Agile BioFoundry

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

BIOENERGY TECHNOLOGIES OFFICE

Agile BioFoundry – Host Onboarding and Development

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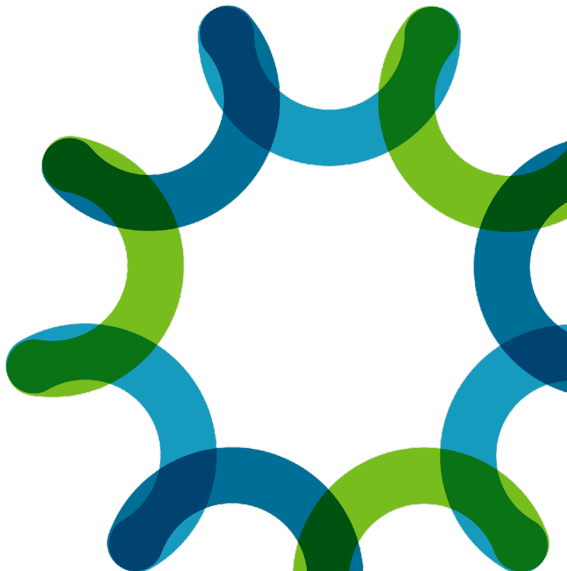
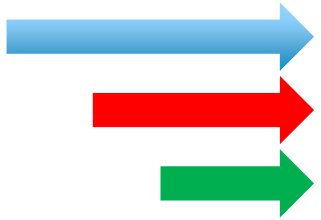


Project overview

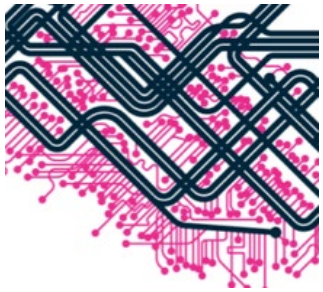
ABF Primary Goal: Enable biorefineries to achieve 50% reductions in time to scale-up compared to the current average of around 10 years by establishing a distributed Agile BioFoundry that will productionize synthetic biology.

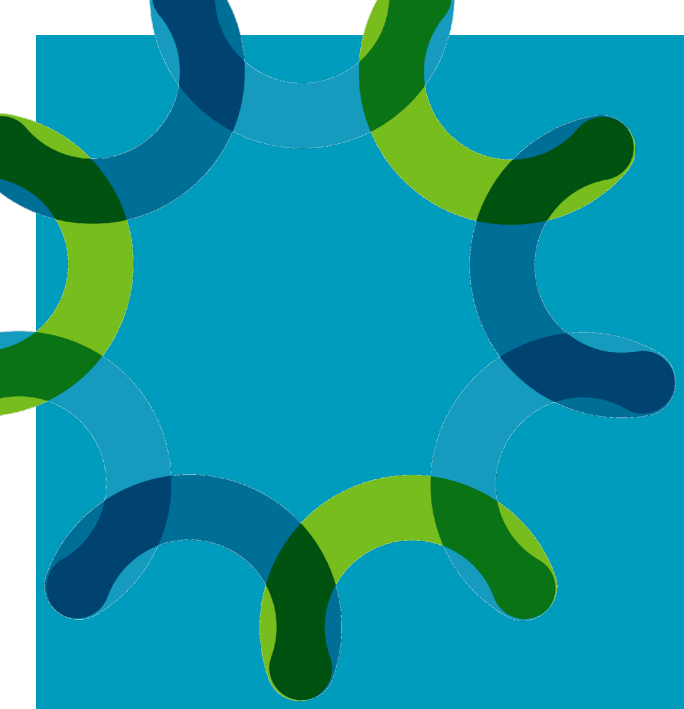


Host Onboarding & Development Goal: Identify, develop, and make available new microbial hosts and metabolic tools to enable industrial bioengineering.

Heilmeier Catechism framing:

- **Goal:** Accelerate synthetic biology through development of microbial hosts and metabolic tools
- **Today:** Most work is done in model systems for a specific target molecule, instead of swaths of metabolic space in non-model hosts
- **Important:** This will directly aid bioeconomy commercialization efforts, by increasing tools and reducing risk with respect to using new organisms
- **Risks:** Judicious molecule and host selection, not achieving TRY goals that meet economic and sustainability goals





1 - Management

Management

Task structure: Roles & Responsibilities

LANL, Co-Lead
- Tier system development
- Tier elevation
- HObT development

ORNL, Co-Lead
- Host onboarding
- Tier elevation
- State of Technology tool development

ANL
- Host onboarding
- Tier elevation

LBNL
- HObT design & development

NREL
- Host onboarding
- Tier elevation

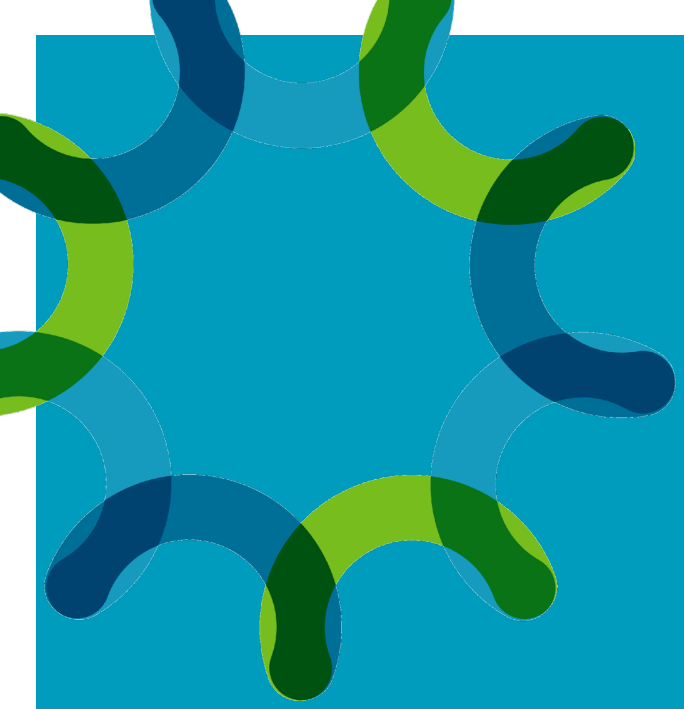
PNNL
- Host onboarding
- Tier elevation

SNL
- Host onboarding
- Tier elevation

- Task structure includes LANL/ORNL as Task Co-Leads and a “lead” Point of Contact for each host organism
- Monthly calls, ad hoc organism meetings, rotating ABF Task Lead call, present and receive feedback from IOE Task, Industry Advisory Board, and DFO process
- **Outcome:** Close interaction across laboratories, along with external stakeholder feedback, leads to collaborative advancement of new non-model microbes

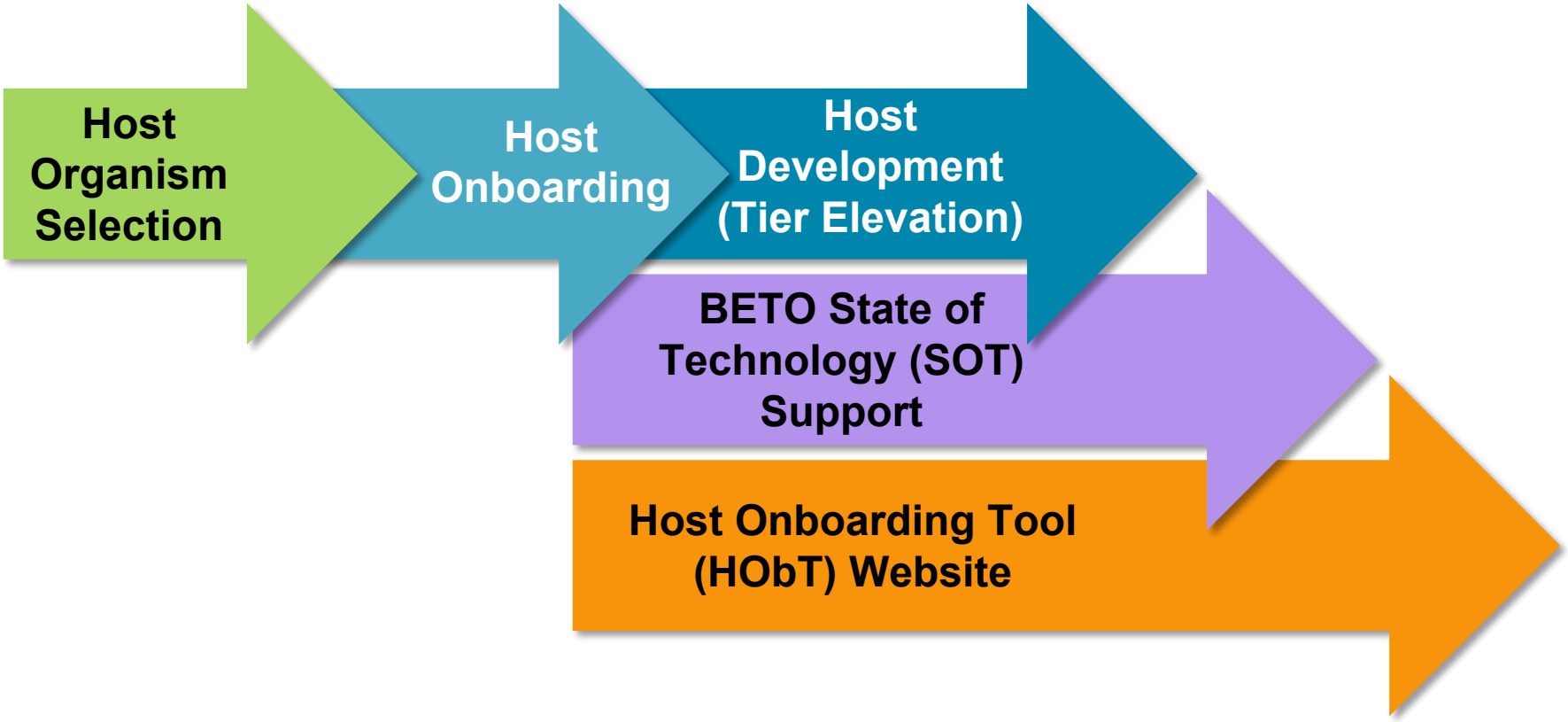
Management

Project Risks	Mitigation
• Challenges in developing genetics for a given organism	• Leverage broad expertise across National Lab system
• Broad metabolic and phenotypic variation in organisms of interest	• Large libraries of in-house tools and genetic parts allows for rapid testing of multiple strategies in parallel
• Tools developed for one organism may not function the same in a closely related organism	• One Lab acts as Lead POC for each microorganism, driving and coordinating host onboarding and development, shares protocols/training for use of strain
• Desired tool/approach from one Lab may need to be ported into a strain whose expertise resides at another Lab	• Frequent communication helps identify barriers and allows collective expertise to suggest solutions
• Tier definitions and criteria may not fit a broad array of organisms	• Built-in flexibility provides structure as well as ability to modify tiers with appropriate justification



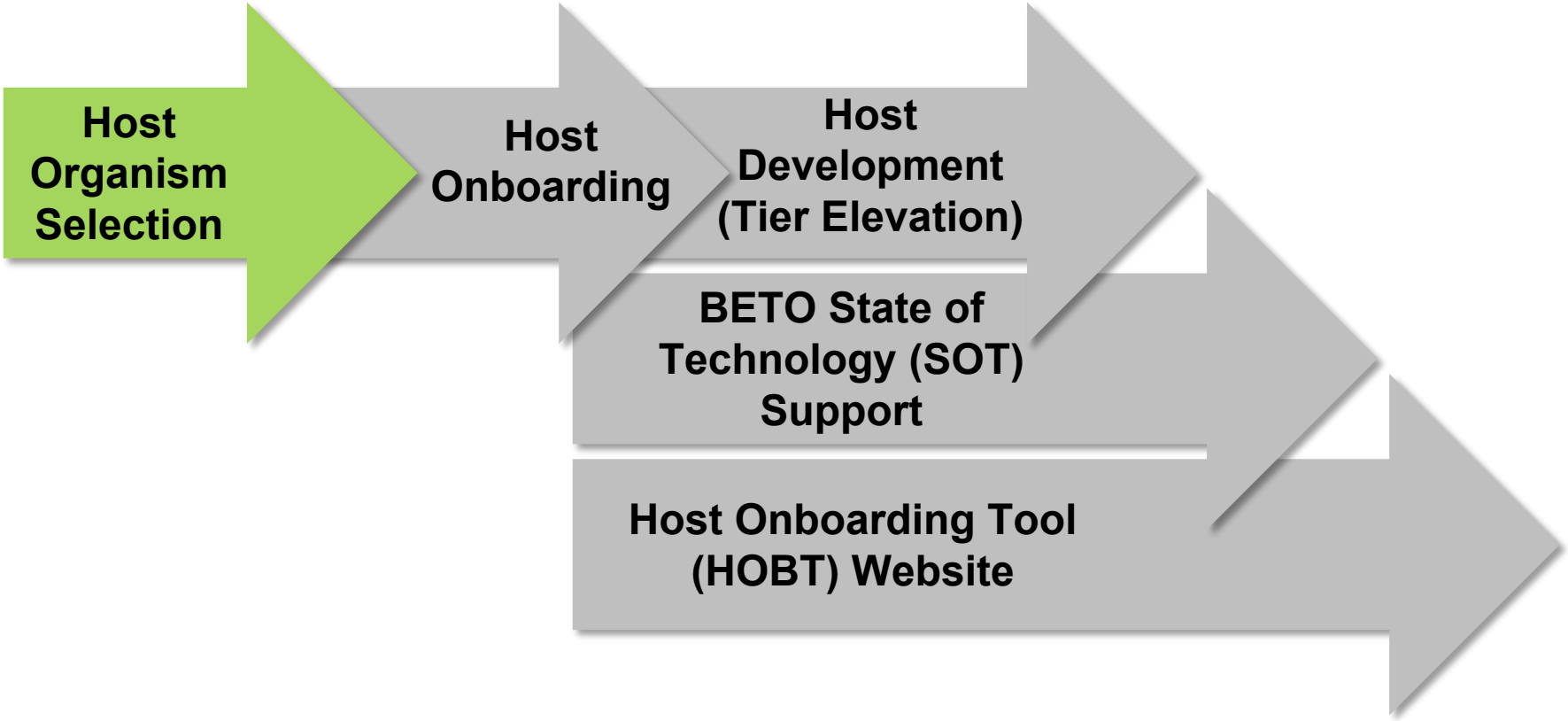
2 - Approach

Approach: Four Primary Activities



- Organisms are ranked and selected, onboarded and developed, and information will be made public using HObT website; also develop tools in SOT strains.
- **Outcome:** Collection of diverse hosts for use by the ABF & external stakeholders

