DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Engineered reversal of the β-oxidation cycle in clostridia for the synthesis of fuels and chemicals

March 5, 2019
Biochemical Conversion

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Quad Chart Overview

Timeline

- Project start date - 10/1/2018
- Project end date - 9/30/2021
- Project complete 7% (1/14 milestones)

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$0	$0	$0	$1,600,000
Project Cost Share*	$0	$0	$0	$400,000

•**Partners:** Northwestern University (34%), LanzaTech (33%), University of South Florida (25%), Georgia Institute of Technology (8%)

Barriers addressed

- Ct-D. Advanced Bioprocess Development
- Ct-H. Gas Fermentation Development
- Ct-L. Decreasing Development Time for Industrially Relevant Microorganisms
- Ct-N. Multiscale Computational Framework toward Accelerating Technology Development
- Ot-A. Availability of Quality Feedstock

Objective

- Our project objective is to develop clostridia to ferment synthesis gas into a range of advanced bioproducts.

End of Project Goal

- We will manufacture one product from engineering a reversal of the β-oxidation cycle in clostridia at a metric of >0.1g/l/h in >80L scalable pilot reactor.
- We will assess environmental, community and rural economic development impacts

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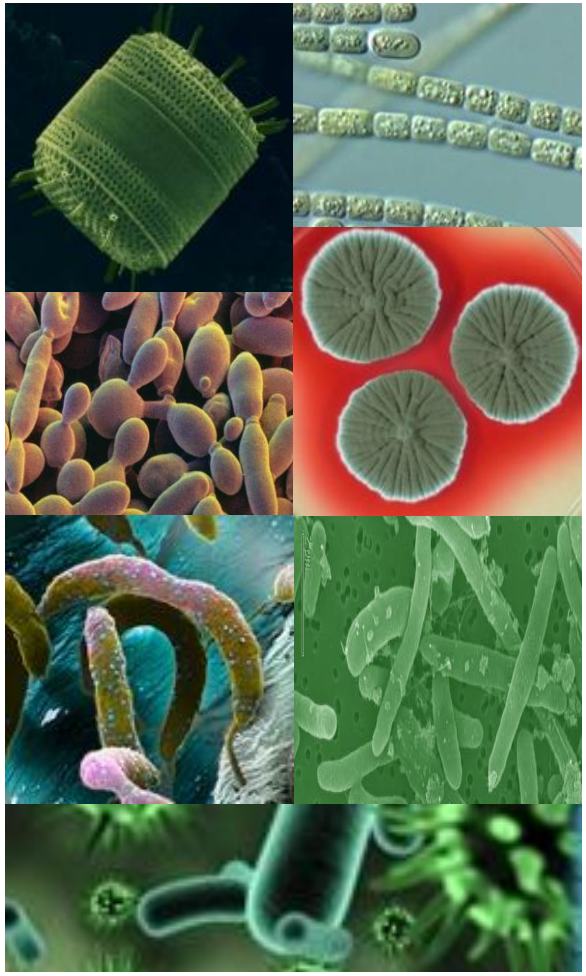
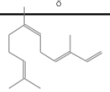
1 - Project Overview

The need for low-cost biofuels and bioproducts from sustainable resources is intensifying



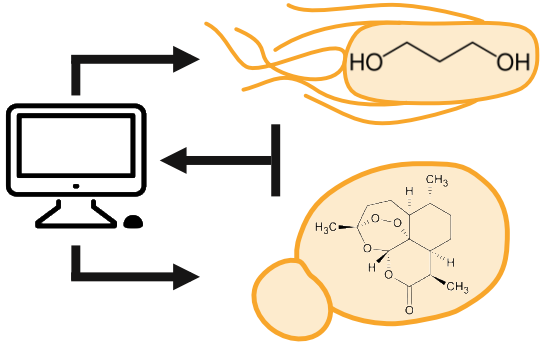
Unfortunately, designing, building, and optimizing biosynthetic pathways in cells remains a complex and formidable challenge

- Development cycles to optimize biosynthetic pathways can be slow, especially for non-model organisms

Molecule	Institutions	Time	Person Years	Cost
1,3-Propanediol 	DuPont, Genencor, Tate & Lyle	15 years (1992-2007)	>550	>$130M
Arteminisin 	UC Berkeley, Amyris, Sanofi	13 years (2000-2013)	>130	>$50M
Farnesene 	Amyris, TOTAL	4 years (2008-2012)	>250	>$30M

Nielsen J & Keasling JD, *Cell* (2016) DOI: 10.1016/j.cell.2016.02.004; Karim AS, Dudley QM & Jewett MC *Industrial Biotechnology* (2017) DOI: 10.1002/9783527807796.ch4

- Platform organisms, accessible feedstocks, target molecules, and stable environments in which to work are limited
- Integrated computational frameworks for biodesign need improvement



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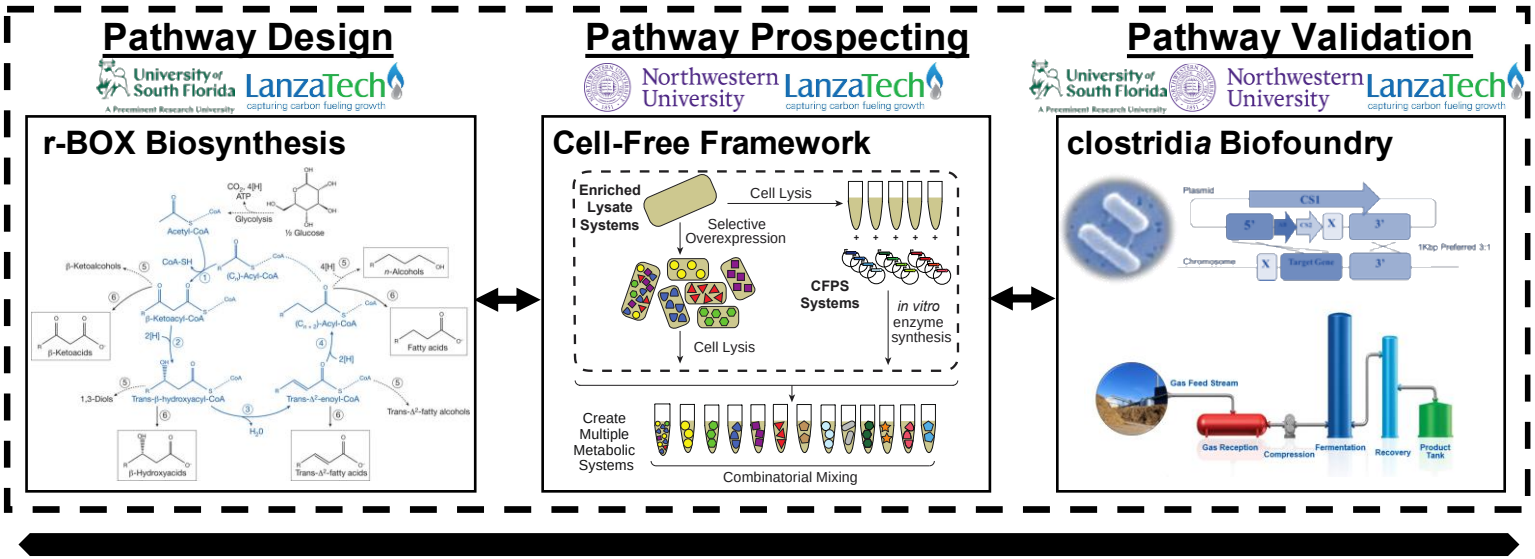
Our project objective is to develop clostridia to ferment synthesis gas produced from cellulosic biomass by established gasification technologies, into a range of advanced bioproducts



We will target products used as **drop-in fuels, fuel additives, and chemical building blocks with a $14Bn US market**.

