for improving the effectiveness of strain engineering

- **Next steps**
  - Development of testing modules
  - Web interface
  - Command-line execution
  - Docker container execution
  - Extension for classification problems; discrete/categorical input variables
  - Incorporation of cost constraints into objective function
  - Using ART's predictions for optimal media additions to DMR for FOH production in *R. toruloides*



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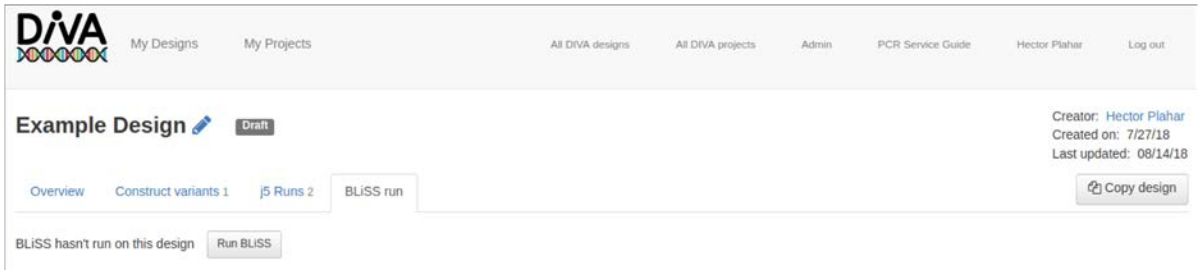
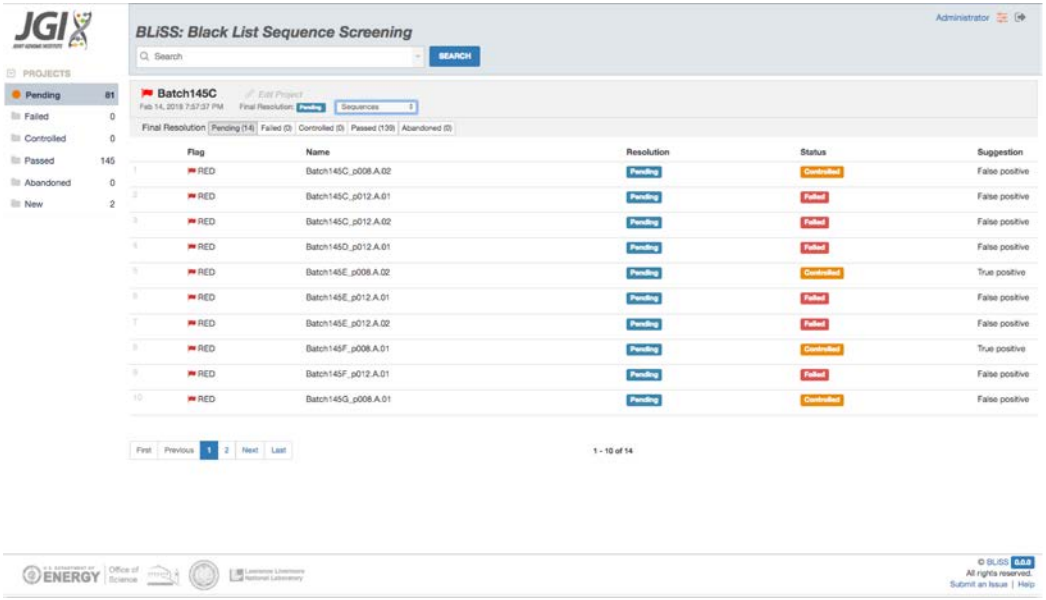
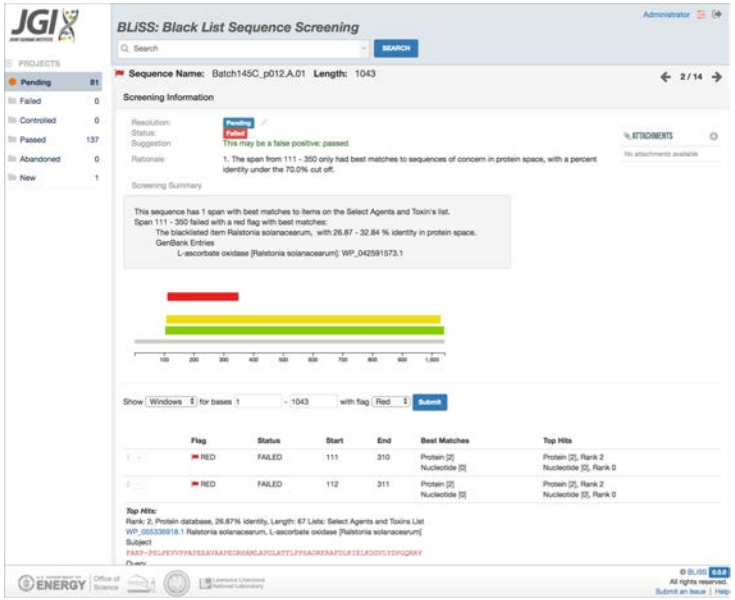
Design/Build Highlight – DIVA Software

- **Design Implementation Validation Automation**
  - Software platform that integrates tools for designing and building DNA constructs

- **Next steps**
  - Public DIVA server (https://public-diva.agilebiofoundry.org)
  - Web of DIVAs
  - BLiSS integration







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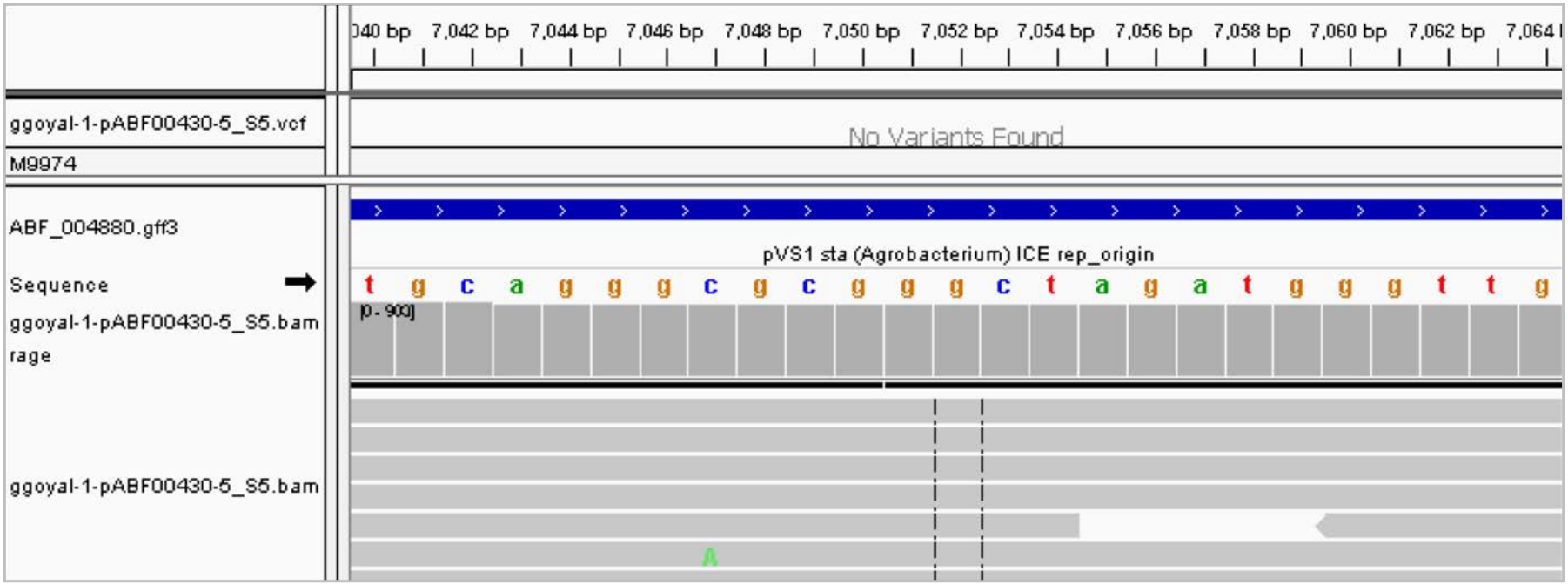
Build Highlight – DNA sequence validation

- **Next steps**
  - Automatically update DNA sequences to match MiSeq data, annotating deviations from intended sequences
  - Embed IGV genome browser within ICE Registry Platform to visualize sequencing data
  - Automated analysis pipeline: alignments, variant calling, de novo assemblies