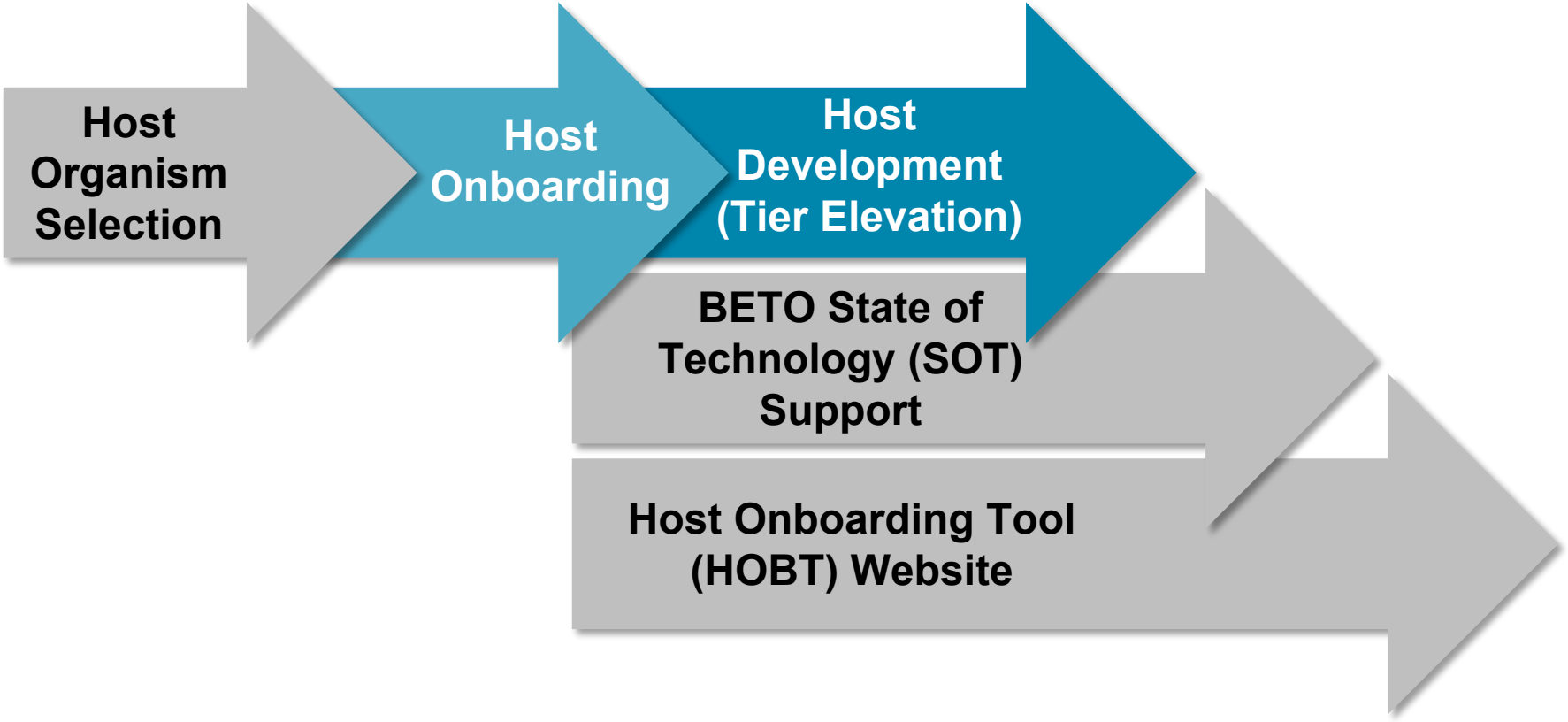
Approach: Host selection



Approach: Host selection

- **Goal**: Strategically select host organisms for HOD team to improve.
- **Criteria**: Require broad phylogenetic and metabolic diversity to enable the use of challenging feedstocks and application of novel metabolic engineering strategies; Align with ABF DBTL efforts in Target/Host exemplars and Beachhead molecules; Consider external stakeholder input.
- **Process:** Develop a defined, transparent approach to reach consensus on the ABF host organism portfolio.
- **Potential challenges**: Unable to reach consensus (mitigated by having an open, collaborative selection process).
- **FY20Q2_Milestone:** Convene a committee consisting of members of the HOD, IA, and Management teams to choose the most strategically important new hosts to onboard. Compile a list of the top 15 most important hosts for the ABF to develop and report on the justification for each.

Approach: Host onboarding & development



Approach: Tier system as a framework for host onboarding and development

- **Goal**: Create a tiered system to organize, standardize (to the extent possible), and communicate HOD activities of the ABF.
- **Onboarding vs development (Tier elevation):**
  Onboarding is the act of meeting the minimum set of criteria that a new organism would need to be used constructively in DBTL (Tier 1).
  Tier Elevation is the act of developing the strain beyond this minimum set (Tiers 2-4).
- **Potential challenges**: Innate physiological and genetic differences between host organisms and the rapidly evolving nature of synthetic biology demands flexibility in the Tier system, which at its heart is aimed at standardization.
- **Related Milestones:** 10 new hosts onboarded by FY21Q4; 20 new hosts onboarded by FY22Q4; 2-3 hosts elevated 1 Tier by FY21Q2

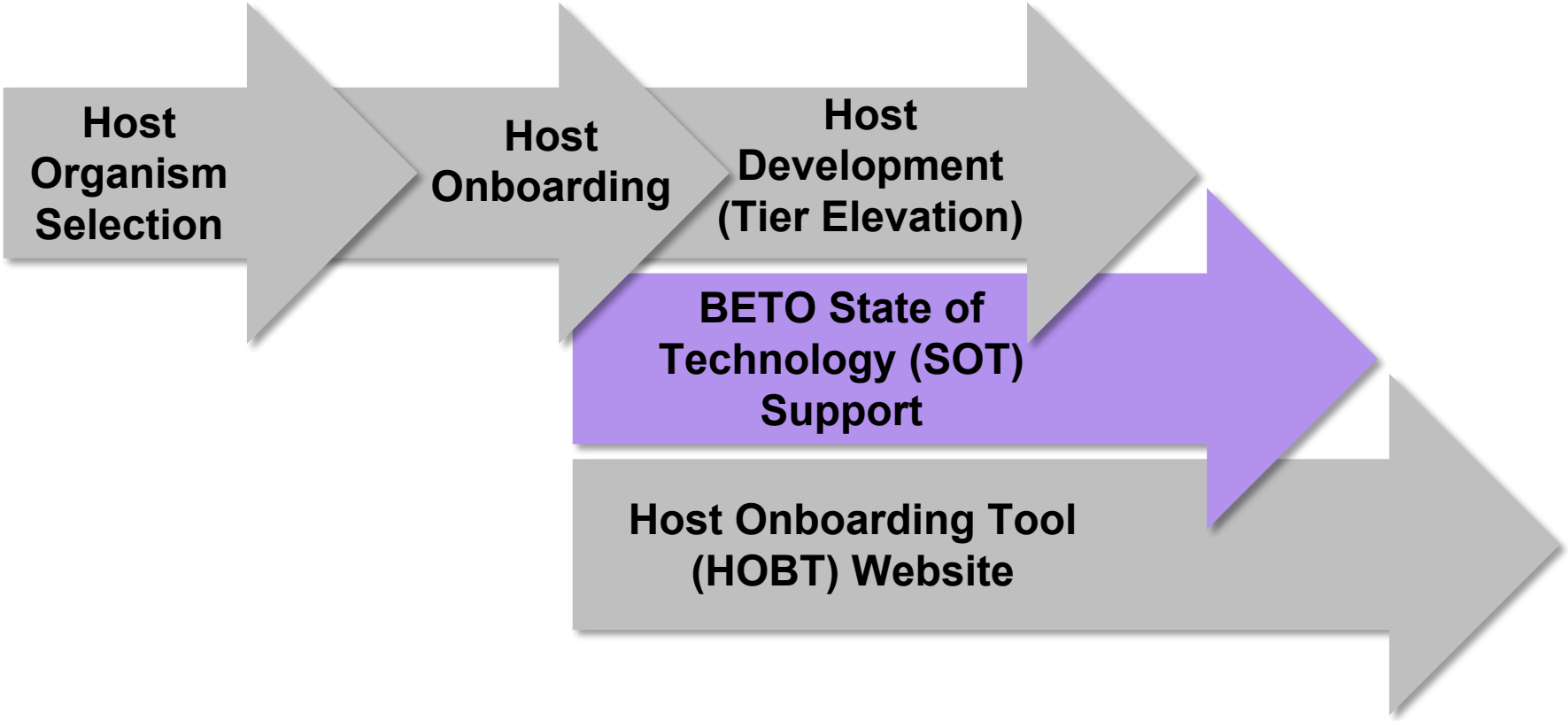
Approach: Host Onboarding

- **Goal**: Acquire or develop tools to enable DBTL use for each target organism, either within the ABF or with external stakeholders.
- **Approach**: Acquire (if available in the literature) or develop tools and methods for genetic manipulation of target organisms, characterize basic genetic parts, determine baseline growth properties, with the goal of handing off to DBTL teams for rational engineering.
- **Potential challenges**: Each organism is unique and could have unique barriers to genetic manipulation; genetic parts from related organisms may not function similarly in the target organism
- **Related Milestones:** 10 new hosts onboarded by FY21Q4; 20 new hosts onboarded by FY22Q4

Approach: Host development & Tier elevation

- **Goal**: Acquire or develop more advanced tools and physiological data to enhance DBTL cycles for each target organism and enable advanced metabolic engineering approaches.
- **Approach**: Acquire (if available in the literature) or develop advanced genetic tools for HTP Build and genome-scale libraries for HTP Test, build models, create omics datasets, etc. that will enable more advanced and higher throughput strain engineering.
- **Potential challenges**: Each organism is unique and could have unique barriers to genetic manipulation; knowledge obtained for one organisms may not always be applicable to another; not every tool/dataset is needed for every organism, so we need to make sure we target the most impactful ones for each organism.
- **Related Milestones:** 2-3 hosts elevated 1 Tier by FY21Q2.