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2 – Approach (Management)

Northwestern:
Michael Jewett
Bioengineering

LanzaTech:
Michael Koepke
Industrial Biotech

Univ. South Florida:
Ramon Gonzalez
Chemical Engineering

LanzaTech:
Robert Conrado
Technoeconomic Analysis

GaTech:
Valerie Thomas
Technology Assessment

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Several activities ensure efficient coordination within the team and reporting to DOE/USDA

- Northwestern and LanzaTech have c**ollaborated on numerous projects since 2015** and have developed successful mechanisms for technical coordination, data sharing, and integration
- Bi-weekly meetings to review the team's progress and discuss any matters requiring action
- Project structure enables multiple, parallel paths to achieve 4 tasks and the milestones
  - **Task 1**. Develop and apply informatics and computer-aided design tools to choose molecules, enzymes, and pathways in clostridia (**USF**, LT)
  - **Task 2**. Establish a cell-free framework for rapid pathway prototyping and analysis (**Northwestern**, LT)
  - **Task 3**. Develop optimized production strains of clostridia (**LanzaTech**, NU, USF, GT)
  - **Task 4**. Rural economic development and sustainability analysis (**GaTech**, LT)
- Interface with other DOE projects to accelerate goals
  - **Complement cBioFab project**, **utilize DOE user facility JGI** for gene synthesis

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2 – Approach (Technical)

To achieve our vision, we will:

- ***Aim 1.*** *Develop and apply informatics and* ***computer-aided design tools*** *to choose molecules, enzymes, and pathways for reverse β-oxidation cycle (r-BOX) in clostridia.*
- ***Aim 2.*** *Establish a* ***cell-free framework*** *for rapid pathway prototyping and analysis*
- ***Aim 3.*** *Develop optimized production strains of* ***gas-fermenting clostridia****.*
- ***Aim 4. Technoeconomic and rural economic development*** *and sustainability analysis.*

Embedded in these aims, are several key innovations that will allow us to combine *in vitro* (cell-free) and *in vivo* work to interweave and advance state-of-the-art pathway design, prospecting, validation, and production in an integrated framework

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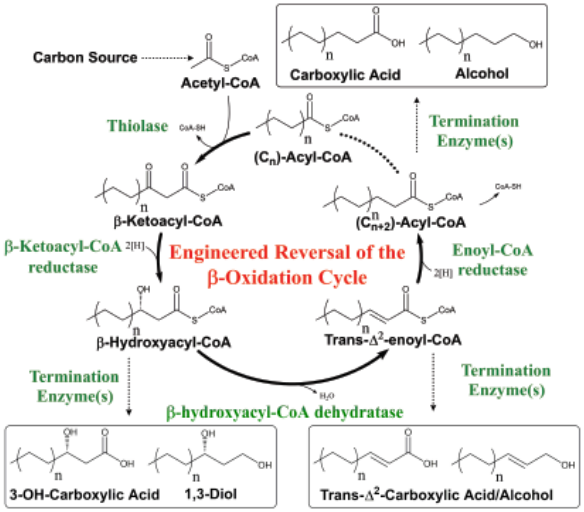
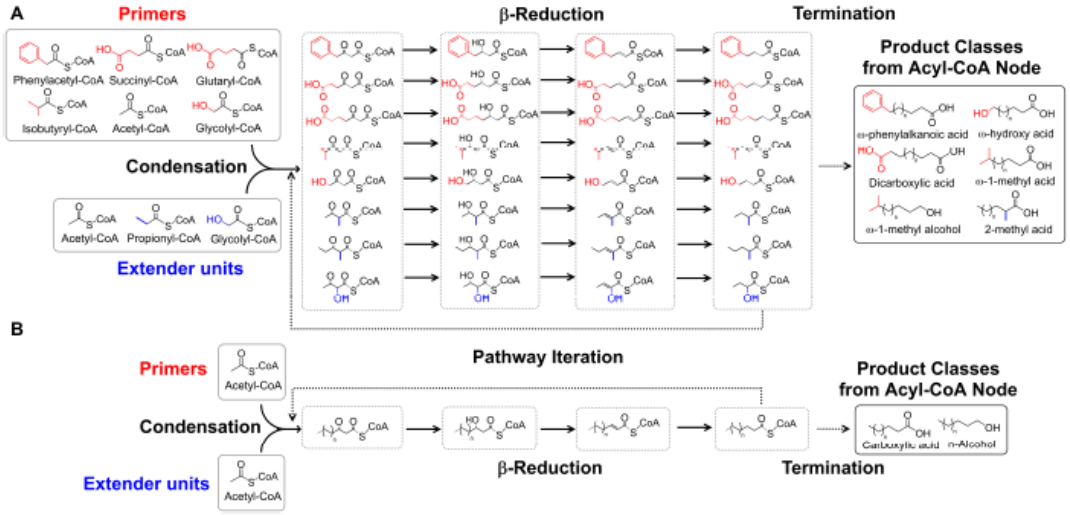
Reversal of the β-oxidation cycle (rBOX) offers unique access to thousands of molecules

- Most bioengineering approaches to date rely on linear pathways specifically designed for a single molecule.
- The cyclic and iterative r-BOX pathway with **highly modular architecture and combinatorial nature** offer the unique advantage of providing **access to thousands of molecules** with different chemistries and chain lengths.