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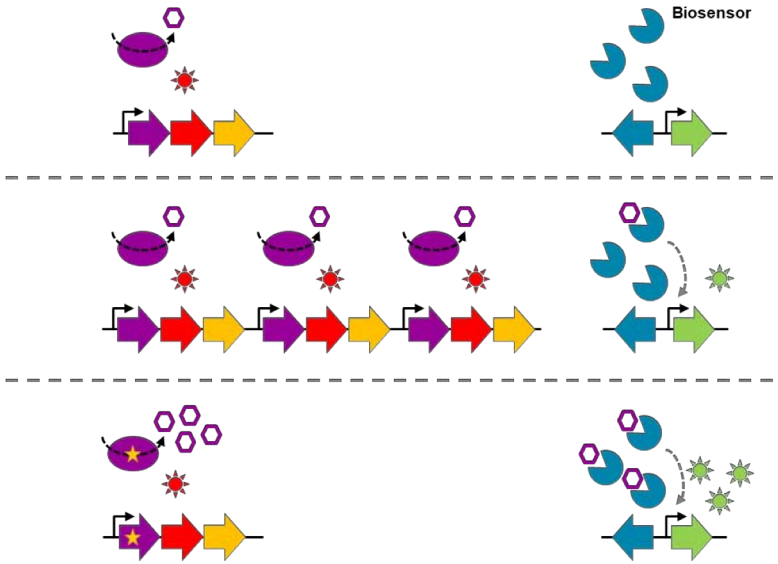
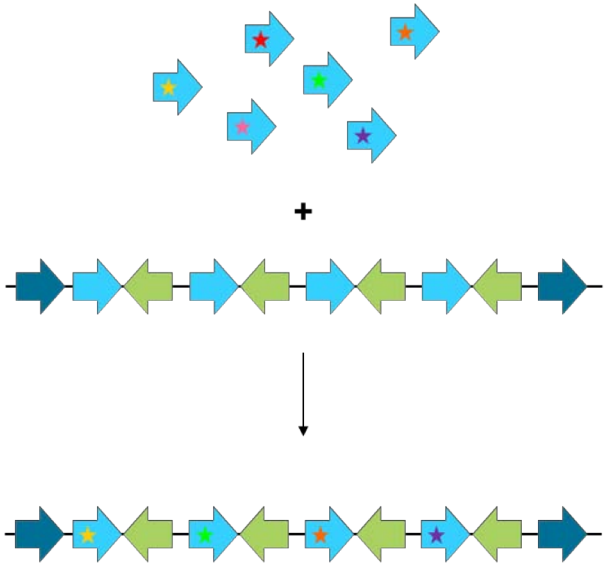
Build/Test Highlight – EASy

- **Next steps**
  - Combine EASy with fluorescence-activated cell sorting and biosensors (ABF-Neidle lab CRADA)
  - Accelerate EASy with mutant DNA libraries for fast screening of beneficial mutations





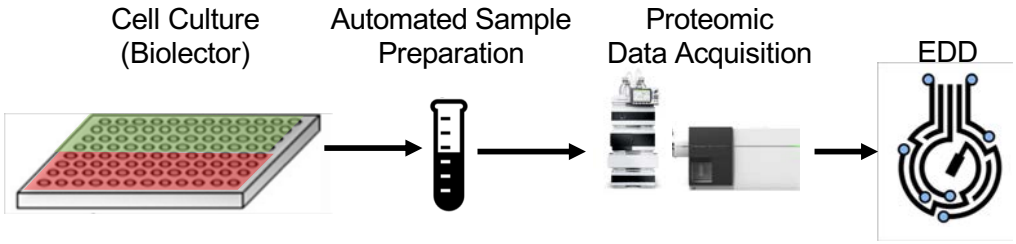
Test Highlight – Multi-omics analyses

- **Global and Targeted Proteomics**
  - Addition of isotopically heavy peptides for more targeted proteomics
  - Close to absolute quantitation for these target protein/peptides
  - Data quality performance metrics and a confidence metric for direct comparisons across all datasets, with more statistical power
  - Automation of pipeline for fast analysis

- **Metabolomics and ^{13}C metabolic flux analysis**
  - Increase of detection coverage with addition of new chemical standards
  - Improvement of metabolic models of target hosts
  - Faster and accurate QA/QC on metabolite identification by improvement of workflow

Test Highlight – Targeted Proteomics Data Quality Assessment and Improvement

- **Next steps**
  - Identify and eliminate sources of error in cell culture, sample preparation, and data acquisition workflows



  - With the Learn team, establish data quality metrics for proteomic and metabolomic workflows

  - Extend automated sample preparation to new ABF host organisms

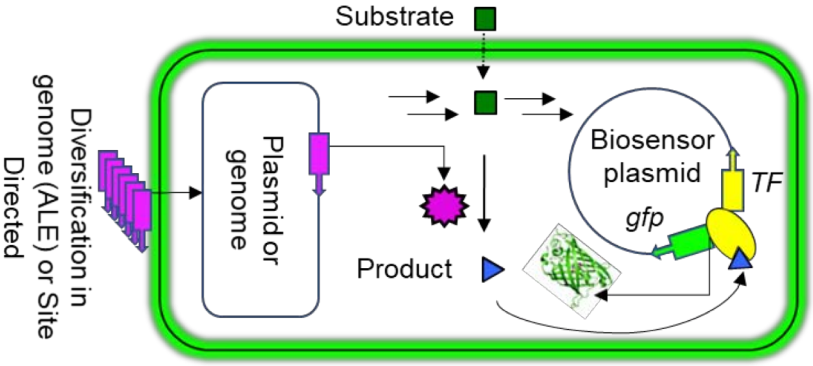
Test Highlight – Biosensors

Biosensor frameworks for strain engineering and pathway optimization

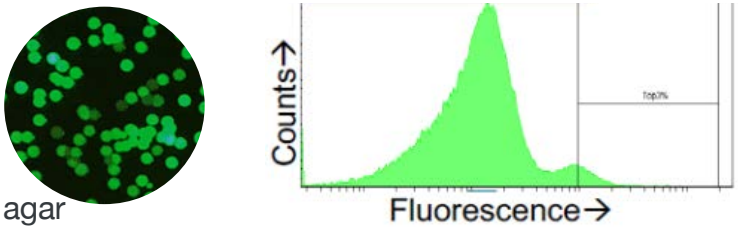
– Versatile platforms for rapid generation of transcription-factor-based and FRET biosensors
– Useful for a range of products and metabolic intermediates of new targets and host pairs of the ABF

TF biosensors for strain optimization

- Reports on *in vivo* metabolic activity
- Can be tuned for different target concentrations
- Coupled to flow cytometry for rapid isolation of top performers.

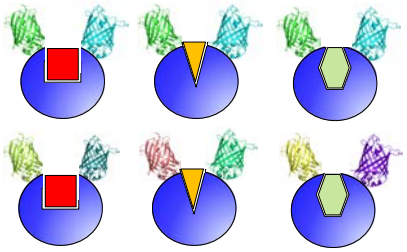


Observe variation in fluorescence level



Expansion of the suite of FRET-based ligand reporting systems

- Tailored ligand binding protein for biocatalyst engineering
- Tailored FP pair driven by application



Scouting for new ligand recognition domains expands sensing of the expanding ABF target repertoire

Next steps

– Expansion of ligand-sensing domains
– Miniaturization of screening methodologies for sensor optimization
– Broad host utility and adaptations for new hosts
– Interface with Test and Learn infrastructures
– Continued optimization of cytometric and microfluidic strategies for high-throughput *in vitro* and *in vivo* screening using transcription factor and FRET-based biosensors