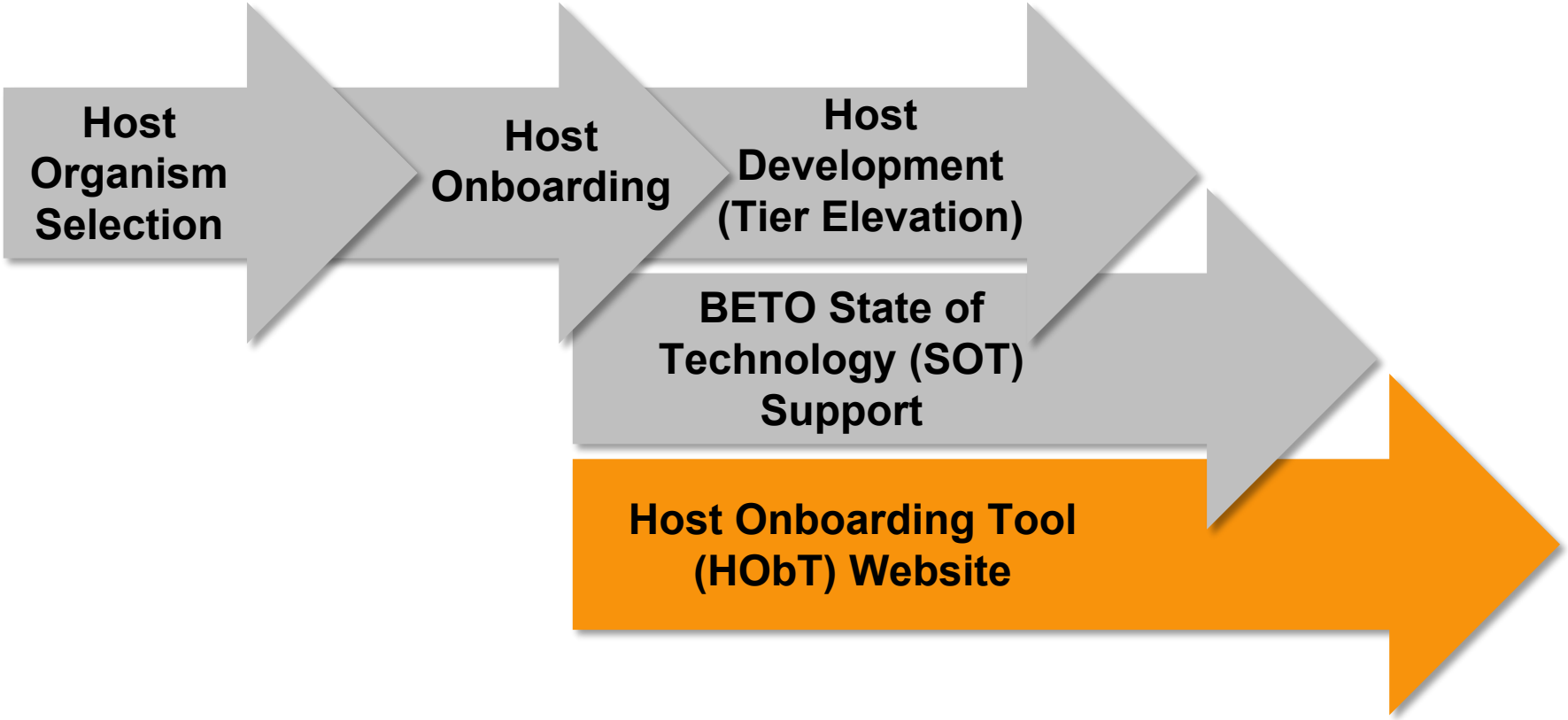
Approach: BETO SOT support



Approach: BETO State of Technology support

- **Goal**: Leverage ABF expertise in genetic tool development to make genetic manipulation of BETO SOT organisms more rapid and reliable
- ***Zymomonas mobilis* technical approach**: increase transformation efficiency by evading restriction systems, develop high efficiency DNA integration tool, develop library of characterized genetic parts
- ***Clostridium tyrobutyricum* technical approach**: increase transformation efficiency, develop high efficiency DNA integration tool, develop library of characterized genetic parts, develop efficient gene deletion system
- **Potential challenges**: organisms often have unique idiosyncrasies that require custom solutions
- **Related Milestones**: FY21Q2 High efficiency gene integration tools developed for both *C. tyrobutyricum* and *Z. mobilis*

Approach: Host Onboarding Tool (HObT)



Approach - Host Onboarding Tool (HObT)

- **Goal:** Establish a website that serves as an intra-ABF and public-facing hub for information, protocols, and tool descriptions of selected non-model organisms.
- **Approach**: Complete the website in two phases, Phase I emphasized the backend implementation and system architecture (setting up framework). Phase II focuses on user interface.
- **Challenges**: Develop a website that functions for both internal ABF use and public use
- **Related Milestones**: FY20Q2. Host Onboarding website phase 1 implemented and operational; HObT web application user-interface completed and deployed to the publicly accessible HObT web site.

Approach: Milestone Summary

Organism selection

FY20Q2. Compile a list of the top 15 most important hosts for the ABF to develop and report on the justification for each.

Host onboarding

FY21Q4. Complete onboarding of at least 10 new / existing Hosts to at least Tier 1, for use in the ABF.

FY22Q4. Bring a total 20 microbial hosts (20 species) to at least Tier 1, & provide corresponding information, resources, and tools via publicly-accessible ABF website.

Tier elevation

FY21Q2. Continue host improvement to elevate 2-3 onboarded hosts by at least one Tier.

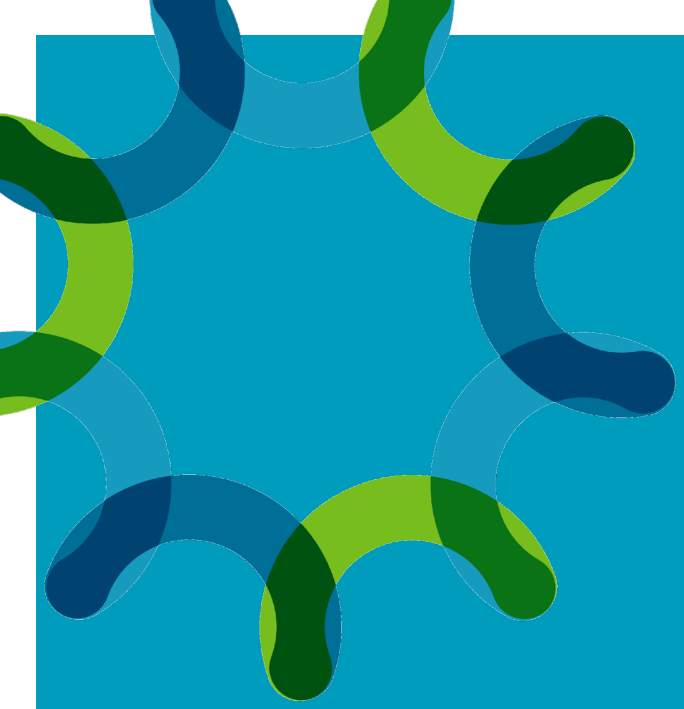
State of Technology support

FY21Q2. High efficiency gene integration tools developed for both *C. tyrobutyricum* and *Z. mobilis* with a minimum efficiency of 1000 cfu/ug DNA.

Host Onboarding Tool

FY20Q2. Host Onboarding website phase 1 implemented and operational.

FY21Q4. HObT web application user-interface completed and deployed to the publicly accessible HObT web site.



3 - Impact

3 – Impact

Reduced barriers to using non-model organisms:

- Reduced risk for adopting strains with atypical feedstock usage and metabolic capacity
- External stakeholder feedback indicates that onboarding new hosts would be beneficial to industry
- Based on needs & described by the IEO Task, during Directed Funding Opportunities, companies are interested in leveraging ABF tool development expertise for non-model organisms

"Expand host range and make new organisms scalable ….Great use of national lab resources"

"Onboard a bunch of organisms with a lot of tools. Avoid companies needing to license and go into different organisms to avoid IP issues"

"Organisms that represent a broad range of metabolic space and manufacturing potential"

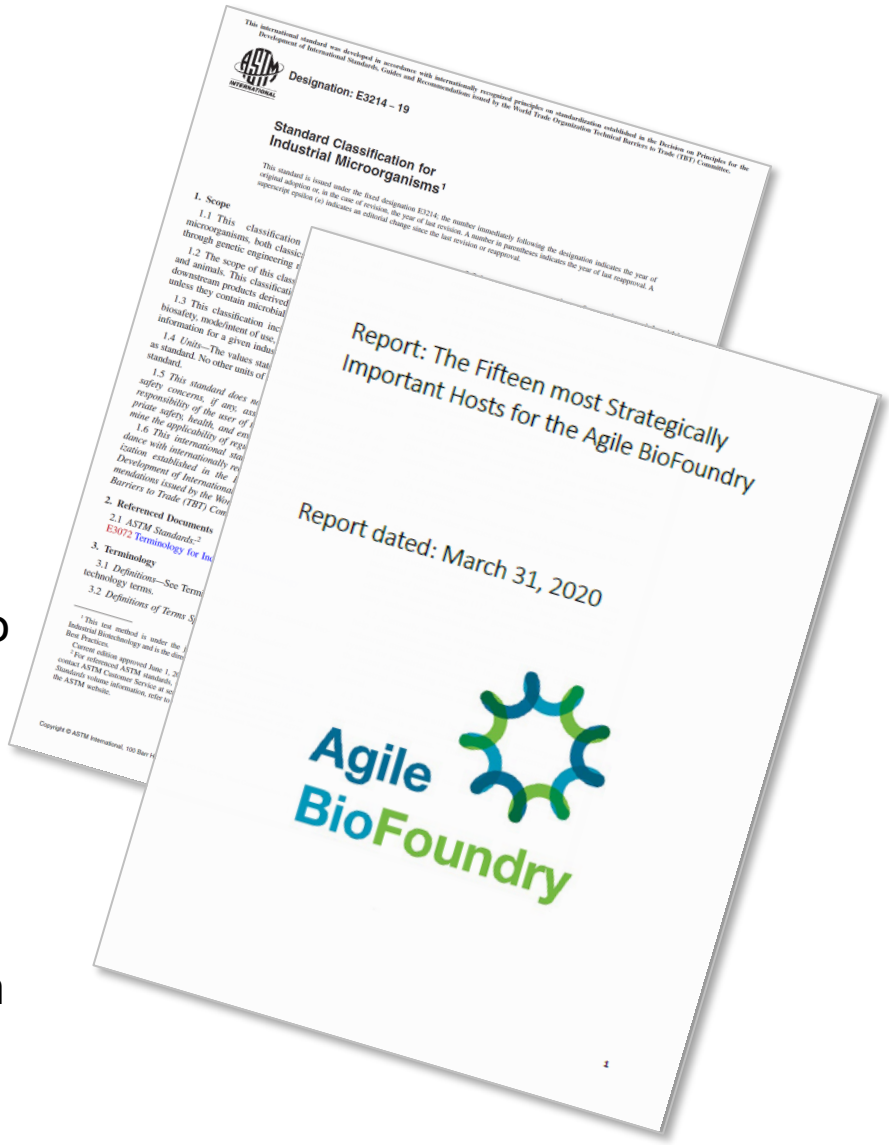
"Make a database. Standardized strain descriptions. Growth conditions. How made. Tools used. Substrate ranges. How transformable."

Quotes from Industry

3 – Impact

Information Dissemination: Public access to strains and tools

- Publications, conference presentations, patent applications
- Agile BioFoundry and HObT websites
- Web portal for each host, with links to protocols and data
- **ASTM E3214 – 19:** We will be integrating classification information, as defined by ASTM E3214 -19 *Standard Classification for Industrial Microorganisms,* into the Tier System



3 – Impact

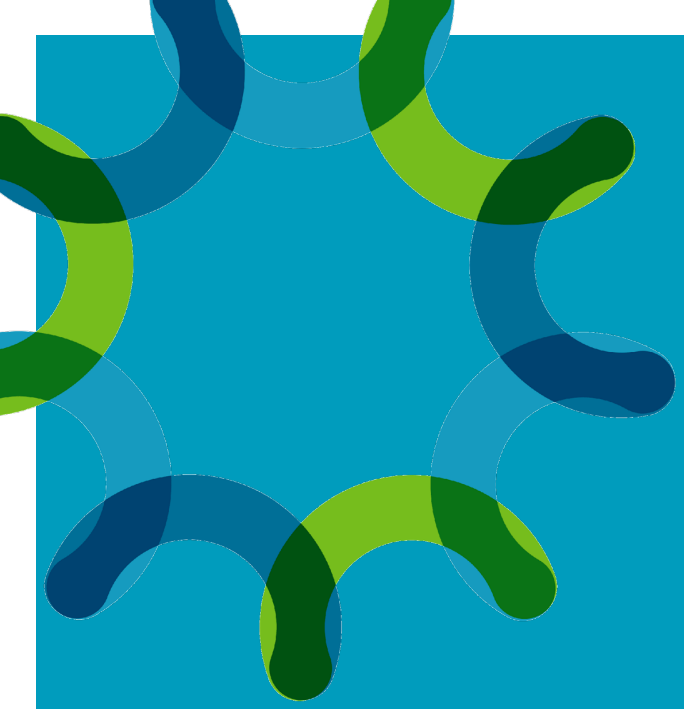
Relevance to BETO mission:

- Assist other BETO projects to accelerate progress across the BETO portfolio, in particular the BETO Conversion State of Technology
- Collaborate with industry via DFO and FOA projects to develop advanced tools in new hosts