Dellomonaco C, Clomburg JM, Gonzalez R, *Nature*(2011) DOI: 10.1038/nature10333

Cheong S, Clomburg JM, Gonzalez R, *Nature Biotech* (2016) DOI: 10.1038/nbt.3505

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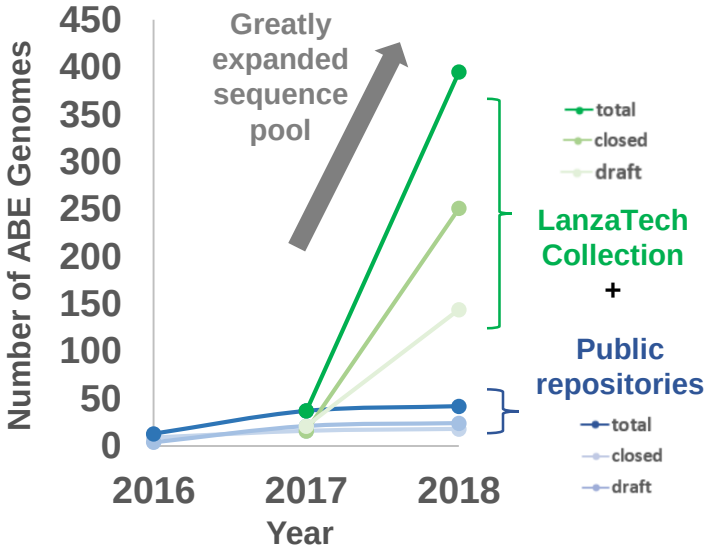
Largest collection of industrial *Clostridium* strains provides unique access to highly-evolved gene variants

- LanzaTech owns the largest collection of industrially-deployed acetone-butanol-ethanol (ABE) clostridia strains
- The collection dates back to 1944 and contains hundreds of highly evolved strains, **spanning several decades of development at several large-scale commercial plants**
- In collaboration with JGI, the entire collection have been sequenced

NCP commercial ABE plant
1935-1982

Jones DT and Woods DR, Microbiol Rev (1986).



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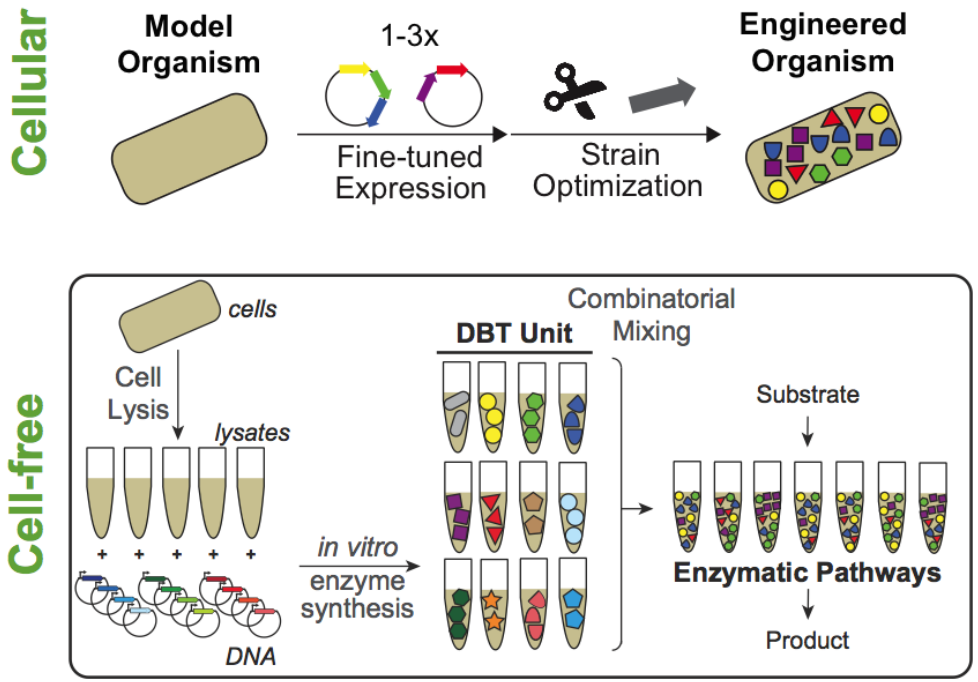
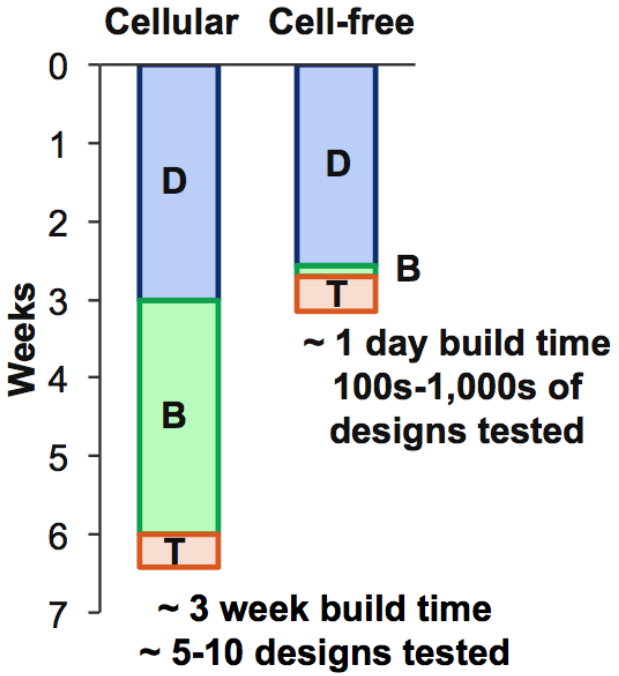
Cell-free Framework allows for rapid pathway prototyping

- Cell-free systems enable **acceleration of new enzyme and pathway assessments**, bypassing transformation idiosyncrasies and low-throughput workflows impeding progress on many non-model microorganisms





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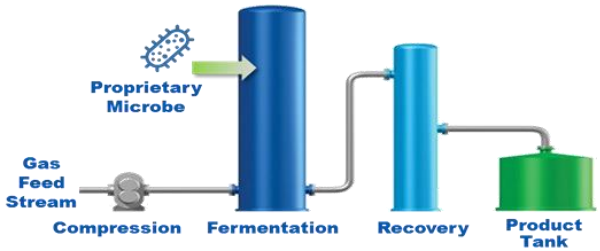
Clostridia gas fermentation allows high yield conversion of lignocellulosic feedstocks

- A hybrid thermochemical (gasification) - biological (gas fermentation) pathway utilizes all biomass components (lignin and cellulose), maximizing yields and overcoming barriers such as biomass recalcitrance.
- Integrated gasification-fermentation has been demonstrated in extended continuous operations using multiple types of lignocellulosic material.
- Syngas fermentation uses the same fermentation process implemented in LanzaTech's first commercial scale gas fermentation facility (started May 3 2018).