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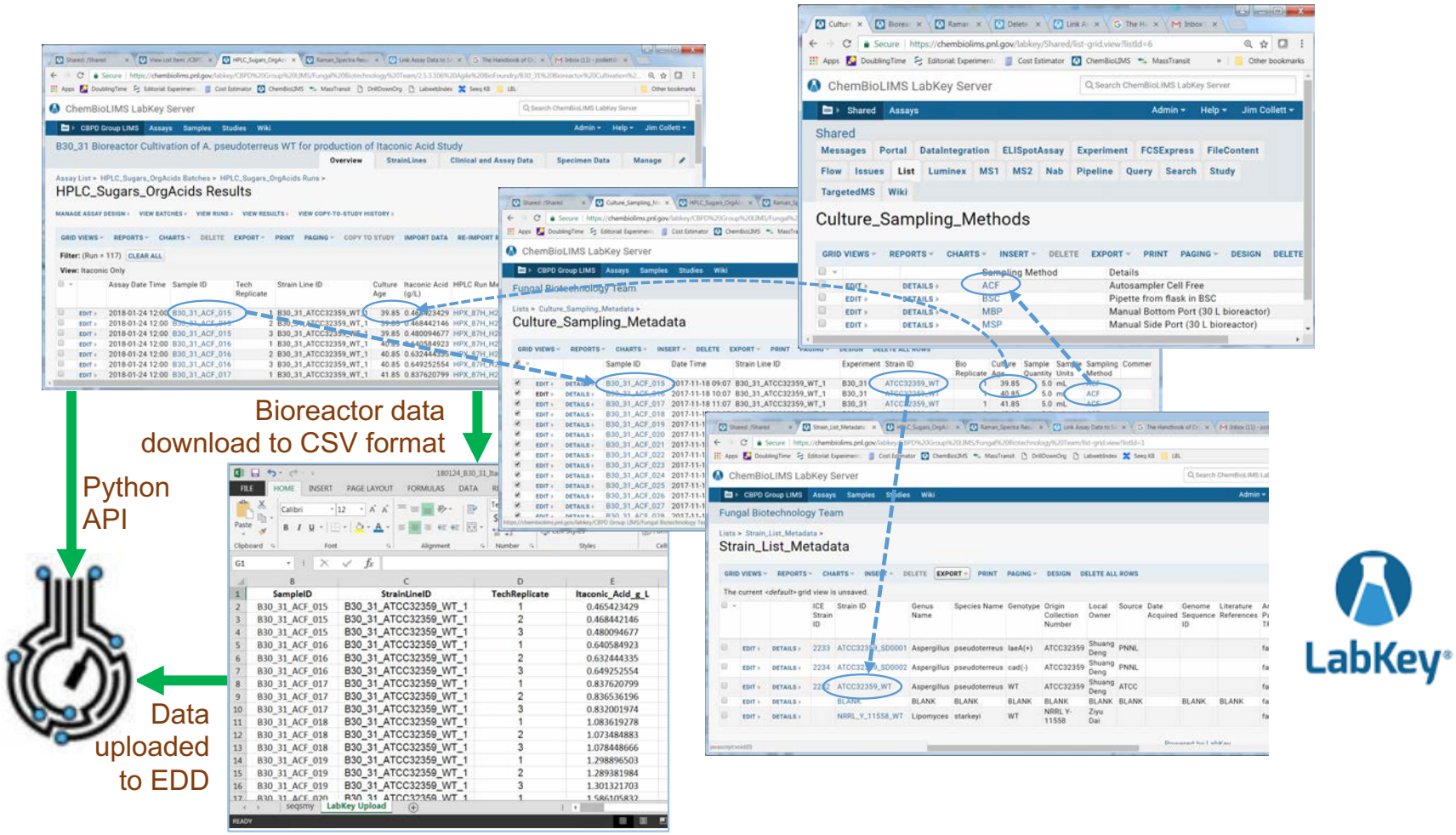
Test Highlight – LabKey Software

- EDD – LabKey Data Integration
  - Bioreactor data and metadata held in LabKey may be easily downloaded into CSV format for uploading into EDD
  - LabKey Python API may also be used to directly access datasets within LabKey



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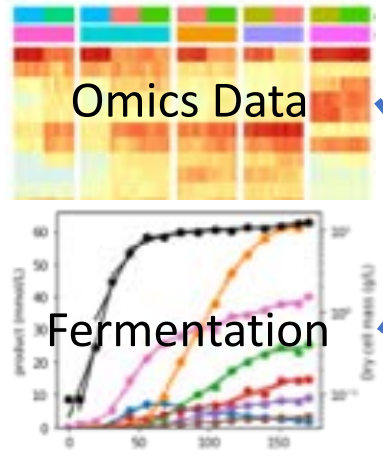
Test/Learn Highlight – EDD Software

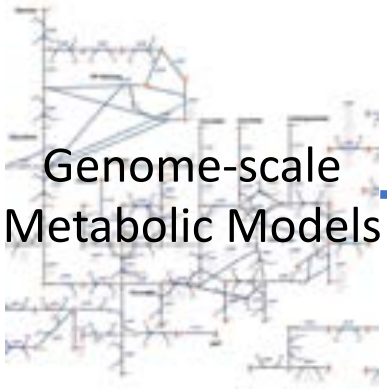
- **Experiment Data Depot**
  - Software platform repository for actionable biological datasets and metadata

- **Next steps**
  - Improve performance – larger datasets, efficient exports, & faster development cycle
  - Complete import redesign by integrating additional workflows
  - Improve metadata tracking to support ABF use cases
  - Productionize transcriptomics pipeline
  - Implement a global proteomics workflow, including LabKey
  - Work with industry partner to integrate support for ABF use cases
  - Improve test coverage and reliability for mature workflows

Learn Highlight – Metabolic network reconstruction and modeling

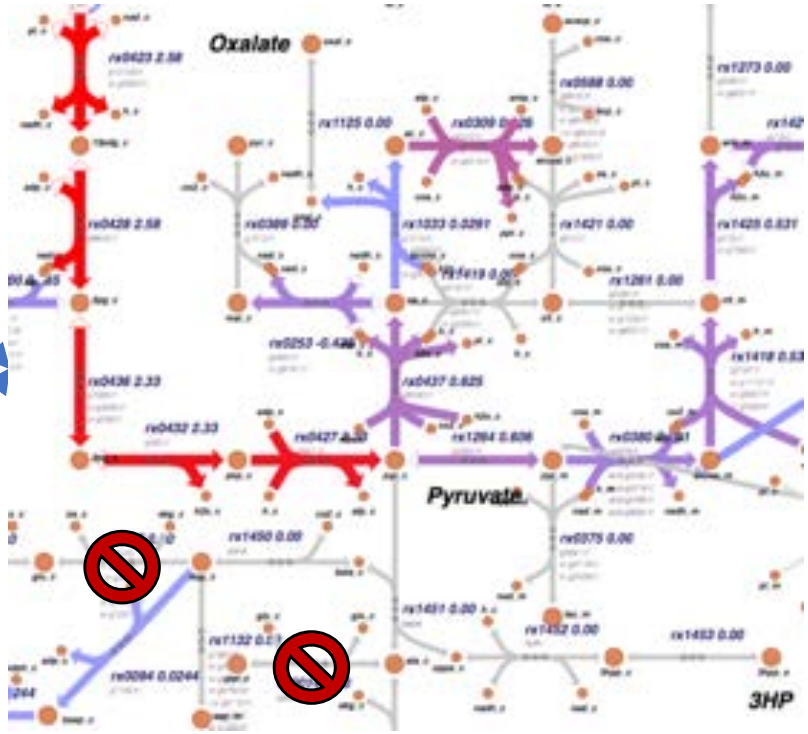
- **Next steps**
  - Multi-omics data integration using metabolic model
  - Develop and improve methods to characterize the metabolic state from data
  - Develop and improve methods to identify non-intuitive engineering strategies





Experimental Data Depot

COBRA Methods



Omics Integration and Target Identification

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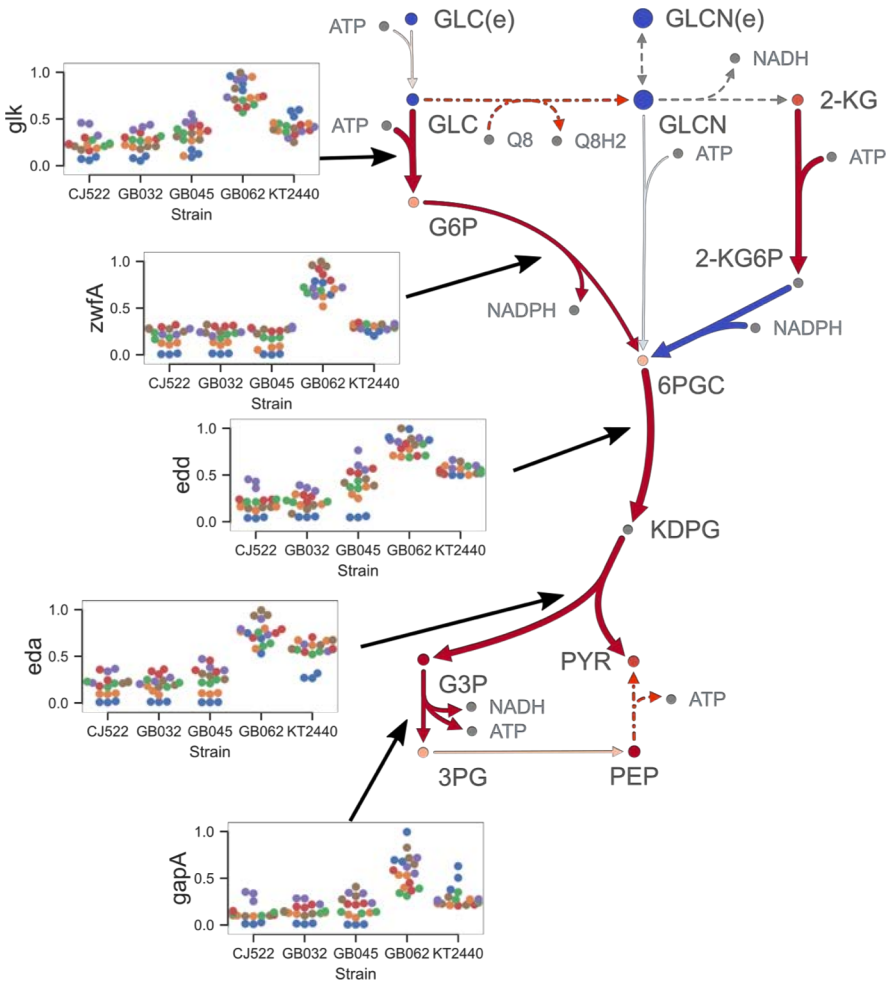
Learn Highlight – Bayesian inference of metabolic kinetics from multi-omics data