Examples of benefits for developing non-model organisms

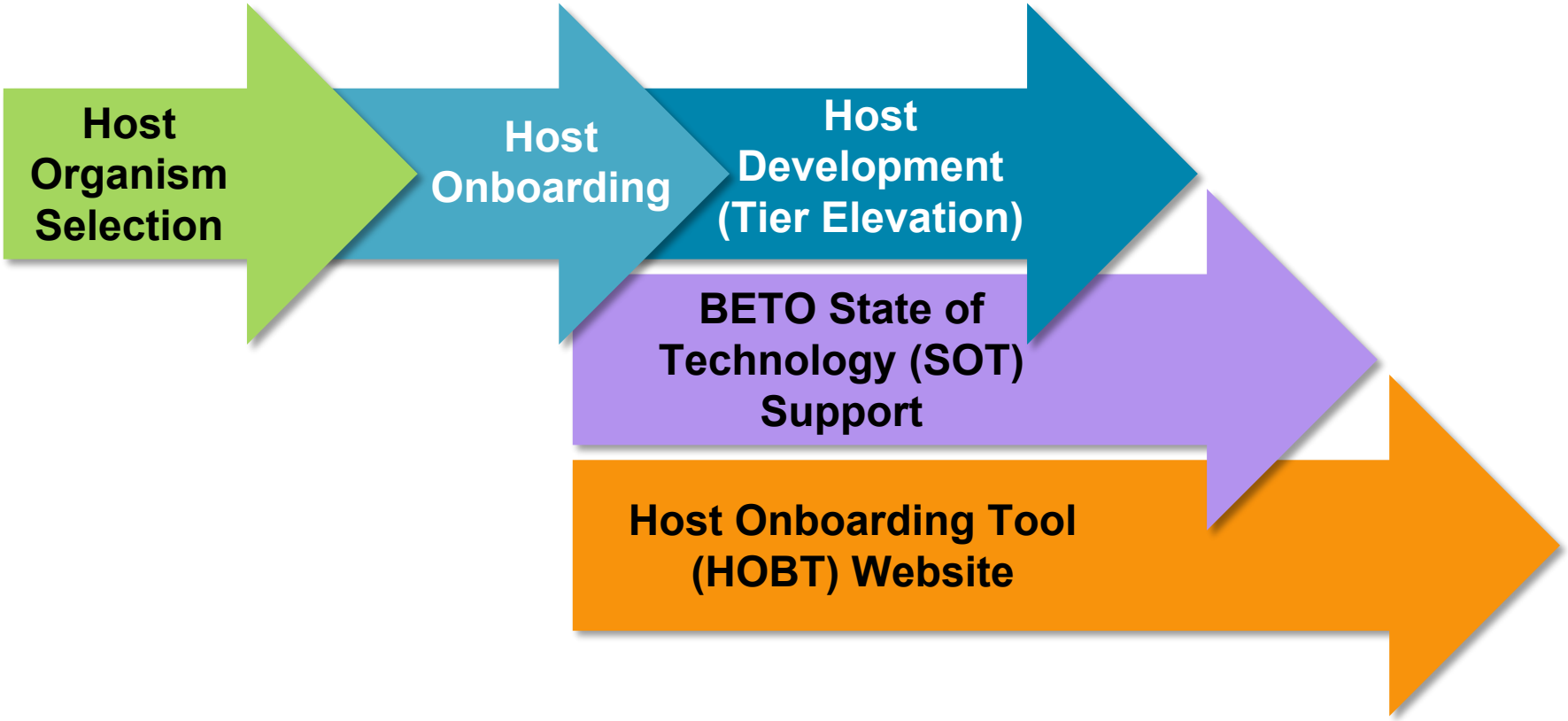
Eliminate costly separations steps by directly producing acids	Engineering of hosts that can consume industrial feedstocks	Higher substrate uptake rates decreasing fermentation times	Accelerate strain construction, allowing rapid screening of pathways in parallel

Enhanced genetic tools enables more rapid engineering of strains, thereby accelerating the advancement of the SOT

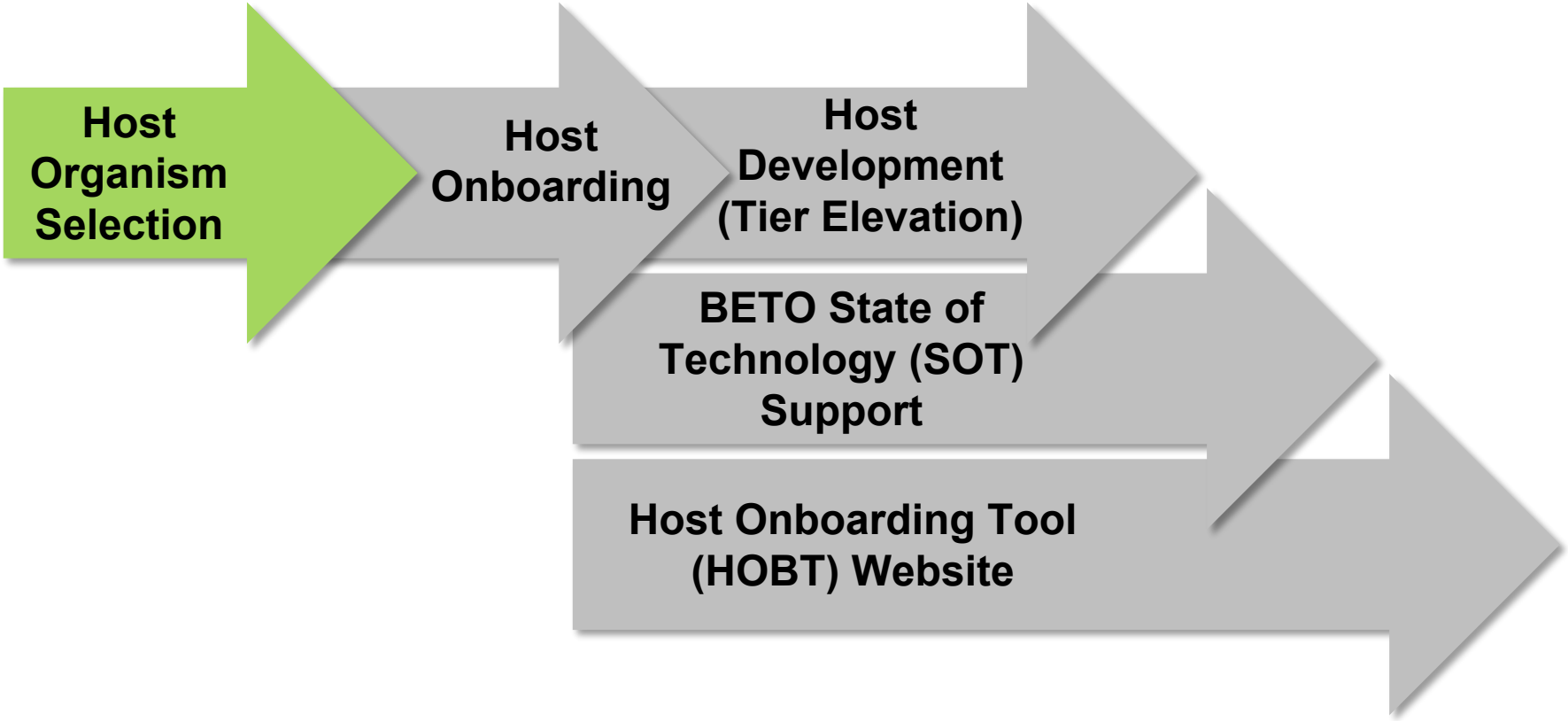


4 - Progress and Outcomes

Progress & Outcomes



Progress & Outcomes: Organism selection



Progress & Outcomes: Organism selection

		Criteria																	
		Metabolic Diversity							Processing Conditions					Feedstock Utilization					
		Beachead							Low pH		Aerobic/Anaerobic			Default	Alternates				
	Organisms	Aromatics	Terpenes	Fatty Acids/ Alcohols (Malonyl-CoA)	Citrate	Pyruvate	Glutamate	Acetoacetyl CoA	Multi-Celled	Single-Celled	Aerobe	Anaerobe	Facultative Anaerobe	Hydrolysate	Syngas	Methane/ MeOH	Formate/ CO2/ H2	Photosynthetic (CO2, sunlight)	Unhydrolyzed Biomass
Existing ABF Organisms	1 *Pseudomonas putida*	■				■		■			■			■					
	2 *Acinetobacter baylyi ADP1*	■			■						■			■					
	3 *Corynebacterium glutamicum*	■			■	■	■						■	■					
	4 *Aspergillus pseudoterreus*				■				■		■			■					
	5 *Aspergillus niger*				■				■		■			■					
	6 *Rhodosporidium toruloides*		■	■							■			■					
	7 *Pichia kudriavzevii*									■			■	partial					
	8 *Zymomonas mobilis*					■		■					■	■					
	9 *Bacillus coagulans*					■							■	■					
	10 *Cupriavidus necator*					■					■			■			■		
	11 *Clostridium tyrobutyricum*							■				■		■					
	12 *Clostridium ljungdahlii*											■			■				
	13 *Clostridium carboxidivorans*											■			■				
Potential Organisms	14 *Rhodococcus jostii*	■		■		■							■	■					
	15 *Lipomyces starkeyi*			■						■			■	■					
	16 *Yarrowia lipolytica*			■	■			■			■			■					
	17 *Rhizopus oryzae*					■					■			■					
	18 *Zygosaccharomyces bailii*					■					■			partial					
	19 *Aspergillus oryzae*				■				■					■					
	20 *Candida boidinii*					■								■		MeOH			
	21 *Methylomicrobium buryatense*					■		■								■			
	22 *Bacillus methanolicus*					■	■									■			
	23 white rot fungus such as *Ganoderma lucidum*																		■
	24 *Myceliophthera thermophila*																		■
	25 *Parageobacillus thermoglucosidasius*					■							■						■
	26 *Synechocystis sp. PCC 6803*		■	■										partial				■	
	27 *Rhodobacter sphaeroides*		■	■									■	partial					

- ABF members proposed hosts and provided justification; committee discussed
- Transparent process with defined criteria; each lab got one vote, as did TEA/LCA
- **Outcome:** Hosts with phenotypic and phylogenetic diversity for use in the ABF

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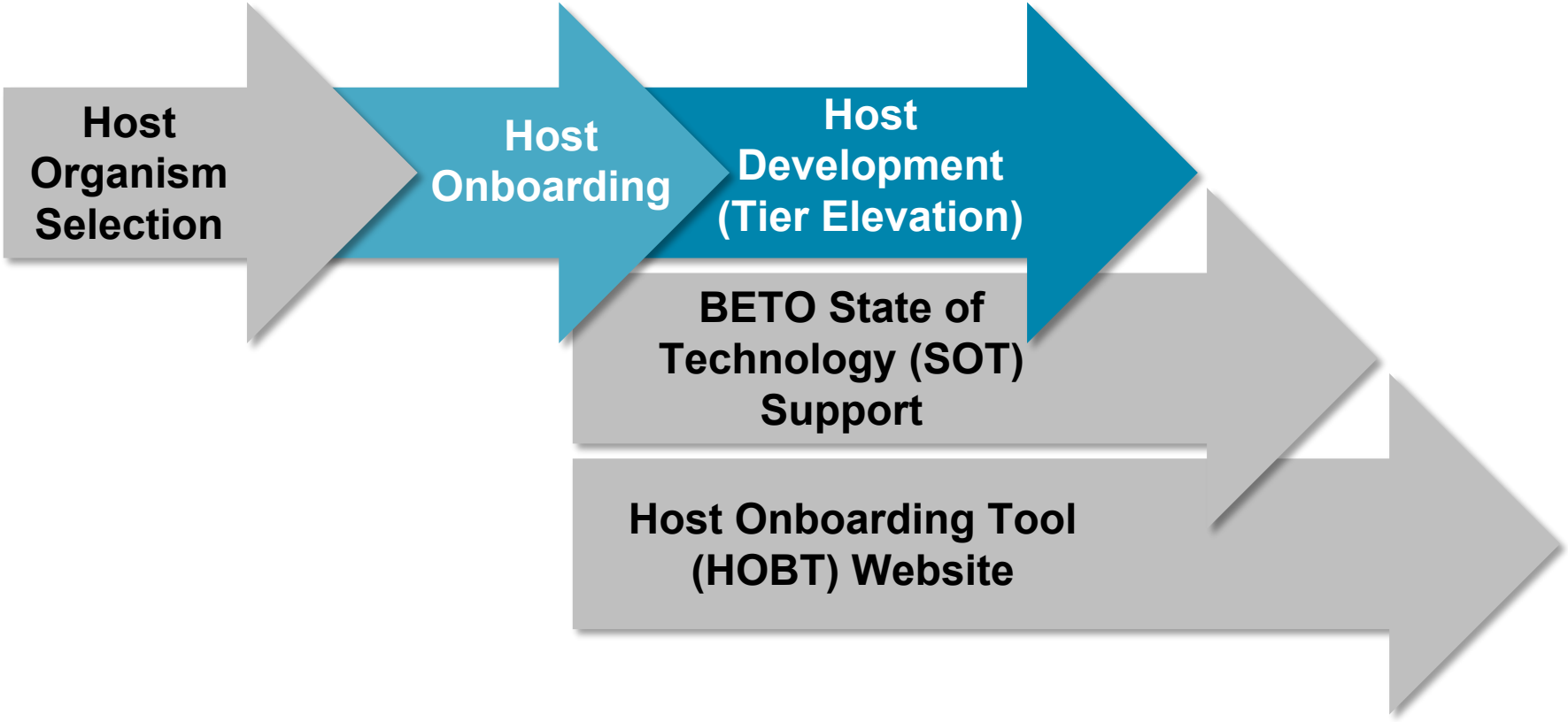
Progress & Outcomes: 15 organisms chosen