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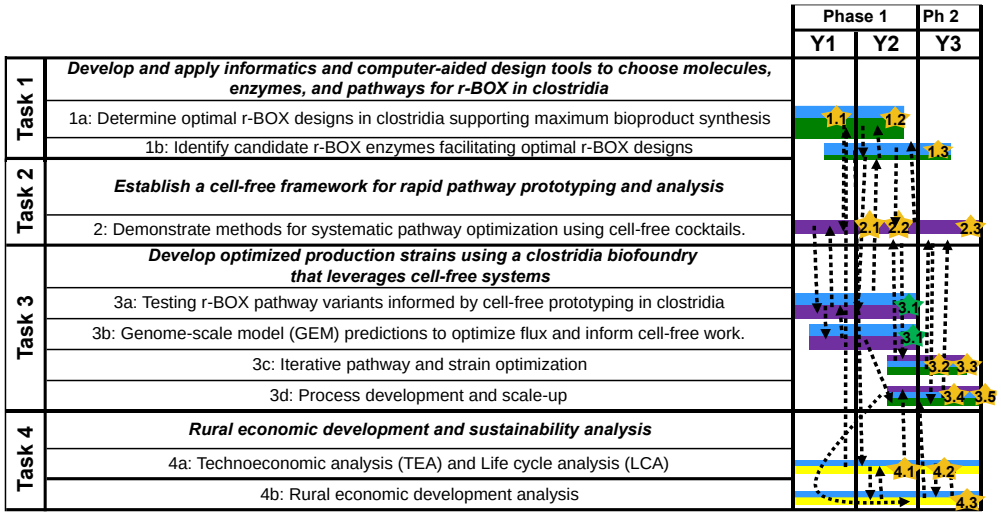
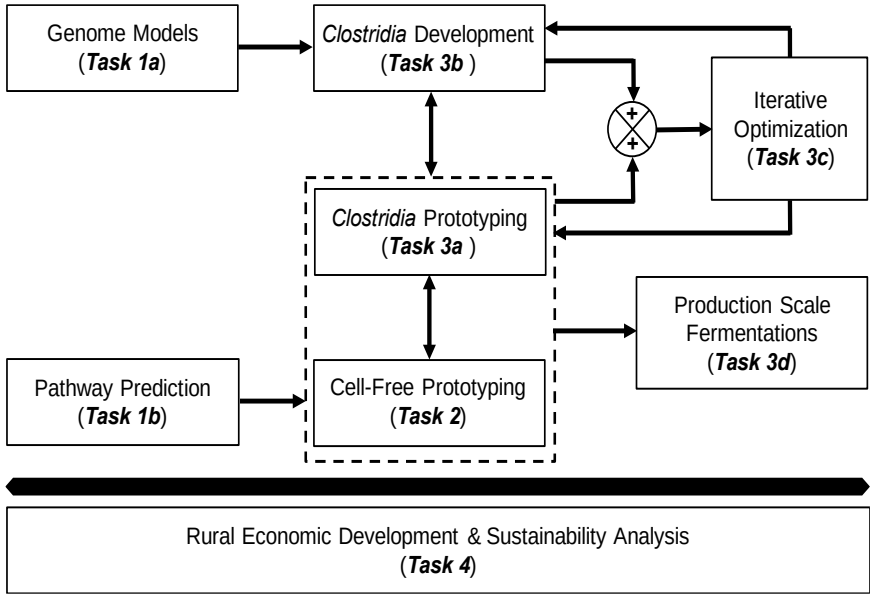
How are we going to achieve our goals?



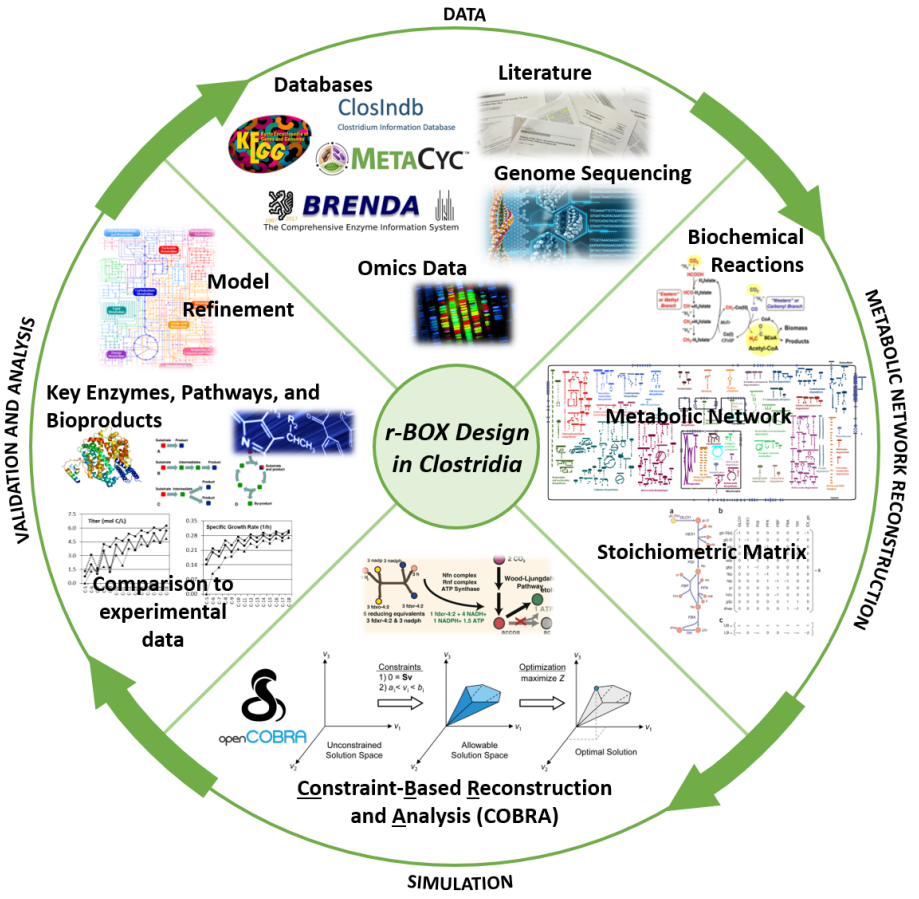


Task 1: Use pathway design to provide a focused set of r-BOX input molecules

- **<u>Milestone 1.1</u>**. Identify and compare r-BOX enzyme variants by data-mining complete *Clostridium* collection and public databases: **>100 additional unique r-BOX and Ptb-Buk putative gene variants over the public domain identified** (Y1/Q4)

- **<u>Milestone 1.2</u>**. Quantify theoretical product yields and **generate optimal strain designs** with ***> 100,000 simulations*** carried out per design (Y2/Q2)

- **<u>Milestone 1.3</u>**. **Optimize computational framework** for generating novel pathways based on feedback from other Tasks and refine pathway design (Y3/Q2)

