DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

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

March 10, 2021
Biochemical Conversion

Michael C. Jewett, Northwestern University (PI)

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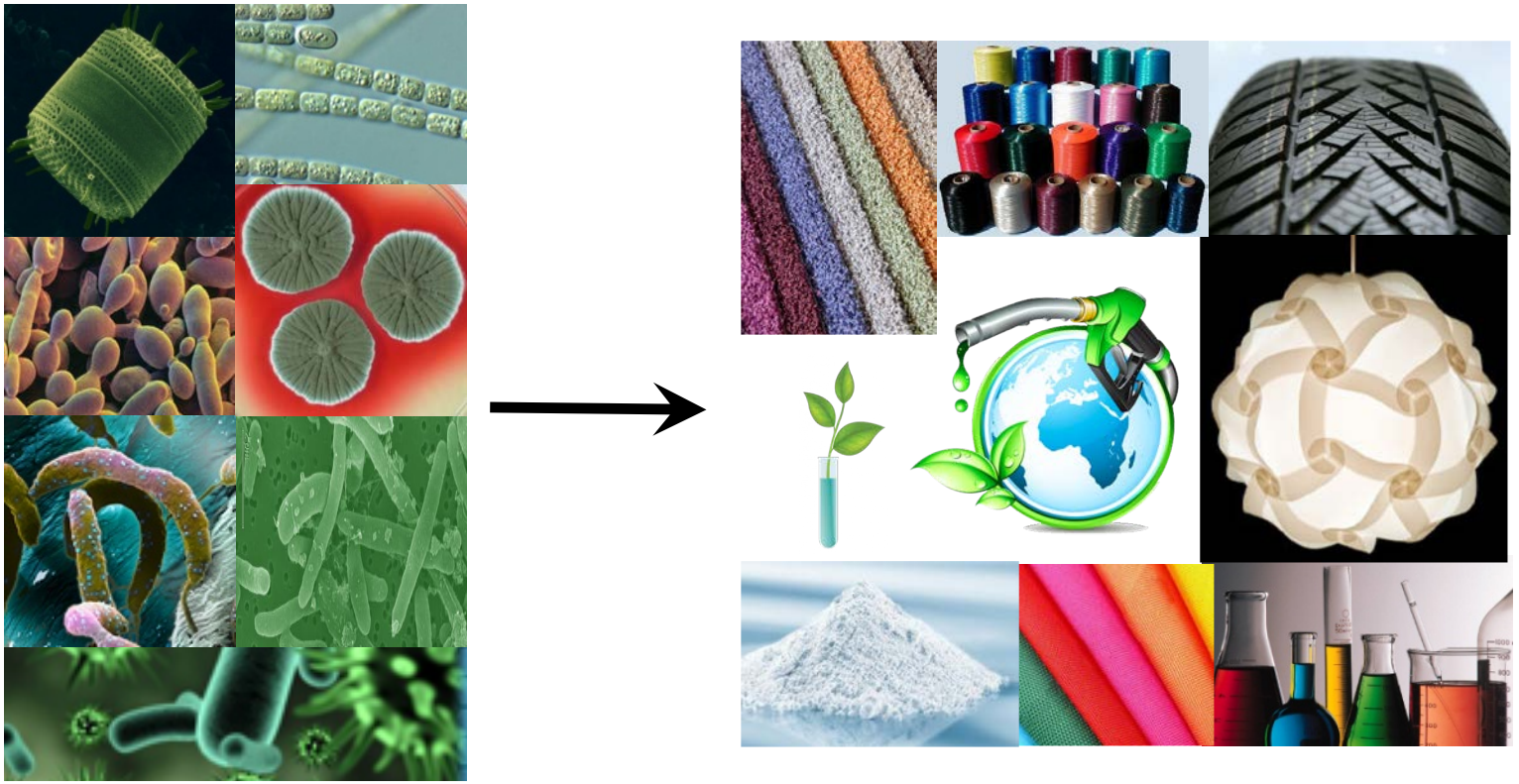
Project Overview

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



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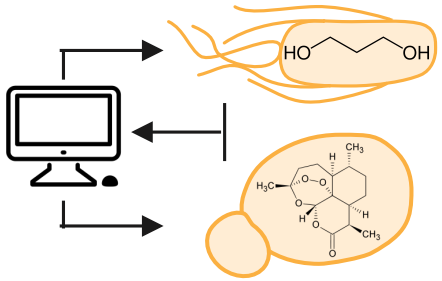
Unfortunately, designing, building, and optimizing biosynthetic pathways in cells remains a complex and formidable challenge