	Organisms
1	*Pseudomonas putida*
2	*Rhodosporidium toruloides*
3	*Aspergillus niger*
4	*Corynebacterium glutamicum*
5	*Cupriavidus necator*
6	*Bacillus coagulans*
7	*Pichia kudriavzevii*
8	*Clostridium tyrobutyricum*
9	*Lipomyces starkeyi*
10	*Rhodobacter sphaeroides*
11	*Aspergillus pseudoterreus*
12	*Zymomonas mobilis*
13	*Clostridium ljungdahlii*
14	*Zygosaccharomyces bailli*
15	*Methylomicrobium buryatense*

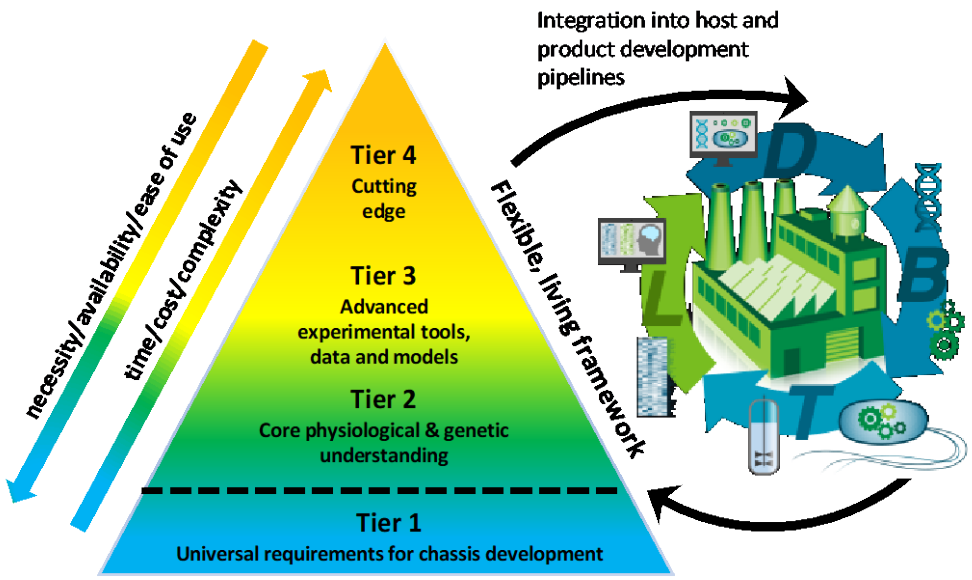
- Selected organisms cover a variety of phylogenetic, phenotypic, and metabolic space
- Use a range of feedstocks and perform under varied processing conditions
- Tied organism selection to beachhead molecules
- **Outcome:** FY20Q2 milestone: 15 organisms selected (complete).

Progress & Outcomes: HOD



Progress & Outcomes: Tier system established

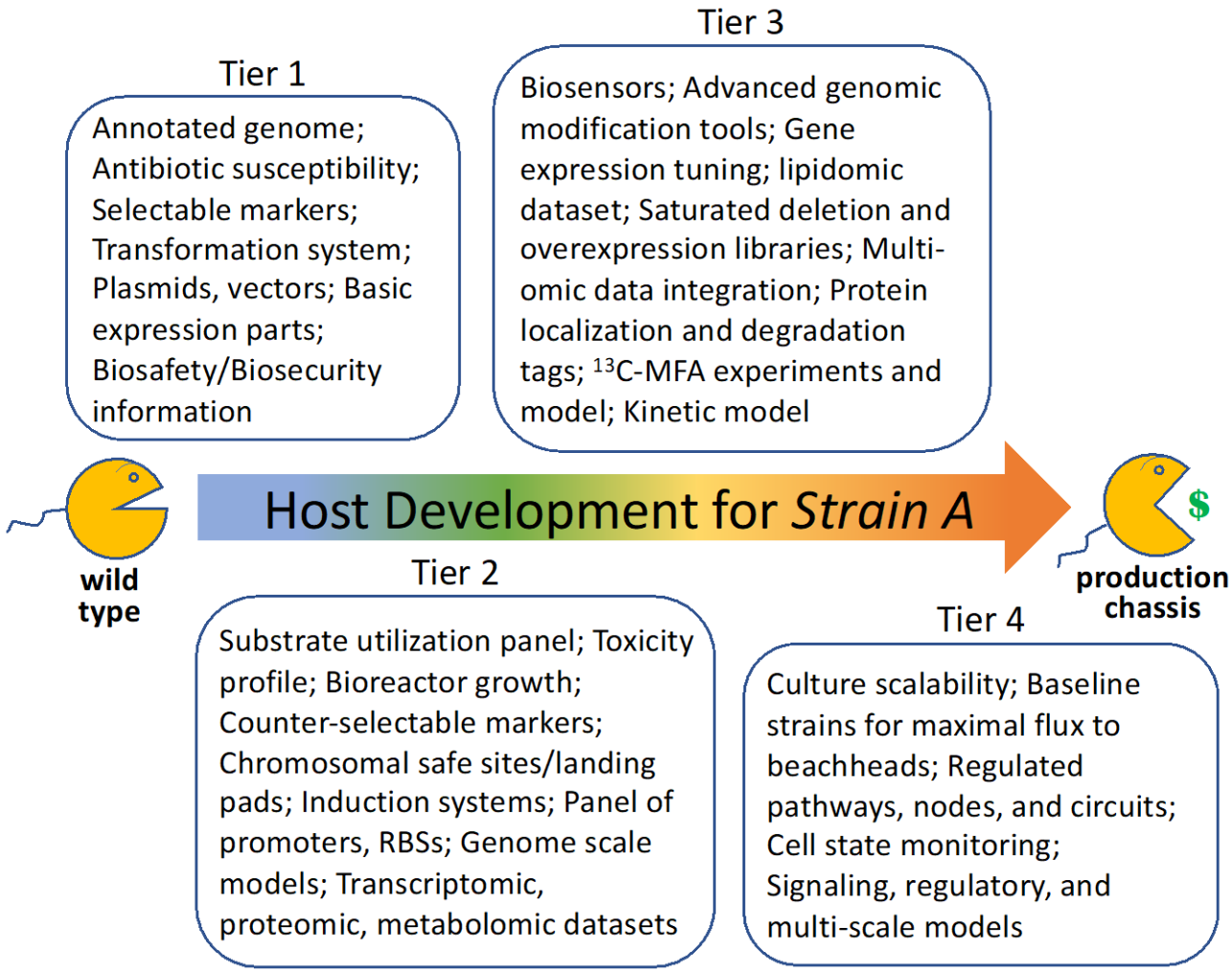
- **Tier 1**: Fundamental tools and information needed for any rational DBTL cycle; these basics must be achieved to be “onboarded”
- **Tier 2**: Tools and knowledge needed for rapid and robust DBTL cycles
- **Tier 3**: More advanced tools, ‘omics data, models, etc
- **Tier 4**: Cutting edge tools, libraries, and data



- Developed a Tier System to focus and organize host development
- Increasing Tier level represents increasing level of tools and baseline knowledge
- **Outcome:** Framework to guide onboarding and development for diverse hosts

Progress & Outcomes: Tier system established

- Criteria were placed in Tiers based on importance for DBTL, and cost/time outlays
- Not hard and fast rules, but rather flexible guidelines for prioritization
- **Outcome:** More advanced tools and capabilities for diverse hosts



Progress & Outcomes: Onboarding status of 15 strains

	Organisms	
1	*Pseudomonas putida*	**Onboarded/elevation**
2	*Rhodosporidium toruloides*	**Onboarded/elevation**
3	*Aspergillus niger*	**Onboarded/elevation**
4	*Corynebacterium glutamicum*	**Onboarded/elevation**
5	*Cupriavidus necator*	**Onboarded/elevation**
6	*Bacillus coagulans*	**Onboarded/elevation**
7	*Pichia kudriavzevii*	**Onboarding**
8	*Clostridium tyrobutyricum*	**Onboarding**
9	*Lipomyces starkeyi*	**Onboarded/elevation**
10	*Rhodobacter sphaeroides*	**Onboarded/elevation**
11	*Aspergillus pseudoterreus*	**Onboarded/elevation**
12	*Zymomonas mobilis*	**Onboarded/elevation**
13	*Clostridium ljungdahlii*	**Onboarding**
14	*Zygosaccharomyces bailli*	**To be onboarded**
15	*Methylomicrobium buryatense*	**To be onboarded**

- 10 hosts already onboarded and undergoing Tier elevation
- 3 actively undergoing onboarding
- 2 selected for future onboarding
- 5 more organisms need to be selected and onboarded for FY22
- **Outcome:** FY21Q4 milestone: 10 hosts onboarded (complete)
- **Outcome:** FY22Q4 milestone: 20 onboarded (on track)

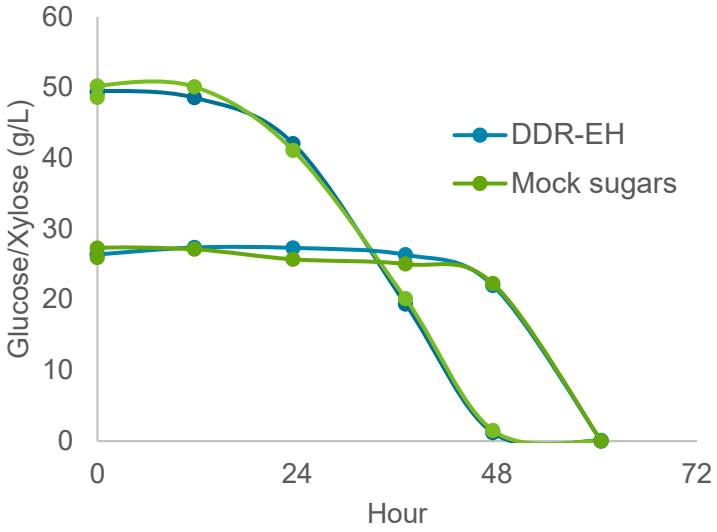
Progress & Outcomes: Example of onboarding an organism, *Bacillus coagulans*

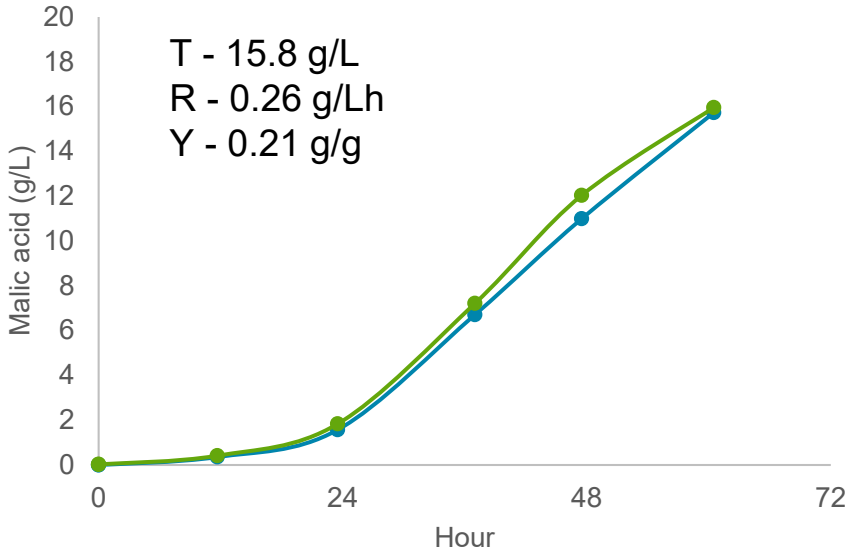
Tier 1

Criteria	Completion	Details
Annotated genome	✓	Sequenced by ABF
Growth conditions	✓	Growth in complex media and DMR-EH hydrolysates has been characterized (BETO – RCFC)
Growth kinetics	✓	Produced kinetic data on sugar utilization and lactic acid production (BETO – RCFC)
Antibiotic susceptibility	✓	ABF tested MIC of 4 antibiotics, 3 are effective (Tet, Cm, Sp)
Selectable markers	✓	ABF tested 4 resistance genes, 3 function for selection
Transformation methods	✓	ABF developed an electroporation method, ~10^4 cfu/ug
Plasmids/vectors	✓	ABF found 2 functional origins of replication
Basic expression parts	✓	ABF demonstrated constitutive reporter gene expression (mCherry) from a replicative plasmid (pMTV80m) using a P*pta* promoter
Biosafety/Biosecurity	✓	BSL1

- Targeted tool development, knowledge generation to enable rational engineering
- Leveraged related tools from related Bacilli and Clostridia
- **Outcome:** *B. coagulans* is now onboarded and ready for ABF DBTL cycles; will proceed to Tier elevation

Progress & Outcomes: Example of onboarding and Tier elevation of an organism, *Lipomyces starkeyi*





- Validated Tier 1 tools to allow use in the ABF
- Targeting malic acid production (via pyruvate and oxaloacetate beachheads)
- **Outcome:** *L. starkeyi* is now onboarded and can be used in DBTL cycles. Tier elevation is currently focused on genome-scale modeling and multi-omics characterization during conversion of hydrolysate to malic acid