- **Next steps**
  - Inferring intracellular flux is the most challenging omics-level data to collect: rigorous benchmarks are needed on ABF-generated data with sparser flux measurements
  - Incorporate thermodynamic constraints on flux and metabolite concentrations; improve uncertainty quantification on larger datasets
  - Include direct transcriptional regulation as an additional layer when transcriptomic measurements are available
  - Develop visualization methods and incorporate tool with easy-to-use interface in the ABF DBTL software stack



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Learn Highlight – Kinetic Learning

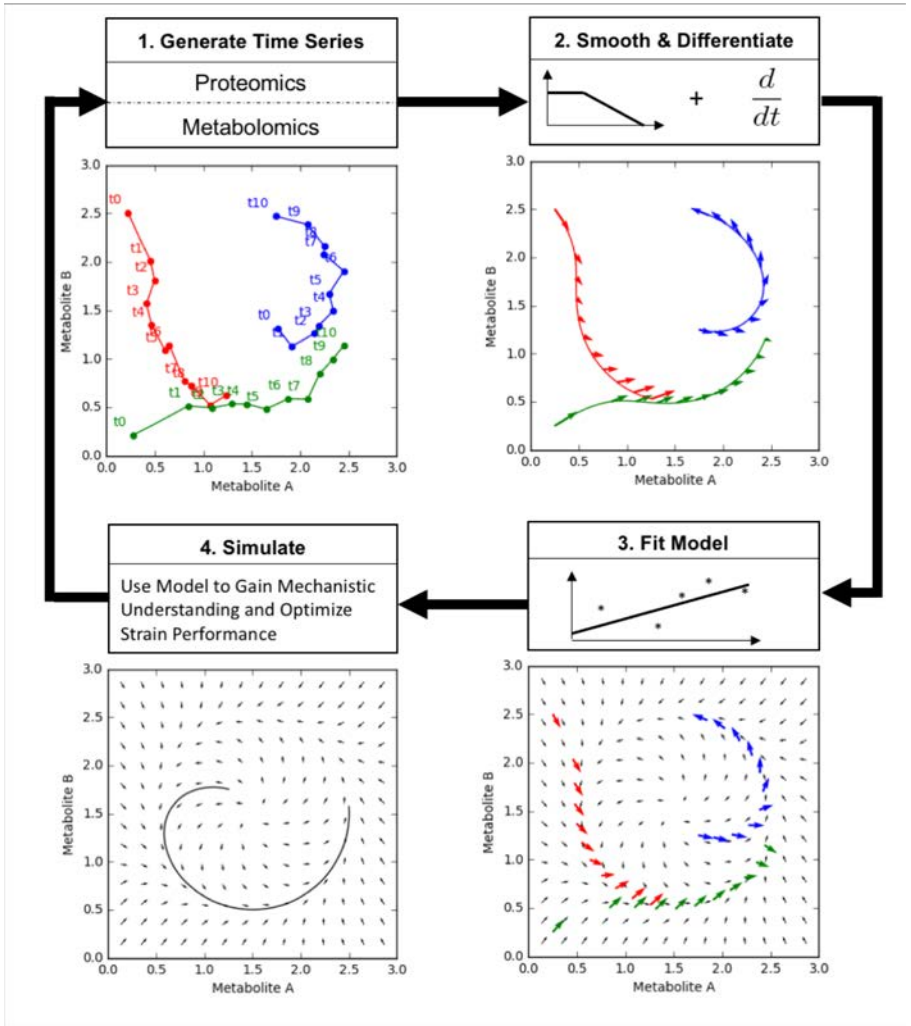
- **A machine learning approach to predict metabolic pathway dynamics from time-series multi-omics data**

- **Next steps**

LYGOS

  - Collaborate with Lygos to learn the dynamics of organic acid-producing strains of *Pichia kudriavzevii* with the goal of improving industrial production.

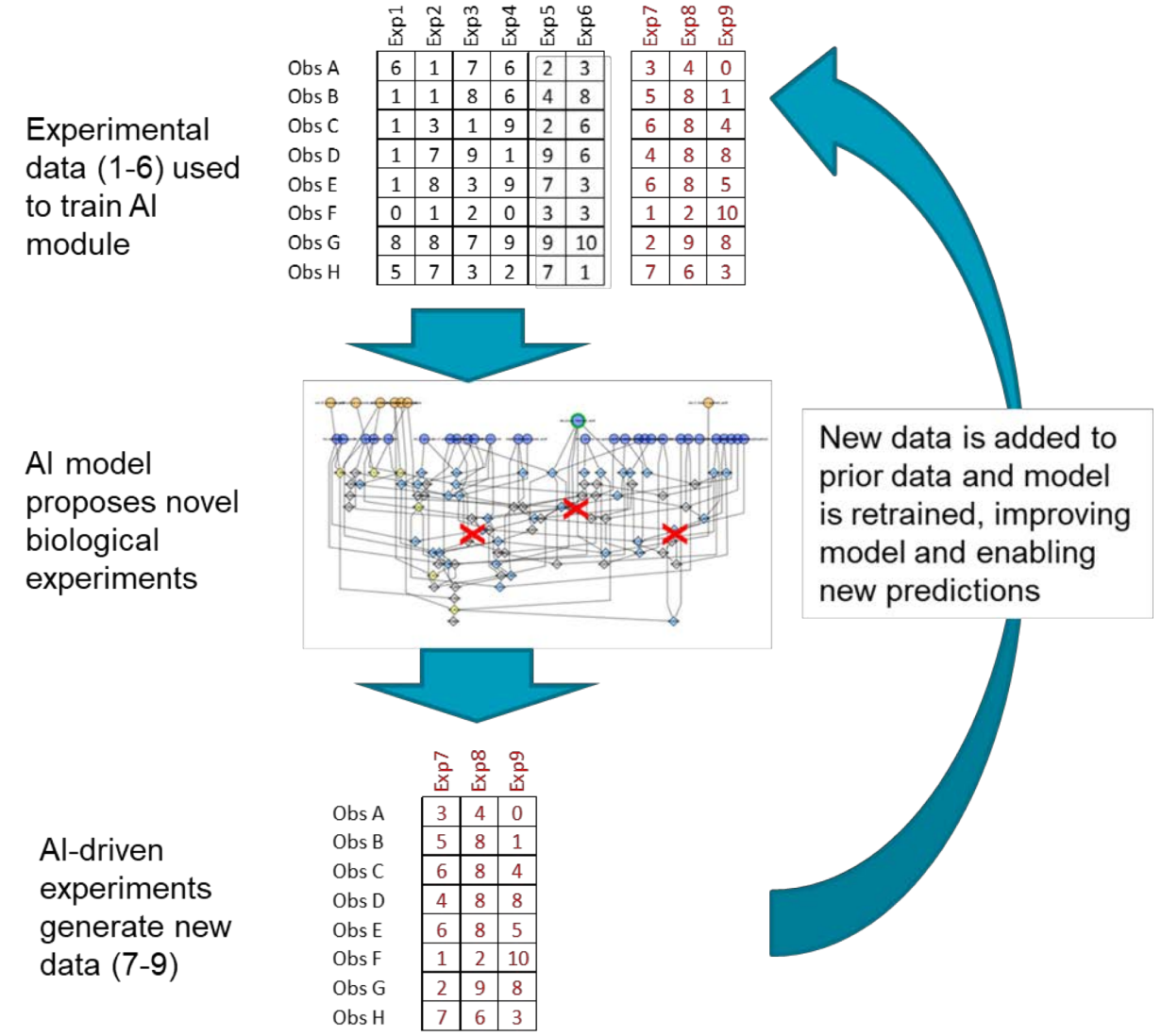
  - Learn dynamics and improve titers of bisabolene-producing strains of *R. toruloides*.