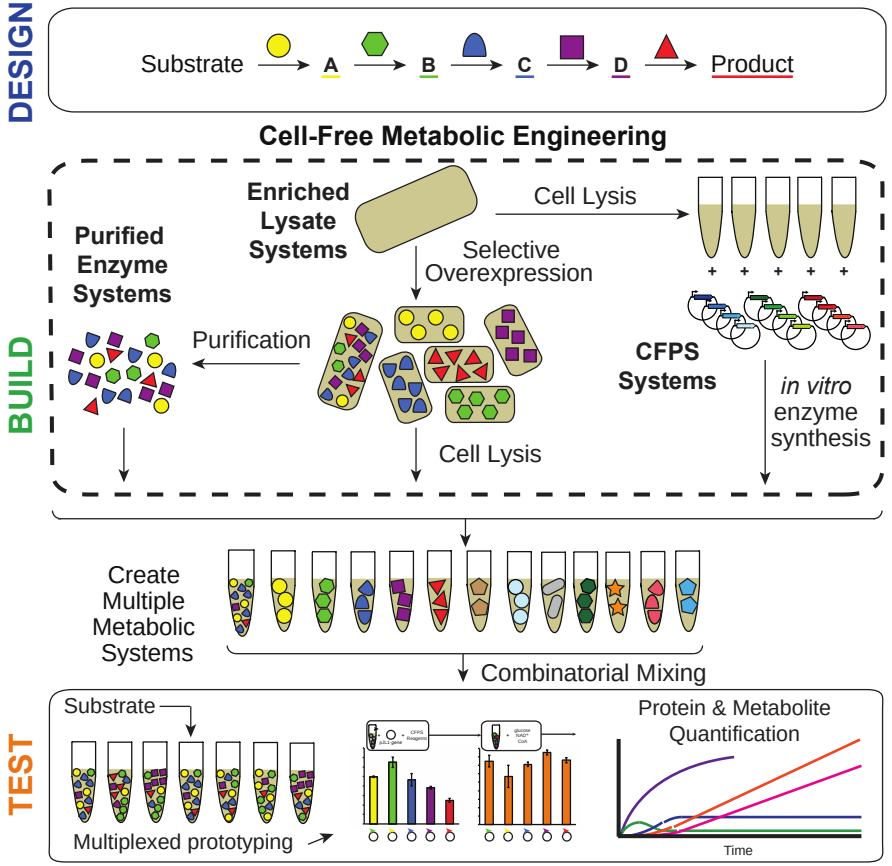


Task 2: Develop methods for accelerating systematic pathway optimization using cell-free cocktails

- **<u>Milestone 2.1</u>**. Develop, implement, and demonstrate methods for the **cell-free mix-and-match approach to optimize biosynthetic pathways** that are ***2x faster than the state of the art*** *in vivo* approach (Y2/Q2).

- **<u>Milestone 2.2</u>.** Demonstrate **expression of pathway enzymes** for at least one r-BOX pathway at levels of ***greater than 50 μg/mL*** using the cell-free framework (Y2/Q3).

- **<u>Milestone 2.3</u>.** Study and **optimize pathways using our cell-free framework**, and refine and optimize pathways with ***at least 2-fold improvement*** (Y3/Q2).



Task 3: Develop optimized strains for production of r-BOX products from biomass syngas

- **<u>Milestone 3.1</u>. (go/no-go decision):** Construct and **evaluate 50 unique pathway designs** for target molecules *in vivo* and *in vitro*. Our ***metric is 100mg/L/d*** of one target product. (Y2/Q4)
- **<u>Milestone 3.2</u>.** Proof of concept for **additional rBOX target products** on syngas. (Y3/Q2)
- **<u>Milestone 3.3</u>.** Construct and evaluate an **additional 150 unique pathway designs** for target molecules *in vivo* and *in vitro*. (Y3/Q3)
- **<u>Milestone 3.4</u>.** Comparison of best performing engineered strain for on **synthetic syngas against real biomass syngas** in 1.5L lab scale reactor and demonstration of a target ***metric of >0.1g/l/h***. (Y3/Q3)
- **<u>Milestone 3.5</u>.** One selected rBOX product at a target ***metric of >0.1g/l/h*** in **>80L scalable pilot reactor**. (Y3/Q4)

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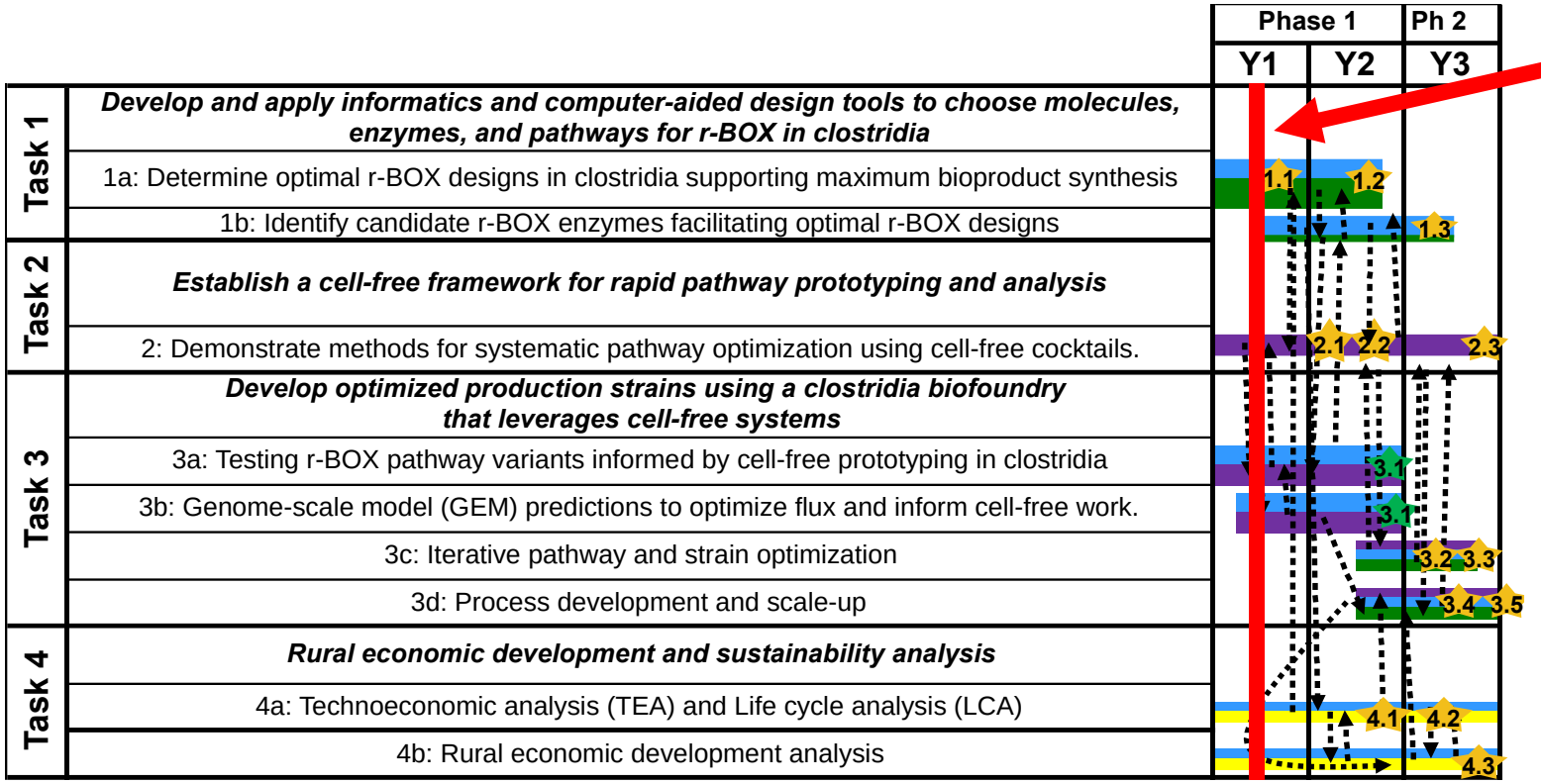
Task 4: Assess environmental, community, and rural economic development impacts of r-BOX synthesis to facilitate design