Progress & Outcomes: Tier elevation status of 15 strains

	Organisms	
1	*Pseudomonas putida*	Tier 1 → 2 complete
2	*Rhodosporidium toruloides*	Tier 1 → 2 complete
3	*Aspergillus niger*	Tier 1 → 2 complete
4	*Corynebacterium glutamicum*	Tier 1 → 2 complete
5	*Cupriavidus necator*	Tier 1 complete
6	*Bacillus coagulans*	Tier 1 complete
7	*Pichia kudriavzevii*	Onboarding
8	*Clostridium tyrobutyricum*	Onboarding
9	*Lipomyces starkeyi*	Tier 1 → 2 ongoing
10	*Rhodobacter sphaeroides*	Tier 1 → 2 ongoing
11	*Aspergillus pseudoterreus*	Tier 1 → 2 ongoing
12	*Zymomonas mobilis*	Tier 1 complete
13	*Clostridium ljungdahlii*	Onboarding
14	*Zygosaccharomyces bailli*	To be onboarded
15	*Methylomicrobium buryatense*	To be onboarded

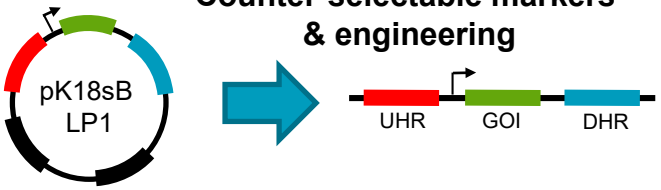
- 4 hosts elevated by 1 tier
- 3 actively undergoing tier elevation
- Tier 3 criteria also partially completed for some strains
- **Outcome:** FY21Q2 milestone: 2-3 hosts elevated by one tier (milestone met)

Progress & Outcomes – Example of Tier elevation: *Corynebacterium glutamicum*

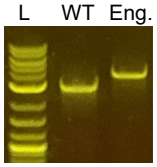
Tier 2

Criteria	Completion	Details
Substrate utilization panel	✓	10+ carbon sources
Toxicity profiles	✓	1 product, 2 substrates
Bioreactor growth	✓	1 bioreactor run
Genetic stability	✓	5 rounds of modifications
Counter-selectable markers	✓	sacB
Genome integration systems	✓	Homologous and site-specific recombination
Chromosomal safe sites/landing pads	✓	3 used, 15 available in literature
Induction systems	✓	Parts available in ABF
Panel of constitutive promoters, RBSs	✓	Promoters and RBS characterized
Genome scale models	✓	Literature available
Transcriptomic, proteomic, metabolomic datasets	✓	Literature available

Counter-selectable markers & engineering

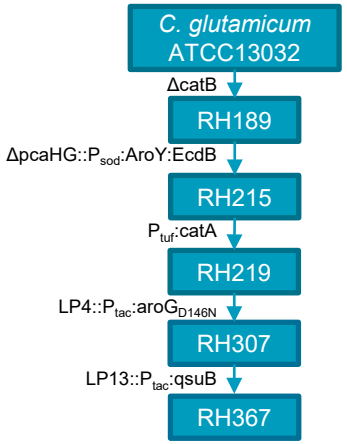


3 landing pads successfully used

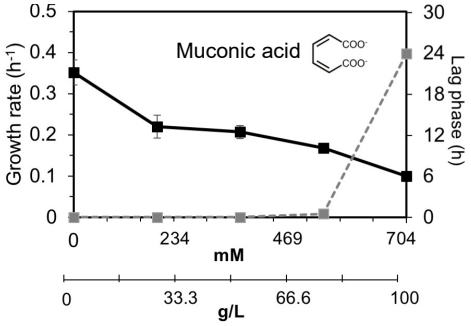


5 genome modifications in a single strain

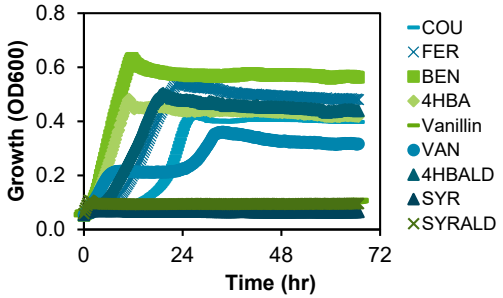
Genetic stability



Toxicity Profiles



Substrate utilization

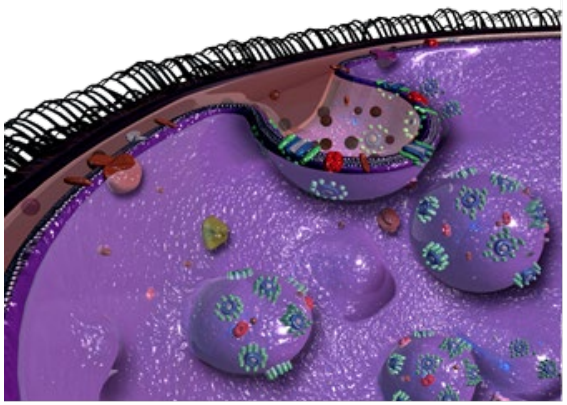


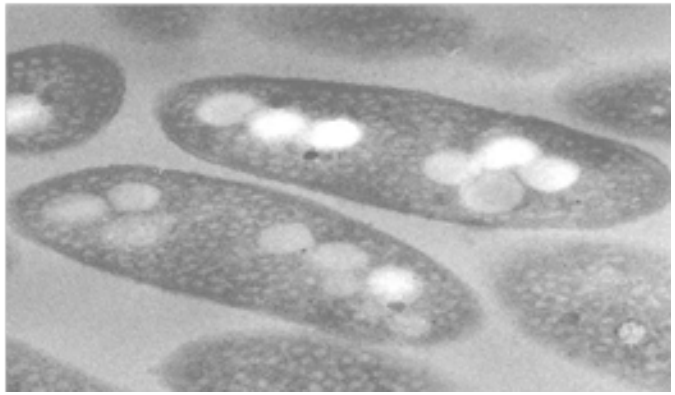
- Culture and transformation procedures onboarded
- Toxicity profiles, substrate utilization, and genetic engineering tools developed
- **Outcome:** *Corynebacterium glutamicum* elevated from Tier 1 to Tier 2 in ABF

Progress & Outcomes: Onboarding and Tier elevation of *Rhodobacter sphaeroides*

Task Tier: 1 2 3 4

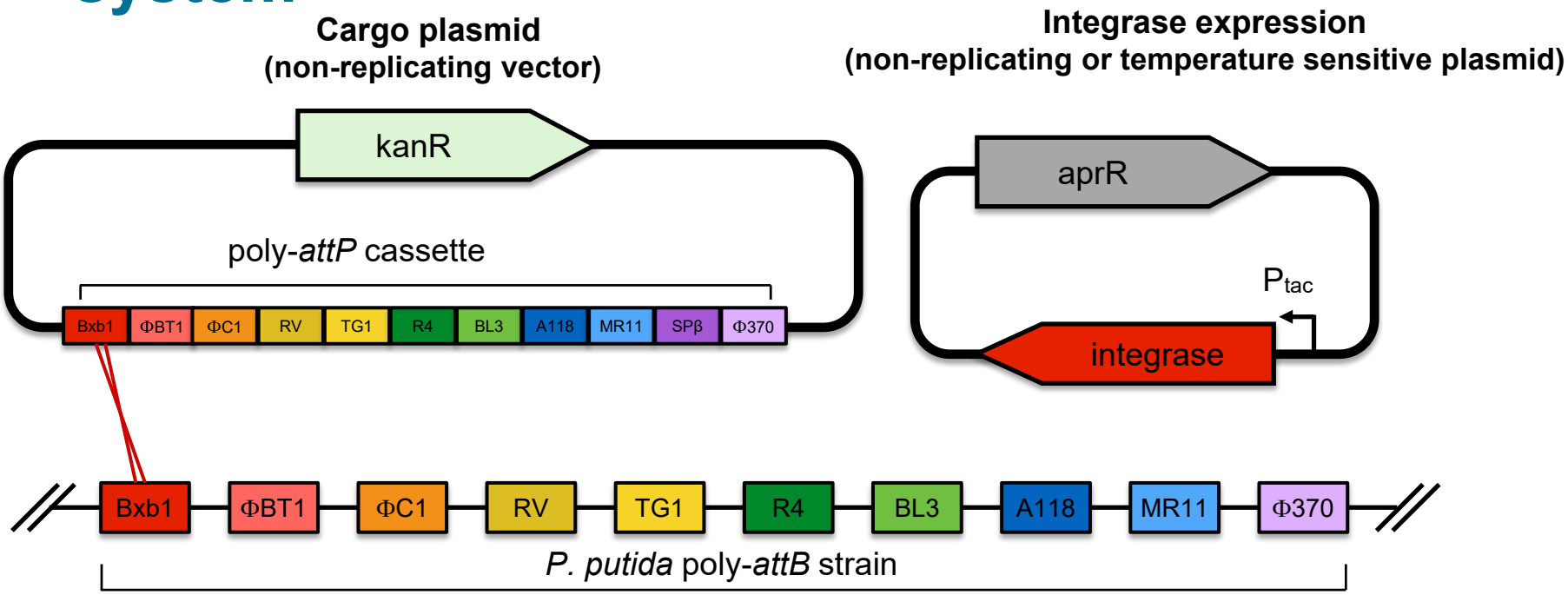
Rhodobacter sphaeroides		**FY21**				**FY22**			
Task	Task Description	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	Growth conditions								
2	Resequence,archive and distribute strains								
3	Transformation methods								
4	Chromosomal safe sites/landing pads								
5	Genome integration systems								
6	Toxicity Profiling								
7	Gene expression tuning								
8	Biosensors								
9	Omics and model building								
10	Bisabolene production								





- Metabolically versatile photosynthetic bacterium for terpene beachhead
- An example of a strain where all Tier 1 tools exist; needed to bring tools in-house
- **Outcome:** *R. sphaeroides* was onboarded this year and is now going through Tier elevation

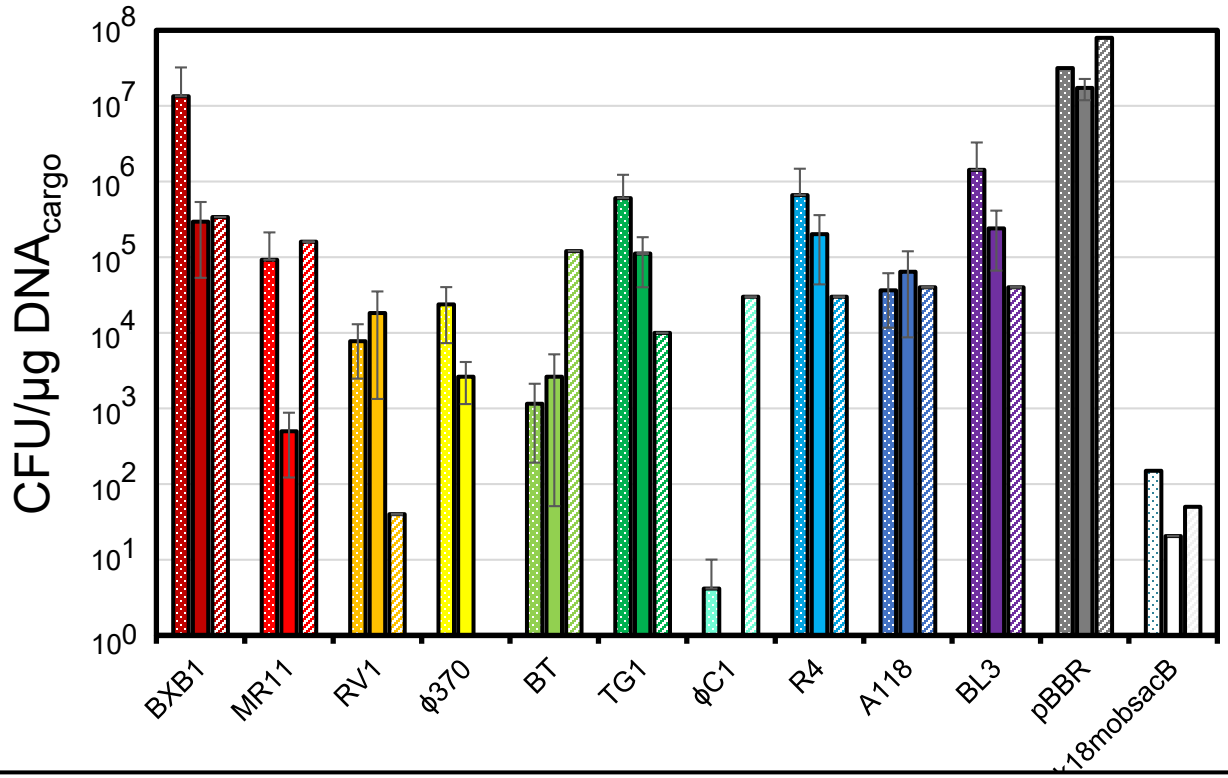
Progress & Outcomes – Example of tool development: Site specific DNA integration system