Learn Highlight - Continuous Model Evolution

Expanded process

- Train 'Second Generation' AI models, building on those generated from initial AI-guided studies
- Continue to steer design of individual experiments to maximize opportunity for AI refinement
- Expand and integrate AI models into ABF ecosystem



	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7	Exp8	Exp9
Obs A	6	1	7	6	2	3	3	4	0
Obs B	1	1	8	6	4	8	5	8	1
Obs C	1	3	1	9	2	6	6	8	4
Obs D	1	7	9	1	9	6	4	8	8
Obs E	1	8	3	9	7	3	6	8	5
Obs F	0	1	2	0	3	3	1	2	10
Obs G	8	8	7	9	9	10	2	9	8
Obs H	5	7	3	2	7	1	7	6	3

	Exp7	Exp8	Exp9
Obs A	3	4	0
Obs B	5	8	1
Obs C	6	8	4
Obs D	4	8	8
Obs E	6	8	5
Obs F	1	2	10
Obs G	2	9	8
Obs H	7	6	3

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(DBTL) FY19 Milestones To Be Completed

Milestone (synopsis)	Task	FY19 Quarter	Type
Selections of new target molecule & existing molecule in different host	Target/Host	Q1	Quarterly (Regular)
4X Build sequence validation capacity increase from FY18 to FY19	DBTL Infrastructure	Q2	Quarterly (Regular)
TEA and LCA on new FY19 target molecule	Integrated Analysis	Q2	Quarterly (Regular)
Deep Learning non-intuitive predictions	DBTL Infrastructure	Q2	Quarterly (Regular)
Titer goals in range of 1 to 10 g/L	Target/Host	Q3	Quarterly (Regular)
Transformation in new organism(s)	Host Onboarding	Q3	Quarterly (Regular)
5X Test capacity increase from FY17 to FY19	DBTL Infrastructure	Q3	Quarterly (Regular)
Promoters in new SOT organisms	Host Onboarding	Q4	Annual (Regular)
10L scale using DMR-EH hydrolysate, with 10 g/L, 100 mg/L/h, 40% yield	Process Integration & Scaling	Q4	Annual (Regular)
SWOT Analysis	Industry Engagement & Outreach	Q4	Annual (Regular)
DBTL Activity, Quarterly/Milestone, and final AOP reports sent to BETO. Updates to ABF website	Management	Q4	Annual (Regular)
Value of non-intuitive Learn predictions demonstrated	Target/Host	Q4	Go/No-Go

Pending FY20-22 Milestones

- **We will put our plans into our FY20-22 AOP proposal.**
  - The following milestones are proposed (will undergo merit review)

- **FY20 Annual Smart**
  - Reproducibility of 3 distributed Test unit operations including bioreactor scale-up quantified through comparison of results post data quality assurance for on-site vs. off-site sample analysis.

  or,

  - Statistics gathered and Industry partner decision making processes analyzed for choice between traditional (exclusive license, shorter option period) and alternative (non-exclusive, longer option period) CRADA IP model that retains incentives for industry yet enables ABF to learn and leverage past experience.

- **Go/No-Go Decision, Q2 FY21**
  - 5 target molecules / tools transferred between host organisms and efficiency gains over prior host organisms assessed

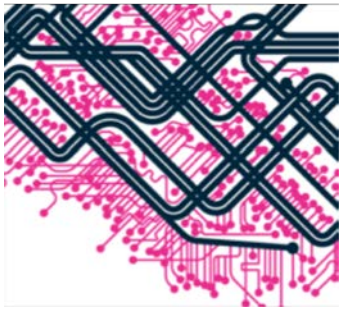
- **FY22 Annual Smart**
  - 5X efficiency improvement in DBTL engineering cycle demonstrated compared to FY19 baseline and 20 host organisms on-boarded to tier 1 or above

Summary – 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 that will productionize synthetic biology.

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

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

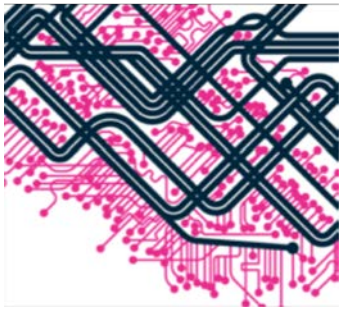


Summary – DBTL Infrastructure

- **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.



Acknowledgements

- Gyorgy Babnigg
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- Dayna Daubaras
- Jay Fitzgerald
- Mark Forrer
- Hector Garcia Martin
- John Gladden
- Adam Gus
- Ramesh Jha
- Christopher Johnson
- JoonHoon Kim
- Young-Mo Kim
- Phil Laible
- Peter Larsen
- Jon Magnuson
- Babs Marrone
- Isabel Pardo Mendoza
- Chris Petzold
- Hector Plahar
- Todd Pray
- Tijana Radivojevic
- Alastair Robinson
- Davinia Salvachua
- Blake Simmons
- Peter St. John
- Deepti Tanjore
- Rosemarie Wilton

Additional Slides

Responses to Previous Reviewers' Comments

- Weaknesses include geographic separation
  - As a distributed effort, we clearly have faced operational challenges, although these have more than been made up for by the Agile BioFoundry's ability to leverage physical and human resources across distributed national laboratories. The Agile BioFoundry's program manager, together with regular communications across the consortium (via teleconferences, webinars, informatics servers, SharePoint, annual in-person meetings), have helped mitigate communications risks. Sample transfer risks (i.e., sample stability, sample loss) will continue to be assessed through local/proximal compared with remote sample analysis, and to date we have not suffered from any notable sample losses. We are continuing to make progress in addressing disconnects in technology adoption, and it continues to be an operational imperative to standardize workflows and data-exchange formats wherever possible.
- Do not yet have a compelling argument as to why and how their approach will be better than other potential approaches to the problem
  - What sets the Agile BioFoundry apart from other foundries is that we develop and distribute publicly available tools, methods, and strains aimed at broadly benefiting the biofuels and bioproducts industry. Whereas private foundries are incentivized to develop proprietary tools and organisms, the Agile BioFoundry is a publicly funded effort aimed at delivering technology that will enable industry to either leverage our resources through partnership or adopt our methodologies for developing bioproducts. In comparison to the publicly funded Defense Advanced Research Projects Agency Living Foundries program, there are distinct programmatic and technical differences between the aims of the two efforts. Where the Living Foundries program is primarily focused on developing biological pathways to materials that cannot be achieved through transformations of petroleum feedstocks, the Agile BioFoundry is focused developing biological pathways for producing advanced biofuels and renewable, high-volume chemicals.

Responses to Previous Reviewers' Comments (cont.)

- Rationale for their choice of product targets needs to be strengthened
  - The Agile BioFoundry is pursuing multiple target/hosts to demonstrate that the methods, software, and technologies can be productively applied across product classes. The process and rationale for selecting the three target/hosts pairs for FY 2017 (and the 15 pairs initially prioritized for FY 2017 – FY 2019) was described during the 2017 Peer Review, and the details were provided to BETO. For our FY 2018 and FY 2019 target/host selection processes, in addition to quantitative technical assessments across multiple categories (TEA and Market, LCA, Strategic Value, Scientific Novelty, DOE Relevance, How Designable, How Buildable, How Hostable, How Testable, How Scalable, and Chemical and Biological Safety), we proactively consulted with the Agile BioFoundry Industry Advisory Board to ensure that our prioritized targets and hosts remain aligned with industry's needs.

- Isn't clear that reducing the cycle time to, say, adipic acid, would be generally applicable to other material
  - As will be / has been presented in the Target/Host ABF presentations at the 2019 Peer Review, we have started to diligently measure cycle times across targets and hosts. This is the pre-requisite step to measuring improvements in (i.e., reductions to) cycle time. It should be noted that we are now pursuing multiple targets in the same host (which could suggest how cycle times for the second target have benefitted from improvements for the first target) and the same target in multiple hosts (which could suggest how cycle times in the second host have benefitted from improvements for the first host). While the former is more directly relevant for this previous reviewer's comment, both are important to capture and understand as they both directly affect the Agile BioFoundry's ability to broadly accelerate biomanufacturing process development across targets and hosts.