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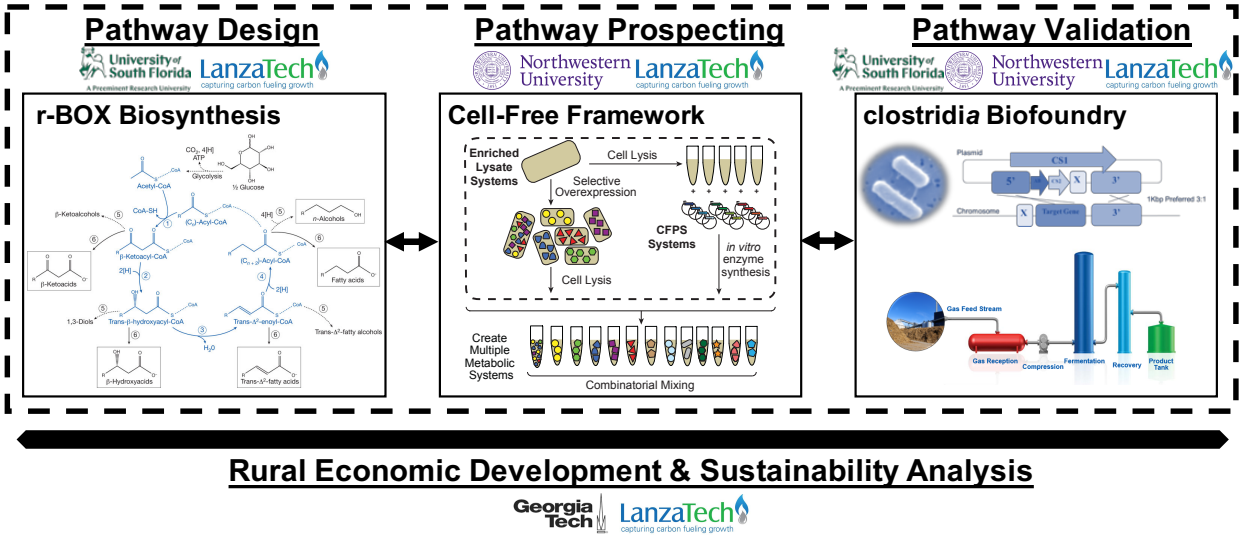
Our project objective is to develop *clostridia* to ferment synthesis gas produced from cellulosic biomass by established gasification technologies, into a range of advanced bioproducts



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

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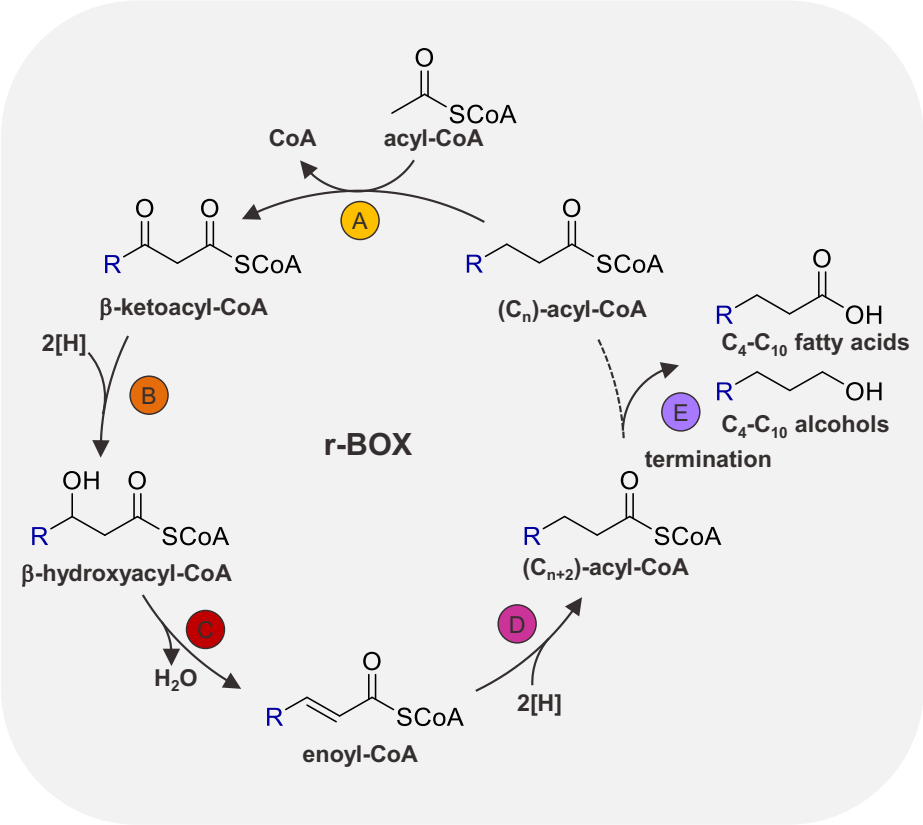
We are engineering reverse β-oxidation (r-BOX) in clostridia for the synthesis of fuels and chemicals