- Originally developed for *P. putida*
- Allows rapid, efficient insertion of heterologous DNA into the chromosome
- **Outcome:** We developed a HTP tool to rapidly screen heterologous expression from the chromosome

Progress & Outcomes – Example of tool development: Integration efficiency in three organisms

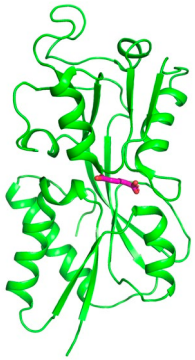
=*P. putida*
=*C. necator*
=*C. glutamicum*

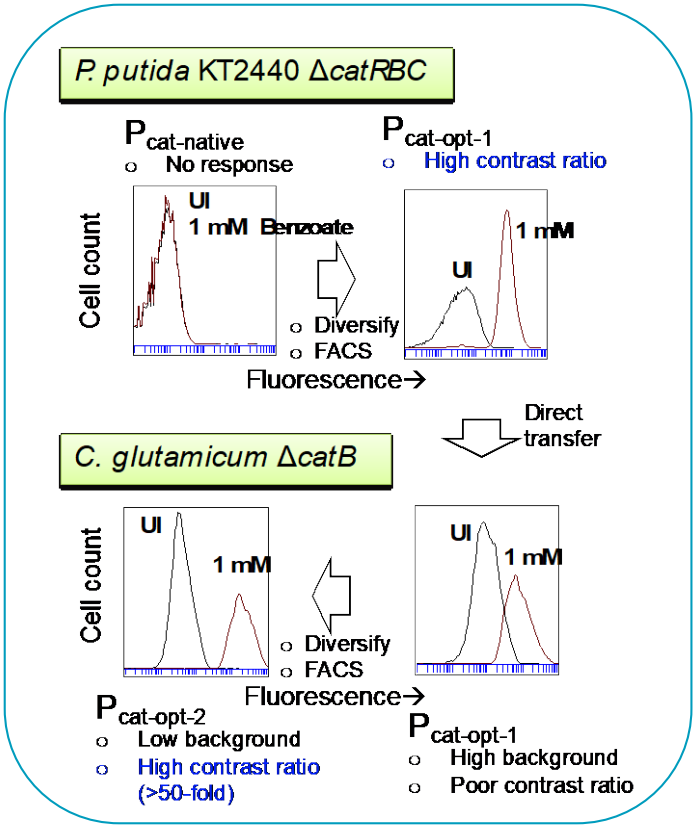


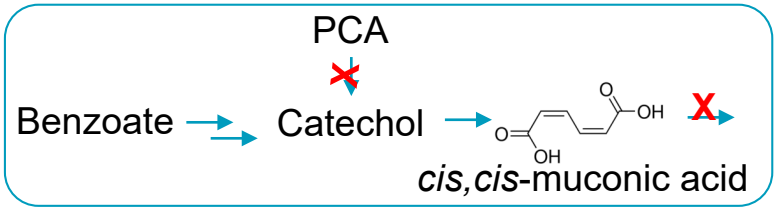
- High efficiency integration of DNA into the chromosome in 3 organisms
- Example of rapid adaptation of tools to new hosts
- **Outcome:** Highly efficient tools that allow parts to be reused in new hosts for rapid prototyping

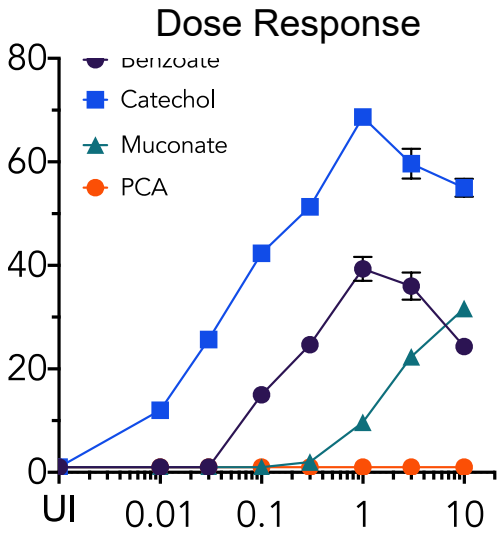
Progress & Outcomes – Example of tool transfer: *C. glutamicum in vivo* biosensor development

CatM (*A. baylyi* ADP1)









- Muconate sensor established in *P. putida* showed weak response in *C. glutamicum*
- Build large promoter library (~35000 diversity); FACS to select top performers
- **Outcome:** Optimized sensor with >50-fold response over baseline in new host

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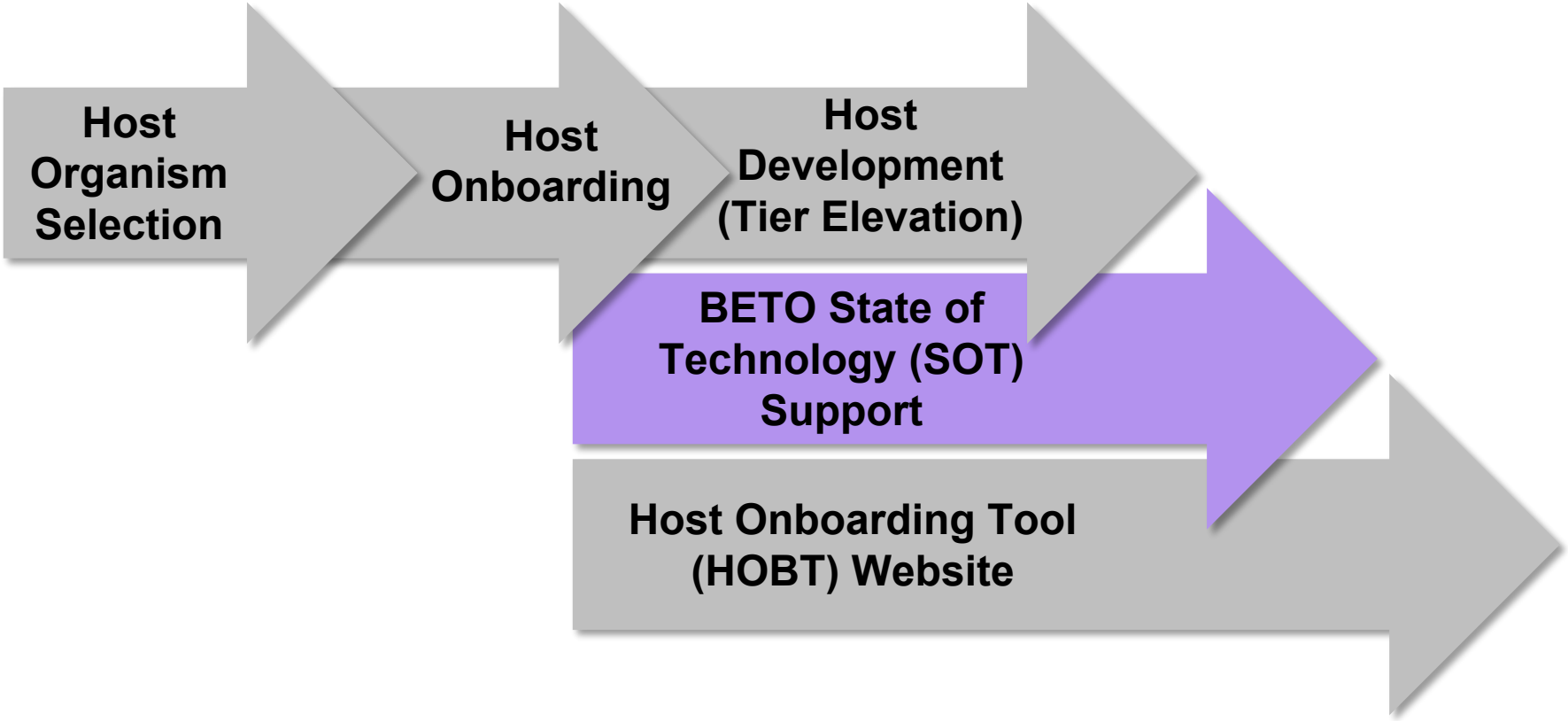
Agile BioFoundry

Progress & Outcomes

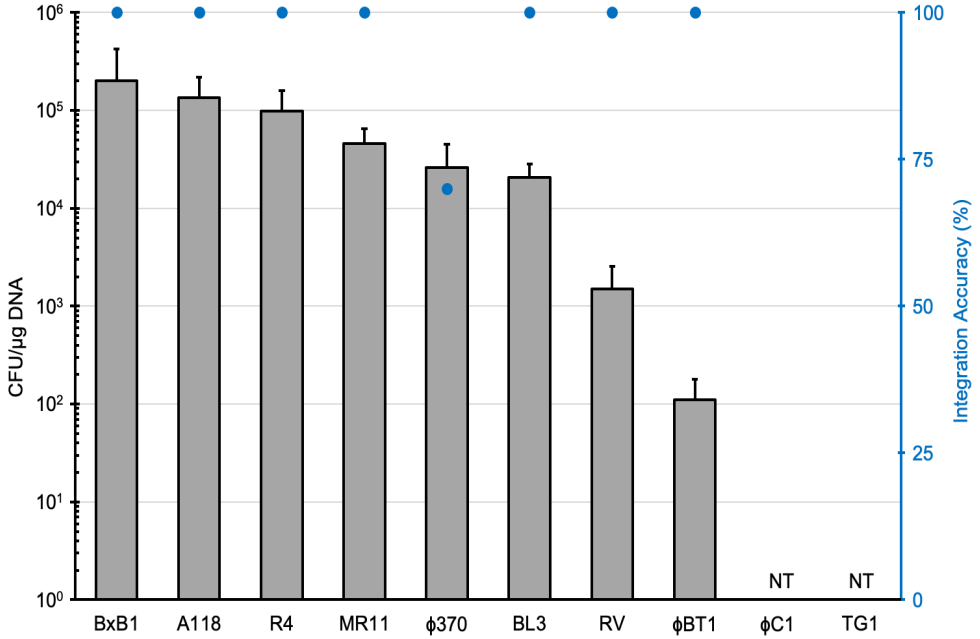


- Other BETO projects use non-model microbes with poorly developed tools
- Building those tools will help advance bioenergy research across the portfolio
- **Outcome:** More rapid strain engineering in BETO SOT projects

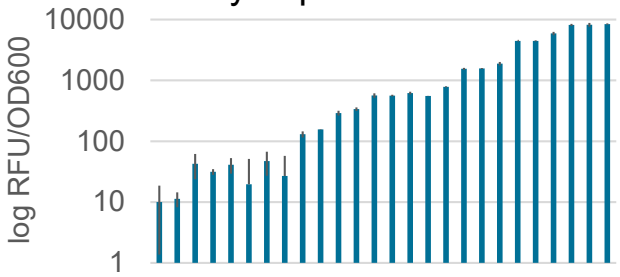
SOT organism improvement: *Zymomonas mobilis*