Responses to Previous Reviewers' Comments (cont.)

- More emphasis should be placed on the performance gap between small-scale culturing and bench-scale fermentation, which is a well-known problem in the field
  - We recognize that there are challenges associated with each increase in process scale, including the transition from high-throughput, small-scale culturing to bench-scale fermentation. Agile BioFoundry workflows leverage design of experiments and small-scale culture to select strains to grow in bench-scale bioreactors. Bench-scale fermentation provides critical data for the "Learn" component of Design-Build-Test-Learn, both to inform future designs and to develop predictive models that may be applied to small-scale experiments. Agile BioFoundry facilities have recently procured Robo/Biolector(Pro) and Ambr250 instrumentation which both serve to bridge the gap between small-scale culturing and bench-scale fermentation.

- PI is encouraged to look deeply into high-throughput fermentation techniques mastered by enzymes and biobased chemicals and fuels companies
  - As mentioned above, towards adopting the techniques practiced and mastered by companies, Agile BioFoundry facilities have recently procured Robo/Biolector(Pro) and Ambr250 high-throughput fermentation instrumentation.

- Encourage the PI to form a strong liaison between fermentation and the high-throughput team
  - There are strong connections between Agile BioFoundry high-throughput and bio-reactor fermentation teams, with staff shared in common between them.

Publications, Patents, Presentations, Awards, and Commercialization

Publications

- Chen Y, Vu J, Thompson MG, Sharpless WA, Chan LJG, Gin JW, et al. (2019) A rapid methods development workflow for high-throughput quantitative proteomic applications. PLoS ONE 14(2): e0211582. https://doi.org/10.1371/journal.pone.0211582
- Garima Goyal, Zak Costello, Jorge Alonso Guitierrez, Aram Kang, Taek Soon Lee, Hector Garcia Martin, and Nathan J Hillson. (2018) "Parallel Integration and Chromosomal Expansion of Metabolic Pathways" ACS Synthetic Biology DOI: 10.1021/acssynbio.8b00243
- Costello, Zak, and Hector Garcia Martin. "A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data." NPJ systems biology and applications 4.1 (2018): 19. https://doi.org/10.1038/s41540-018-0054-3
- Oyetunde, Tolutola, et al. "Leveraging knowledge engineering and machine learning for microbial bio-manufacturing." Biotechnology advances (2018). https://doi.org/10.1016/j.biotechadv.2018.04.008
- Amin Zargar, Jesus F. Barajas, Ravi Lal, Jay D. Kealsing. “Polyketide Synthases as a Platform for Chemical Product Design” AIChE (2018) https://doi.org/10.1002/aic.16351
- Jha RK*, Bingen JM, Johnson CW, Kern TL, Khanna P, Trettel DS, Straus CEM, Beckham GT, Dale T* (2018). A protocatechuate biosensor for Pseudomonas putida KT2440 via promoter and protein evolution. Metabolic Engineering Communications (6) 33-38. https://doi.org/10.1016/j.meteno.2018.03.001
- Mitchell G. Thompson, Nima Sedaghatian, Jesus F. Barajas, Maren Wehrs, Constance B. Bailey, Nurgul Kaplan, Nathan J. Hillson, Aindrila Mukhopadhyay & Jay D. Keasling. (2018) "Isolation and characterization of novel mutations in the pSC101 origin that increase copy number". Scientific Reports 8, 1590 doi:10.1038/s41598-018-20016-w
- Jesus F. Barajas, Amin Zargar, Bo Pang, Veronica T. Benites, Jennifer Gin, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, and Jay D. Keasling. (2018) "Biochemical Characterization of β-Amino Acid Incorporation in Fluvirucin B2 Biosynthesis". ChemBioChem 10.1002/cbic.201800169
- Denby, Charles M., et al. "Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer." Nature communications 9.1 (2018): 965
- Garber ME, Rajeev, Kazakov AE, Trinh J, Masuno D, Thompson M, Kaplan, N, Novichkov PS and Mukhopadhyay A. (2018) "Multiple signaling systems target a core set of transition metal homeostasis genes using similar binding motifs” Mol Microbiol. 107(6):704-717. doi: 10.1111/mmi.13909
- Ando, D., Garcia Martin, H. (2018) "Two-Scale 13C Metabolic Flux Analysis for Metabolic Engineering". In "Synthetic Metabolic Pathways - Methods and Protocols", Springer Protocols - Methods in Molecular Biology, Jensen, Michael Krogh, Keasling, Jay D (Eds.) ISBN 978-1-4939-7295-1 http://www.springer.com/us/book/9781493972944
- Backman TWH, Ando D, Singh J, Keasling JD, García Martín H. (2018) “Constraining Genome-Scale Models to Represent the Bow Tie Structure of Metabolism for (13)C Metabolic Flux Analysis”. Metabolites. 2018 Jan 4;8(1). pii: E3. doi: 10.3390/metabo8010003
- Yuzawa S, Bailey CB, Fujii T, Jocic R, Barajas JF, Benites VT, Baidoo EEK, Chen Y, Petzold CJ, Katz L, Keasling JD. Heterologous Gene Expression of N-Terminally Truncated Variants of LipPks1 Suggests a Functionally Critical Structural Motif in the N-terminus of Modular Polyketide Synthase. ACS Chem Biol. 2017 Nov 17;12(11):2725-2729. doi: 10.1021/acschembio.7b00714

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

Publications (cont.)

- Morrell, W., Birkel, G., Forrer, M.,; Lopez, T., Backman, T.W.H, Dussault, M., Petzold, C., Baidoo, E., Costello, Z., Ando, D., Alonso Gutierrez, J., George, K., Mukhopadhyay, A., Vaino, I., Keasling, J., Adams, P., Hillson, N., Garcia Martin, H. "The Experiment Data Depot: a web-based software tool for biological experimental data storage, sharing, and visualization" (2017) ACS Synthetic Biology DOI: 10.1021/acssynbio.7b00204
- Eng, C.H.*, Backman, T.W.H.*, Bailey, C.B., Magnan, C., Garcia Martin, H.G., Katz, L., Baldi, P., Keasling, J.D. "ClusterCAD: a computational platform for type I modular polyketide synthase design." (2017) Nucleic Acids Research DOI: 10.1093/nar/gkx893 *Contributed equally
- Barajas, J.F., Blake-Hedges, J., Bailey, C.B., Curran, S., Keasling, J.D. (2017). "Engineered polyketides: Synergy between protein and host level engineering" Synthetic and Systems Biotechnology doi.org/10.1016/j.synbio.2017.08.005
- Shymansky, Christopher M., et al. "Flux-enabled exploration of the role of Sip1 in galactose yeast metabolism." Frontiers in Bioengineering and Biotechnology 5 (2017)

Presentations

- Gregg Beckham, Hybrid biological and catalytic processes to manufacture and recycle plastics, Princeton University, November 28th, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Ginkgo Bioworks, Boston, MA, November 12, 2018
- Nathan J. Hillson. "DIVA (DNA Design, Implementation, Validation Automation) Platform". Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 8, 2018
- Nathan J. Hillson. "Recent developments at the U.S Department of Energy Agile BioFoundry". Invited Talk, 2nd Darmstadt RoboWorkshop, Darmstadt, Germany, November 7, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". AIChE annual meeting, Pittsburgh, PA, October 31 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". Thermo Fisher, San Jose, CA, October 19, 2018
- Garcia Martin, H. "Towards a predictive synthetic biology enabled by machine learning and automation". DTRA Tech Watch, Ft. Belvoir, VA, October 10, 2018
- Nathan J. Hillson. "DOE Agile BioFoundry Overview". Invited Talk, SynBioBeta 2018 visit to ESE, Emeryville, CA, October 1, 2018
- Nathan J. Hillson. "ABF Organization, Progress, and FY19 Plans". Invited Talk, ABF All Hands Annual Meeting 2018 (Industry Day), Emeryville, CA, September 12, 2018
- Nathan J. Hillson. "Agile BioFoundry Overview". Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garcia Martin, H. "A new approach to flux analysis". Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018

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

Presentations (cont.)