- **Milestone 4.1**. Complete **2 workshops to inform lifecycle impact analysis** using TRACI model. All stakeholders will be invited to both workshops; aims are to gather input from multiple parties on potential economic, community, and environmental impacts. (Y2/Q4)

- **Milestone 4.1**. Completed LCA for two rBOX molecules. (Y3/Q2)

- **Milestone 4.1**. Completed assessment of infrastructure and supply chains for biomass feedstock supply of two rBOX molecules in the US southeast. (Y3/Q4)

3 – Technical Accomplishments/ Progress/Results



Legend				
1 -Milestones	NU	Northwestern University: (Jewett)	GT	Georgia Tech (Thomas)
-Interactions between tasks	LT	LanzaTech (Köpke, Conrado)		
	RU	Rice University (Gonzalez)		-Go/No-go decision

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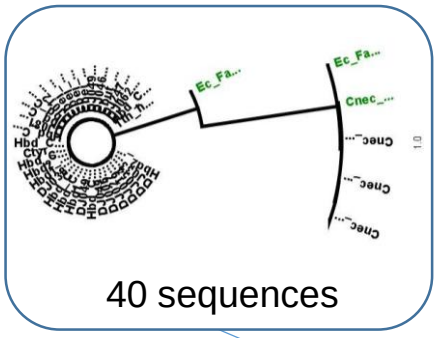
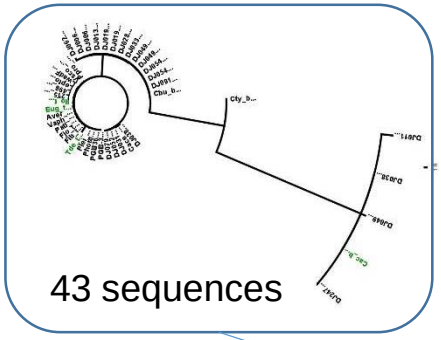
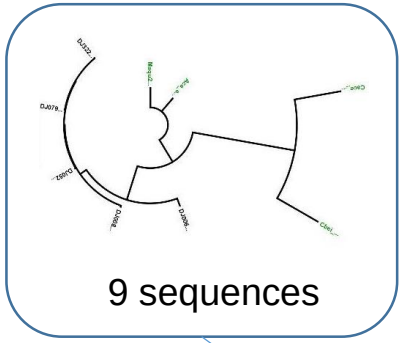
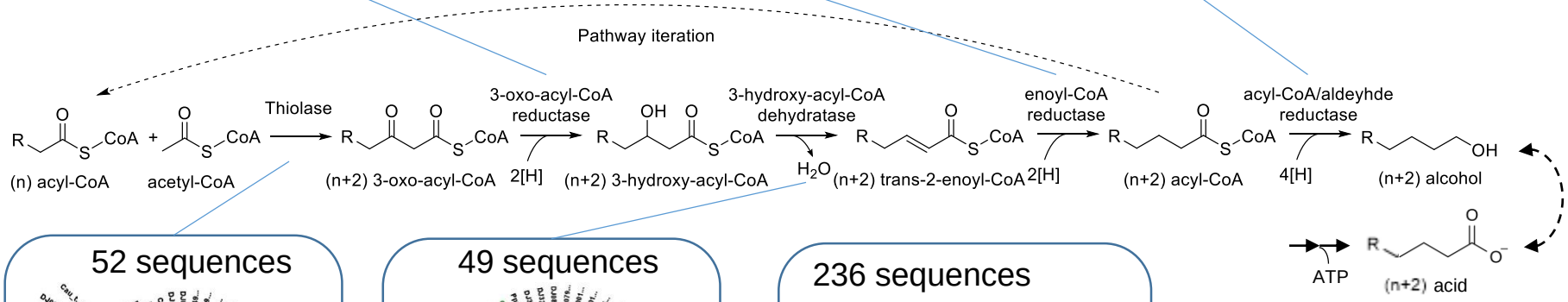
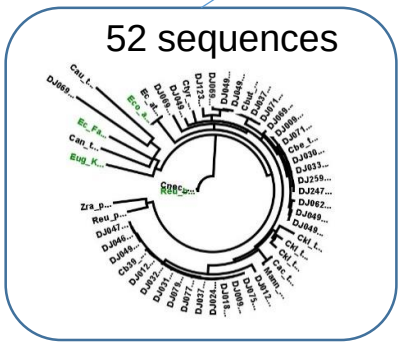
Task 1: Based on initial enzyme set, mined enzyme candidates for r-BOX in *Clostridium* collection

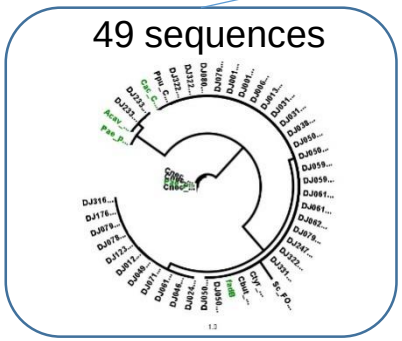


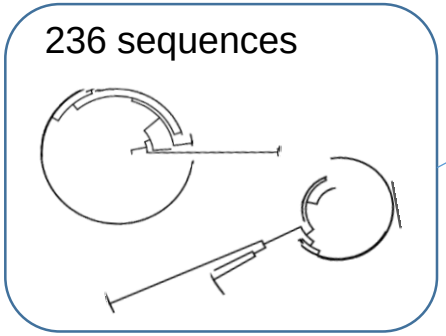












More than 200 unique rBOX gene variants identified that have been codon adapted and are currently being synthesized through the JGI DNA synthesis program