Increased transformation efficiency

DNA integration efficiency and accuracy

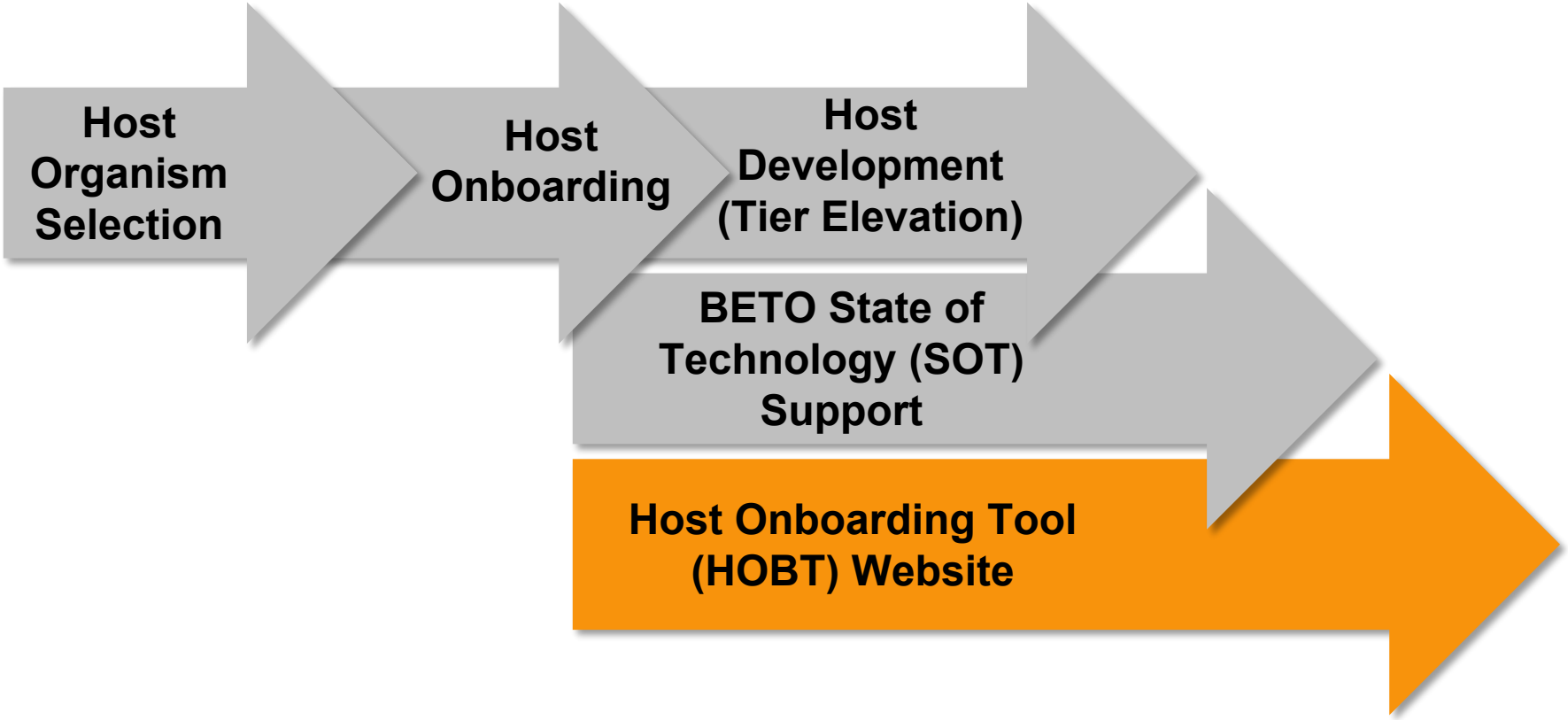


Library of promoter fusions

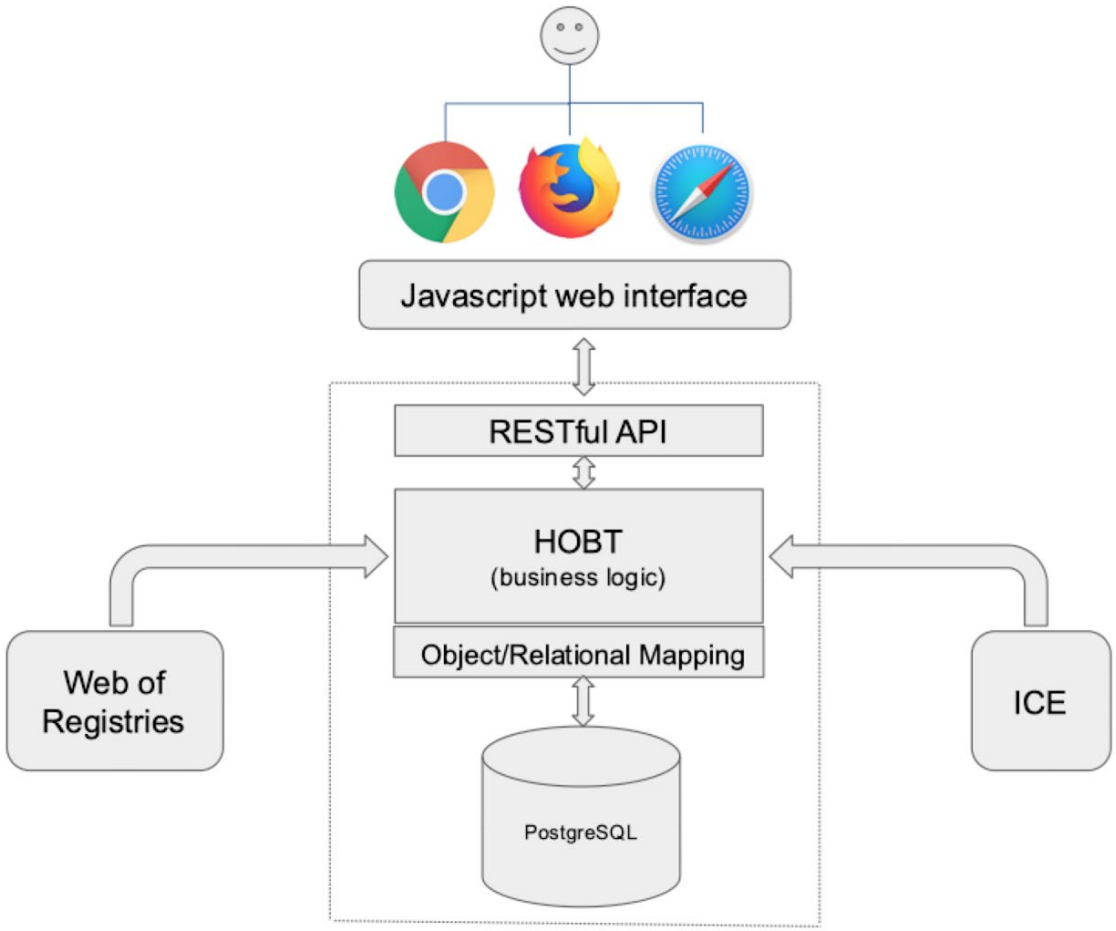


- Improved transformation efficiency by evading restriction systems
- Site-specific recombination system allows rapid and efficient DNA
- **Outcome:** Tools have been transferred to NREL team; similar ongoing efforts with *Clostridium tyrobutyricum*; will accelerate progress across the BETO portfolio

Progress & Outcomes

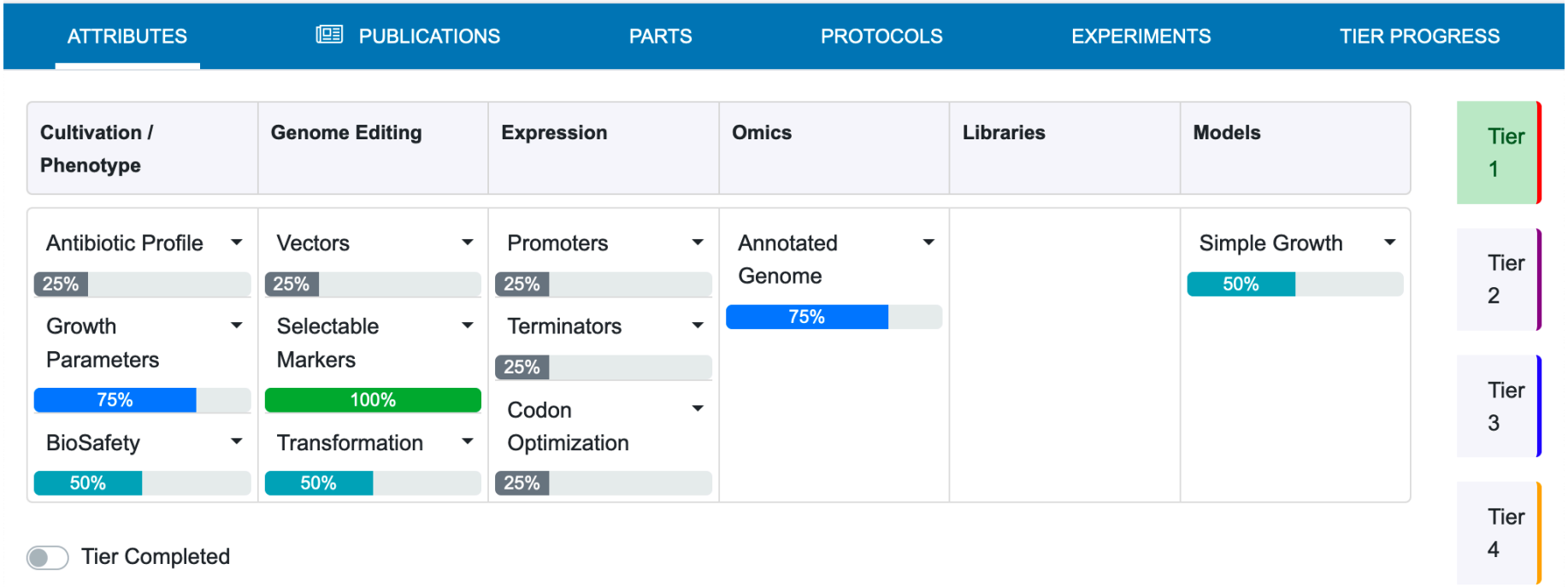


4 – Progress & Outcomes: HObT Phase I



- Integrated with ABF ICE & web of registries instances to retrieve strain host data
- Potential for integration with third-party software applications
- **Outcome:** Web based application for maintaining and managing knowledge and data-set information on ABF selected hosts

4 – Progress & Outcomes: HObT Phase II



- User stories implementation; Emphasis on user interface development
- Integrate more data sources (e.g. EDD, publication data); Additional data input for information that doesn't fit other applications (e.g. Tier system metrics and status)
- **Outcome:** Data warehouse for strain information, protocols, and tie-backs to ABF systems such as DIVA (designs for parts)

Summary

- New microbial hosts and metabolic engineering tools are needed to enable industrial bioengineering
- We have developed a framework to organize and prioritize the development of tools and datasets for new hosts
- We are applying this framework to develop 20 hosts that will be available for use within the ABF and by external stakeholders
- We are also using our expertise to help advance non-model hosts used within other BETO projects to accelerate SOT host engineering
- We are developing an online portal to centralize and organize host development status, data, protocols, and tools, both for internal use and for the community at large

Quad Chart Overview

Timeline

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

	FY20	FY21	FY22	Total Active
DOE Funding	$1.9M	$2.0M	$2.0M	$5.9M

Project Partners

- LBNL (0%), SNL (10%), PNNL (10%), NREL (10%), ANL (9%), LANL (23%), ORNL (38%)

Barriers addressed

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

Project Goal

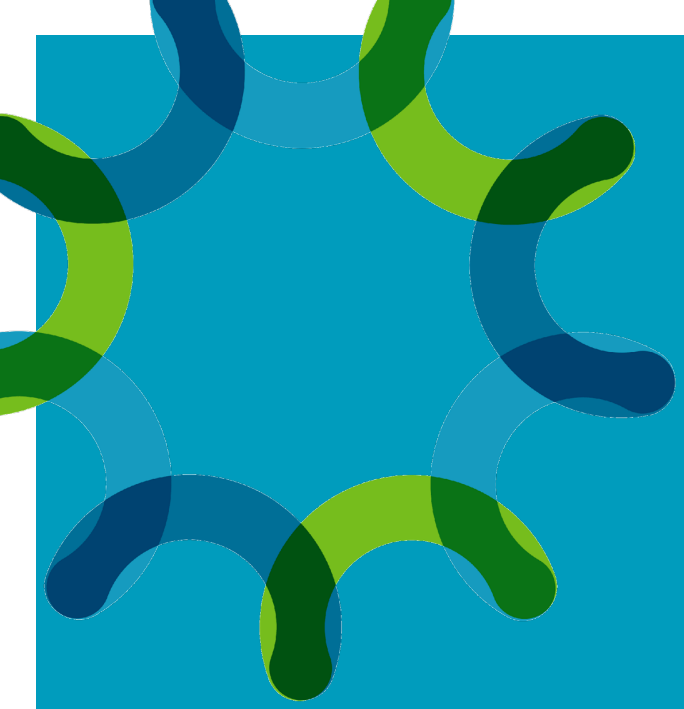
- Onboard new hosts for use in the ABF
- Develop more advanced tools and datasets to enable faster and higher throughput DBTL
- Enhance genetic tools for BETO SOT organisms to accelerate progress across the BETO portfolio

End of Project Milestone

- Bring a total 20 microbial hosts (20 species) to at least Tier 1, & provide corresponding information, resources, and tools via publicly-accessible ABF website.

Funding Mechanism

AOP



Additional Slides

Responses to Previous Reviewers' Comments

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

Reviewers: The new host onboarding program is creating significant value and ideally will expand the pool of industrially relevant hosts (and host-target pairs). A sensible tiered set of criteria to downselect to approprite selections has been developed and a good, diverse set of initial host-targets have been put into play. Consideration to upgrading biosafety/regulatory criteria to the selection process, as well as a biosafety board, should be considered.
HOD Team: Thanks for these comments. We do have a biosafety plan in place, wherein any new organism used needs to be evaluated by our home institutions, which have rigorous regulations. We also utilize LBNL-developed software called BLiSS to identify biosecurity/biosafety concerns of synthetic DNA in advance. Our current goal is to only use BSL-1 organisms, and so the biosafety risk is inherently low. However, the reviewer's point is an important one, and we will work on formalizing a process within the ABF itself, particularly taking into consideration how biosafety might affect use of such organisms by industry.

Responses to Previous Reviewers' Comments

Reviewers: Not a lot of progress has been made with regards to tool development.
HOD Team: As noted above, the experimental component of Host Onboarding is just ramping up in FY19, so progress is on track with expectations. That said, we have now demonstrated genetic transformation of B. coagulans, and work is proceeding with other target hosts.

Reviewers: Potential applications are not clearly defined or prioritized. Milestones are vague.
HOD Team: Potential applications are largely defined by the phenotypes that we use to select organisms. For instance, C. carboxidivorans was chosen for the application of "syngas conversion". Our milestones are SMART, and we apologize for not presenting this clearly enough.

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