- Hector Plahar. “DIVA Software Platform”. Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Tijana Radivojevic. “Automatic Recommendation Tool”, Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Jennifer Chiniquy. “DIVA DNA-Seq and DNA Construction”, Invited Talk, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garcia Martin, H. “A New Approach to Flux Analysis". ABF Annual Meeting, Berkeley CA, September 7, 2018
- Garcia Martin, H. “Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, Machine learning for science workshop, Berkeley, CA, September 5, 2018
- Nathan J. Hillson. “Agile BioFoundry Overview”. Invited Lightning Talk, LBNL BioSciences Area Retreat 2018, Lafayette, CA, August 30, 2018
- Garcia Martin, H. “Modeling from molecules to ecosystems : opportunities, challenges and vision". Invited talk, BioEpic meeting, Berkeley, CA, August 23, 2018
- Garima Goyal “DIVA DNA Construction”. Invited Talk, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- Tijana Radivojevic. “Automatic Recommendation Tool”, Invited Talk, JBEI Annual Meeting 2018, Sonoma, CA, August 22, 2018
- Garcia Martin, H. “Opportunities in the intersection of synthetic biology, machine learning and automation". Invited talk, JBEI Annual Meeting, Berkeley, CA, August 20, 2018
- Garcia Martin, H. “Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, SIMB, Chicago, IL, August 15, 2018
- Garcia Martin, H. “Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, International Workshop for BioDesign and Automation (IWBDA), Berkeley, CA, August 2nd, 2018
- Garcia Martin, H. “Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, Biocruces, Bilbao, Spain, July 20, 2018
- Garcia Martin, H. “Machine Learning to Predict Metabolic Pathway Dynamics from Multiomics Data". Invited talk, AI for synthetic biology, Stockholm, Sweden, July 15, 2018
- Garcia Martin, H. “Towards a predictive synthetic biology enabled by machine learning and automation". Invited talk, BCAM, Bilbao, Spain, July 3, 2018
- Nathan J. Hillson, "Berkeley (and other) National Lab(s): Current Biosecurity Frameworks and Strategies in Action", Invited Talk, EBRC meeting - Improving Security Considerations in Engineering Biology Research, Emeryville, CA, June 26, 2018
- Nathan J. Hillson and Hector A. Plahar, "ICE Software Platform", Invited Talk, Software for Synthetic Biology Workflows Workshop, SEED 2018, Scottsdale, Arizona, June 7, 2018
- Gregg Beckham. Developing new processes to valorize lignin and sugars to building-block chemicals and materials, RWTH Aachen University, May 28th, 2018

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

Presentations (cont.)

- Gregg Beckham. Adventures in engineering Pseudomonas putida for expanded substrate specificity and improved tolerance, RWTH Aachen University, May 28th, 2018
- Hillson, N.J. “Berkeley Lab project activities, biosecurity practices, and their roles within the larger biosecurity landscape”. Invited Talk, Working Group on Automation in SynBio, Gryphon Scientific, Takoma Park, MD, May 23, 2018
- Hillson, N.J. “Recent developments at the Agile BioFoundry”. Invited Talk, Diligence Ventures/Suzhou Government visit to ABF, Emeryville, CA, May 2, 2018
- Gregg Beckham. Hybrid biological and catalytic processes to manufacture and recycle plastics, MIT, April 27th, 2018
- Hillson, N.J. “Recent developments at the Agile BioFoundry”. Invited Talk, 2018 Life Science Symposium - Synthetic Biology and Metabolic Engineering, MilliporeSigma Innovation Center, St. Louis, MO, April 27, 2018
- Garcia Martin, H. " A Machine Learning Approach to Predict Metabolic Pathway Dynamics from Time Series Multiomics Data". Invited talk at Madison Microbiome Meeting at University of Wisconsin, Madison, WI, April 25, 2018.
- Jennifer Chiniquy, Cindi Hoover, Joel Guenther, Nurgul Kaplan, Garima Goyal, Mark Kulawik, Hector Plahar, Zachary Costello, Brian Bushnell, Samuel Deutsch, and Nathan J. Hillson. “Overcoming Challenges in MiSeq DNA Construct Sequence Validation”. Invited Poster, DOE JGI User Meeting 2018, San Francisco, CA, March 14, 2018
- "Test" and "Learn" in process research informs design strategy Sundstrom, E. R.,, M. Mirsiaghi, F. Tachea, N. Sun, T.R. Pray, D. Tanjore. ECO-BIO, Dublin, Ireland, March 5, 2018.
- Garcia Martin, H. "EDD as a data warehouse and Learn facilitator". Invited talk at Argonne National Lab, St. Louis, Lemont, IL, March 5, 2018
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Hector A. Plahar, Annabel Large, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA Services: PCR, Full DNA Construction, and MiSeq Validation”. Invited Poster, DOE BER GSP Contractor’s Meeting 2018, Tysons Corner, VA, February 27, 2018
- Hillson, N.J. “Three synthetic biology design challenges we face, and how we are approaching them”. Invited Talk, Dagstuhl Seminar 18082, Wadern, Germany, February 19, 2018
- Jennifer Chiniquy, Nurgul Kaplan, Garima Goyal. “DIVA DNA-Seq Service”, JBEI User Meeting presentation, February 12, 2018.
- Garcia Martin, H. "Metabolic Modeling of –omics Data for Biofuel Production". Invited talk at Bayer, Sacramento, CA, February 2, 2018.
- Garcia Martin, H. " Machine Learning and Mechanistic Models to Predict Biological Outcomes using ‘omics Data". Invited talk at Environmental Genomics and Systems Biology retreat, Berkeley, CA, January 19, 2018
- Jesus F. Barajas. “Current progress towards engineered PKS lactam pathways”. JBEI/BBD group meeting presentation, December 13, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, iSynBio/SIAT visit to JGI, Walnut Creek, CA, December 9, 2017
- Jennifer Chiniquy, Nurgul Kaplan. “DIVA DNA-Seq Service”. ESE User Meeting presentation, November 20, 2017

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

Presentations (cont.)

- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Cargill visit to ESE, Emeryville, CA, November 17, 2017
- Hillson, N.J. “Flanking Homology DNA Assembly, Protocol Design Software, and Synthetic DNA”. Invited Talk, Bitesize Bio Webinar, November 15, 2017
- Simmons, B.A. and Hillson, N.J. “The BioDefense Foundry”. Invited Talk, DTRA Tech Watch Briefing, Springfield, VA, November 8, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, University of Wyoming, Laramie, WY, November 3, 2017
- Hillson, N.J. “Parallel Integration and Chromosomal Expansion of Metabolic Pathways”. Invited Talk, University of Wyoming, Laramie, WY, November 3, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Braskem Zoom Teleconference, November 1, 2017
- Hector Garcia Martin. “Modeling of -omics data for Biofuel Production through Synthetic Biology”. EECE Department seminar, Washington University, St. Louis MO, October 20th, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, ABLC Next Tour of ESE (ABF/ABPDU/JBEI), Emerville, CA, October 16, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Berkeley Lab Workshop: Industrialization of engineering biology: from discovery to scale-up, SynBioBeta SF 2017, UCSF Mission Bay, San Francisco, CA, October 3, 2017
- Hillson, N.J. “How the Agile BioFoundry Thinks About Paths to Commercialization”. Invited Talk, SynBio for Defense, Arlington, VA, September 27, 2017
- Hillson, N.J. “BioDefense – the Agile BioFoundry and Predictive Biology”. Invited Talk, Presentation for Dimitri Kusnezov (Chief Scientist, DOE NNSA), Berkeley, CA, September 21, 2017
- Hillson, N.J. “Sustainable development through a synthetic biology foundry”. Invited Talk, CellPress LabLinks - Basic to Applied Science for Sustainable Development, Berkeley, CA, September 18, 2017
- Plahar, H.A. “Software Session: Recent DeviceEditorjs/DIVA/ICE improvements”. Invited Talk, JBEI Annual Meeting, Monterey, CA, September 15, 2017
- Costello, Z. “Software Session: The Automatic Recommendation Tool”. Invited Talk, JBEI Annual Meeting, Monterey, CA, September 15, 2017
- Backman, T.W.H. “ClusterCAD: a computational platform for type I modular polyketide synthase design.” Invited Talk, JBEI Annual Meeting, Monterey, CA, September 14, 2017
- Hillson, N.J. “Agile BioFoundry Update”. Invited Talk, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Plahar, H.A. “ICE/DIVA Software Tutorial”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 29, 2017
- Hillson, N.J. “Agile BioFoundry Overview”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- De Paoli, H.C. “A. pseudoterreus 3HP Design and Build”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017.
- Chiniquy J., “DIVA DNA-Seq Service”. Invited Talk, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017

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

Presentations (cont.)