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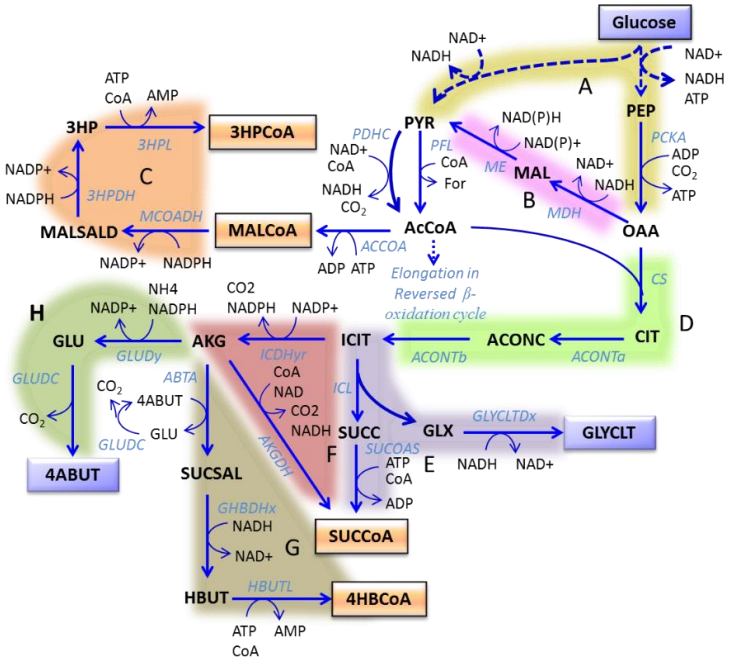
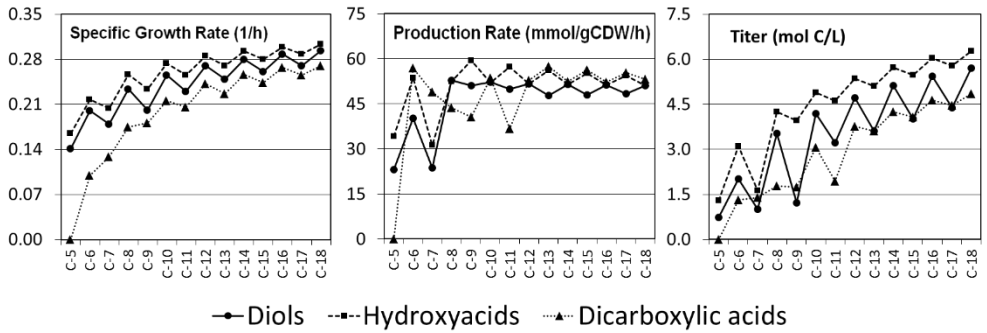
Task 1: Genome-scale modeling (GEM) and metabolic flux balance analysis to define optimal r-BOX inputs





- *E. coli* GEM has previously been exploited to determine optimal routes for omega-functionalized product synthesis and input molecules (left) to maximize target product synthesis from glucose (right)

Reactions from *E. coli* model have been integrated to *Clostridium* GEM to enable modeling of target product synthesis in desired genetic background

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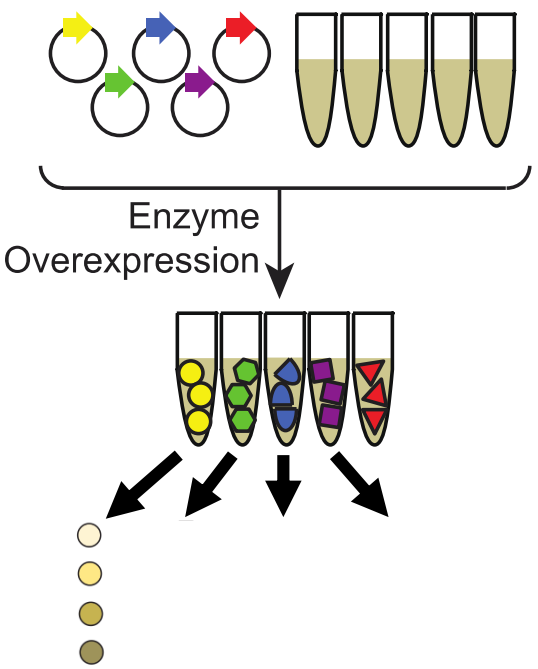
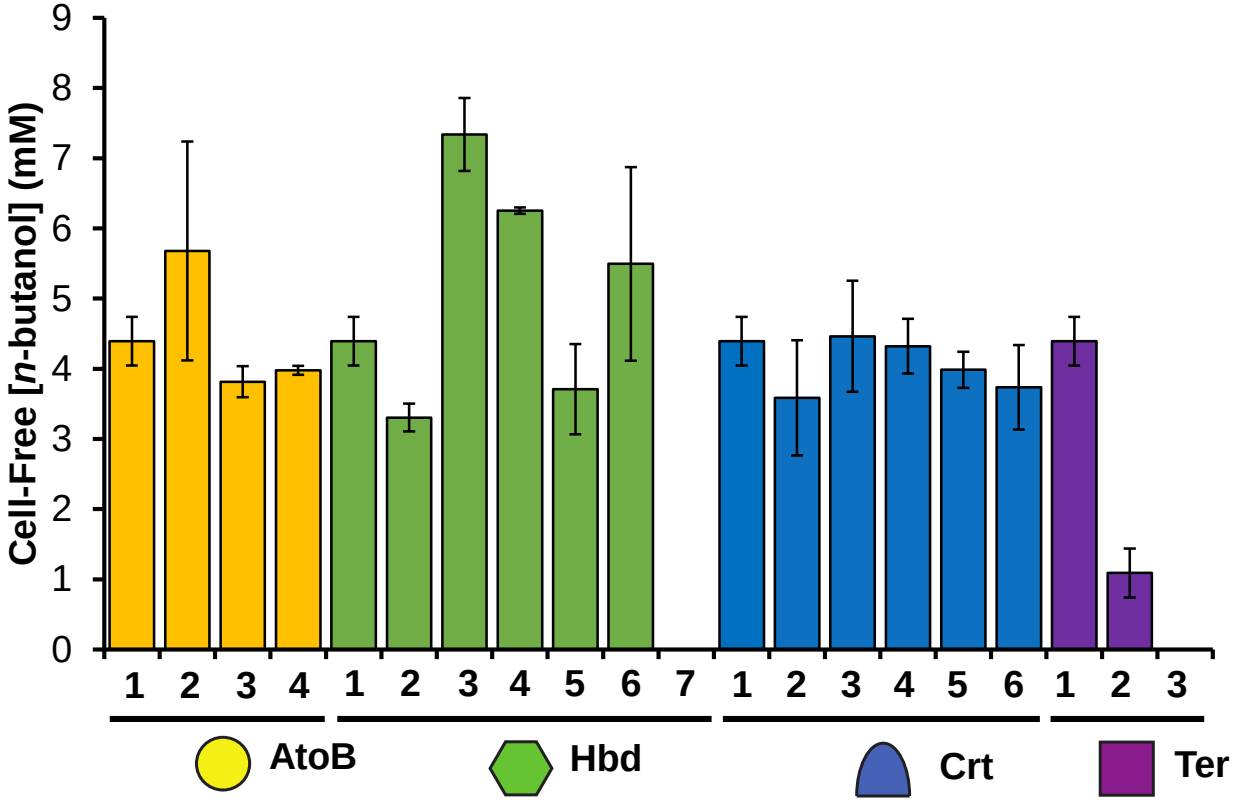
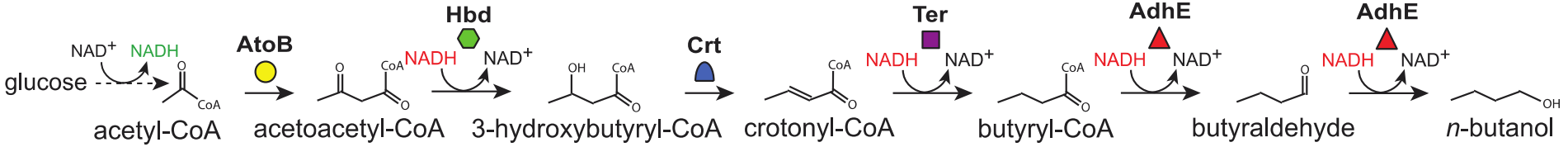
Task 2: Developed cell-free platform to test homology activity and beginning to apply it to r-BOX

(example for butanol pathway below)







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Task 4: We plan two workshops to guide the project's strategic direction and product emphasis

- *Workshop 1.* Atlanta, October 2019. Invitees will include Georgia Rural Economic Development Authority representatives, industry representatives, environmental NGO representatives, experts on community impacts, ethanol manufacturers

- *Workshop 2.* Soperton area, April 2019. Invitees will include: Treutlen County Development Authority, Heart of Georgia Altamaha Regional Commission, Site Tour

4 – Relevance

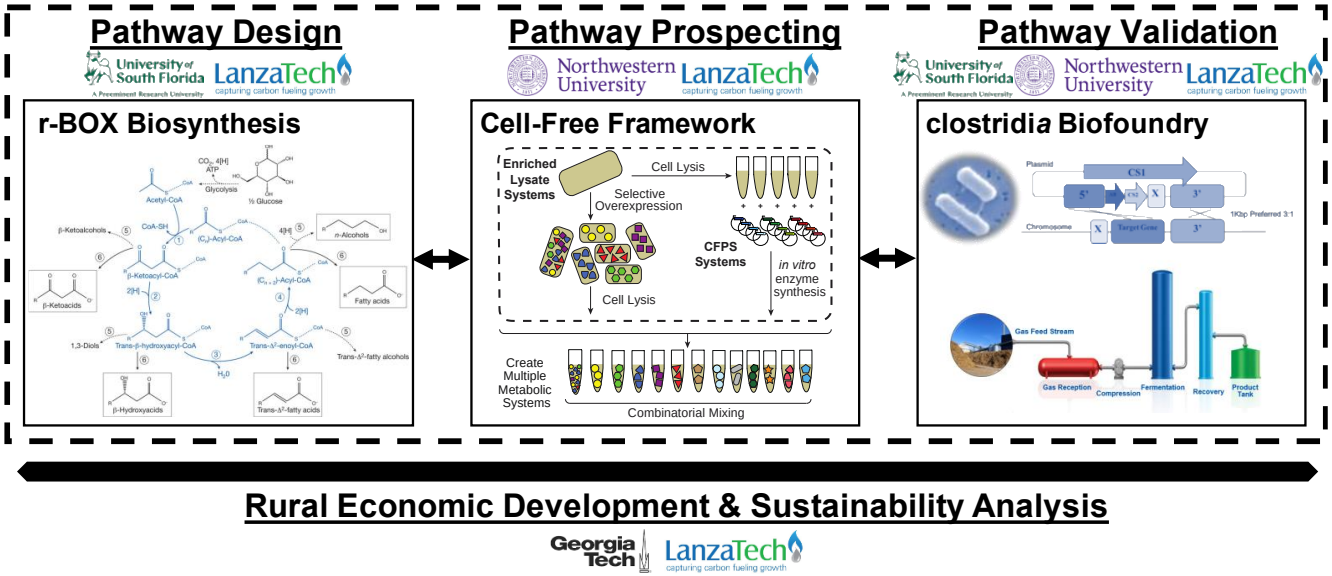
- Enable high-level, sustainable synthesis of next-generation biofuels and bioproducts by **developing clostridia to ferment synthesis gas**
  - Advance syngas fermentation, a cost-effective technology for the use of cellulosic biomass that is broadly applicable in the production of biofuels and biobased products (Ct-H)
  - Expand the diversity of products that can be produced and co-produced via syngas fermentation (Ct-H)
  - Gain new knowledge of metabolism in obligate anaerobes and traits related to “industrial fitness” of clostridia (Ct-H, Ct-D)
- Create a cell-free framework to **decrease development time for industrially relevant microorganisms**
  - Advance bioprocess development by reducing the time to new biosynthetic pathways (Ct-D, Ct-L, Ct-N)
  - Provide a key case study for the bioenergy industry by establishing r-BOX for production of advanced biofuels and value-added chemicals
- Expand the scope of biomanufacturing practice, **enabling regional and global economic growth**
  - Develop rural economic and sustainability analysis frameworks to guide product selection
  - Accelerate commercialization of new gas fermentation products from lignocellulosic biomass, with specific application to forestry residues in the Southeast (At-A)
  - Demonstrate pilot scale synthesis of one r-BOX product (Ct-H, Ct-D)

5 – Future Work

- **Key upcoming milestones (18 months):**
  - Use genome-scale metabolic models for r-BOX pathway design
  - Establish a cell-free framework for rapid pathway prototyping of r-BOX pathways to accelerate design in clostridia
  - Complete workshops to inform life cycle analysis
- **Go/No-Go Point (9/30/2020):**
  - Construct and evaluate 50 unique pathway designs for target molecules *in vivo* and *in vitro* to enable 100mg/L/d of one r-BOX product in clostridia
    - *Already created compatible vector system to facilitate evaluation*
- **Project completion steps**
  - Demonstrate synthesis of one r-BOX product in clostridia at a metric of >0.1g/l/h in >80L scalable pilot reactor.
  - Complete rural economic development & sustainability analyses

Summary

- We will develop a **new technology platform for engineering r-BOX in clostridia for fuel and chemical synthesis from biomass syngas**
- Our approach to integrate pathway design, pathway prospecting, pathway validation, and economic analysis will **enable a new paradigm of biological design**.



- Our current project progress is **ahead of schedule.**
- The impact will be to **reduce the time required to develop new biosynthetic products** and **expand the scope of biomanufacturing practice through gas fermentation**

Thank you!

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

BIOENERGY TECHNOLOGIES OFFICE

Technology Manager: Jay Fitzgerald
Project Monitor: Clayton Rohman
Grants Management Specialist: Nicholas Oscarsson

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