- Garcia Martin, H. "Predicting Metabolic Pathway Dynamics by Combining Multiomics Data with Machine Learning and Kinetic Modeling". Invited talk at "Multi-omics for Microbiomes" conference, Pasco, WA, July 31, 2017.
- Johnson, C.W. “Metabolic engineering of Pseudomonas putida KT2440 for production of muconic acid from sugar”, SIMB Annual Meeting, July 31, 2017
- Hillson, N.J. "j5 Software Through the Years: Insights from Aggregate Public Usage Metrics". Invited lightning talk, World Metrology Day Symposium, Stanford, CA, May 22, 2017.
- Beckham, G.T. "The Agile BioFoundry: Investing in Biomanufacturing Infrastructure”, TechConnect World, May 16, 2017
- Derek Vardon. Potential commercialization opportunities for valorization of biomass to polymer precursors. Invited Seminar. Alliance Commercialization and Deployment Committee Meeting, NREL. May 2017.
- Gregg Beckham. The Agile BioFoundry: Investing in Biomanufacturing Infrastructure, TechConnect World, May 16, 2017
- Hillson, N.J. "Overview of the Agile BioFoundry". Invited talk, IMP (Mexican Petroleum Institute) Visit to JBEI, Emeryville, CA, April 21, 2017.

Posters

- J. Meadows, C. Johnson, S. Notonier, YM. Kim, S.Tripathy, K. Burnam-Johnson, M. Burnet, J. Magnuson, G. Beckham, N. Hillson, J. Gladden. “Engineering Pseudomonas putida KT2440 to produce adipic acid from lignocellulosic components”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Jesus F. Barajas, Jingwei Zhang, Amin Zargar, Bo Pang, Huaxiang Deng, Veronica T. Benites, Edward E. K. Baidoo, Christopher J. Petzold, Nathan J. Hillson, Jay D. Keasling. “Development of Valerolactam and Caprolactam Biosynthetic Routes”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Jonathan Diab, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA (Design Implementation Validation Automation) DNA Construction”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Jonathan Diab, Jennifer Chiniquy, Cindi Hoover, Joel Guenther, Nurgul Kaplan, Garima Goyal, Mark Kulawik, Hector Plahar, Zachary Costello, Brian Bushnell, Samuel Deutsch, and Nathan J. Hillson. “MiSeq DNA Construct Sequence Validation”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Edward E.E.K. Baidoo and Veronica Teixeira Benites. “High throughput analysis of isoprenoid pathway intermediates by HILIC-QTOF-MS”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018.
- Isaac Wolf, Carolina Barcelos, Shawn Chang, Nilufer Oguz, Matt Dorsey, Davinia Salvachua, Robert Nelson, Todd Pray, Eric Sundstrom and Deepti Tanjore. “Harmonization of Fermentation for Production of P. putida-derived Muconic Acid”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018

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

Posters (cont.)

- J. Prahl, S. Coradetti, D. Liu, G. Geiselman, T. Pray, J. Gladden, E. Sundstrom, and D. Tanjore. “Insights from Bioreactors make Scale-Down Modeling more Effective”. Invited Poster, ABF All Hands Annual Meeting 2018, Emeryville, CA, September 10, 2018
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Jonathan Diab, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA (Design Implementation Validation Automation) DNA Construction”. Invited Poster, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- William Morrell, Mark Forrer, Garrett Birkel, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. “Collaboration with the Experiment Data Depot”. Invited Poster, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- Jonathan Diab, Jennifer Chiniquy, Cindi Hoover, Joel Guenther, Nurgul Kaplan, Garima Goyal, Mark Kulawik, Hector Plahar, Zachary Costello, Brian Bushnell, Samuel Deutsch, and Nathan J. Hillson. “MiSeq DNA Construct Sequence Validation”. Invited Poster, JBEI Annual Meeting 2018, Sonoma, CA, August 20-22, 2018
- Sarah A LaFrance, Jacob Coble, Thomas Rich, Hector Plahar, Joshua Nixon, Nathan J. Hillson. “VectorEditor: Freely Open-Source Javascript Webapp for DNA Visualization, Annotation, and Editing”. Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Annabel Large, Nurgul Kaplan, Jennifer Chiniquy, Garima Goyal, and Nathan Hillson. “Expansion and Optimization of DIVA DNA Sequence Validation Services”. Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA (Design Implementation Validation and Automation) DNA Construction”. Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “Using DIVA, DeviceEditor, and j5 for DNA Construction”. Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- William Morrell, Garrett Birkel, Mark Forrer, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. “The Experiment Data Depot platform”. Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Backman, T.W.H., Eng, C.H., Bailey, C.B., Keasling, J.D., Garcia Martin, H. “Software for polyketide synthase (PKS) design”. Invited Poster, JBEI Annual Meeting, Monterey, CA, September 13, 2017
- Garima Goyal, Nurgul Kaplan, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA (Design Implementation Validation and Automation) DNA Construction”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Manjiri Tapaswi, Nina Stawski, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “Using DIVA, DeviceEditor, and j5 for DNA Construction”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017

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

Posters (cont.)

- Jennifer L. Chiniquy, Cindi A. Hoover, Joel M. Guenther, Nurgul Kaplan, Christopher W. Beitel, Samuel Deutsch, and Nathan J. Hillson. “Towards a High-Throughput Low-Cost Automated DNA Sequence Validation Workflow”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- William Morrell, Garrett Birkel, Mark Forrer, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. “The Experiment Data Depot platform”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Hector A. Plahar, Elena Aravina, Oge Nnadi, Joanna Chen, Paul D. Adams, Jay D. Keasling, and Nathan J. Hillson. “ICE: A Distributed and Interconnected Biological Part Registry”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Jha, R., Narayanan, N., Johnson, C., Beckham, G., Dale, T. “Whole cell biosensing in Pseudomonas putida KT2440”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- Pandey N., Krishnamurthy, M., Jha, Ramesh., Hennelly, S., Dale, T. “Riboregulator Development To Increase Metabolic Flux Towards Muconate Production”. Invited Poster, Agile BioFoundry Annual Meeting, NREL IBRF, Golden, CO, August 28, 2017
- John Meng, Angela Tarver, Matthew Hamilton, Robert Evans, Lisa Simirenko, Nathan J. Hillson, Jan-Fang Cheng, and Samuel Deutsch. “SynTrack 2: A Scalable DNA Assembly Production Workflow Management”. Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Sarah A LaFrance, Jacob Coble, Thomas Rich, Hector Plahar, Joshua Nixon, Nathan J. Hillson. “VectorEditor: Freely Open-Source Javascript Webapp for DNA Visualization, Annotation, and Editing”. Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- William Morrell, Garrett Birkel, Mark Forrer, Traci Lopez, Nathan J Hillson, Hector Garcia Martin. “The Experiment Data Depot platform”. Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Nina Stawski, Manjiri Tapaswi, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA (Design, Implementation, Validation Automation) DNA Construction: Wet-Lab Workflow and Software Platform”. Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Philip C. Gach, Manasi Raje, Nurgul Kaplan, Sangeeta Nath, Samuel Deutsch, Jay D. Keasling, Paul D. Adams, Nathan J. Hillson and Anup K. Singh. “A Microfluidic Platform for Combinatorial Gene Assembly, Transformation, Culture and Assay”. Invited Poster, 2017 Synthetic Biology: Engineering, Evolution & Design (SEED), Vancouver, British Columbia, Canada, June 20-23, 2017.
- Hillson, N.J. "j5 Software Through the Years: Insights from Aggregate Public Usage Metrics". Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.
- Jennifer L. Chiniquy, Cindi A. Hoover, Joel M. Guenther, Nurgul Kaplan, Christopher W. Beitel, Samuel Deutsch, and Nathan J. Hillson. “Towards a High-Throughput Low-Cost Automated DNA Sequence Validation Workflow”. Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.

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

Posters (cont.)

- G. Goyal, Z. Costello, J.A. Gutierrez, A. Kang, T.S. Lee, H.G. Martin, and N.J. Hillson. “PIACE: Parallel Integration and Chromosomal Expansion of Biofuel Pathways in E. coli”. Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.
- Nurgul Kaplan, Garima Goyal, Jennifer L. Chiniquy, Joel M. Guenther, Hector A. Plahar, Joanna Chen, Nina Stawski, Manjiri Tapaswi, Lisa Simirenko, Samuel Deutsch, and Nathan J. Hillson. “DIVA (Design, Implementation, Validation Automation) DNA Construction: Wet-Lab Workflow and Software Platform”. Invited Poster, World Metrology Day Symposium, Stanford, CA, May 22, 2017.