- Cyclic pathway allows for the synthesis of a diverse set of compounds
- Energy efficient C-C bond formation
- Thermodynamic bottleneck and iterative nature requires fine-tuned enzyme levels
- Requires specific termination enzymes



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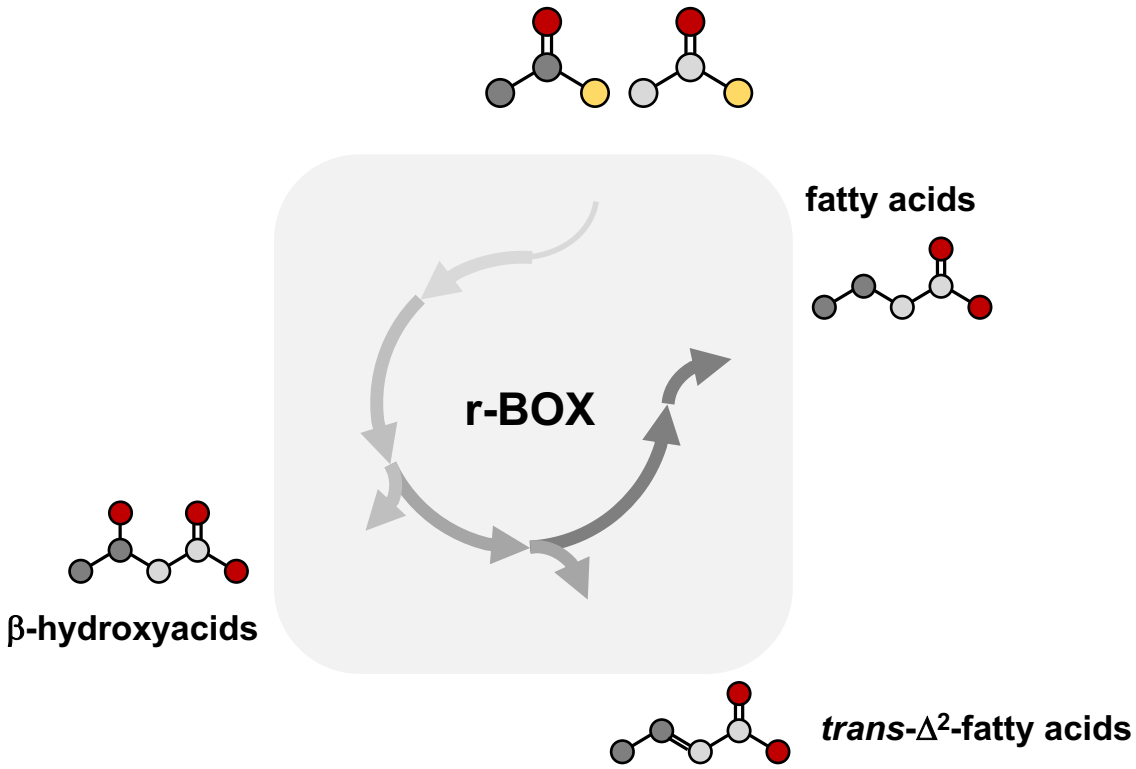
r-BOX gives access to a diverse set of products



- Most bioengineering approaches to date rely on linear pathways specifically designed for a single molecule.
- This is not true for r-BOX.

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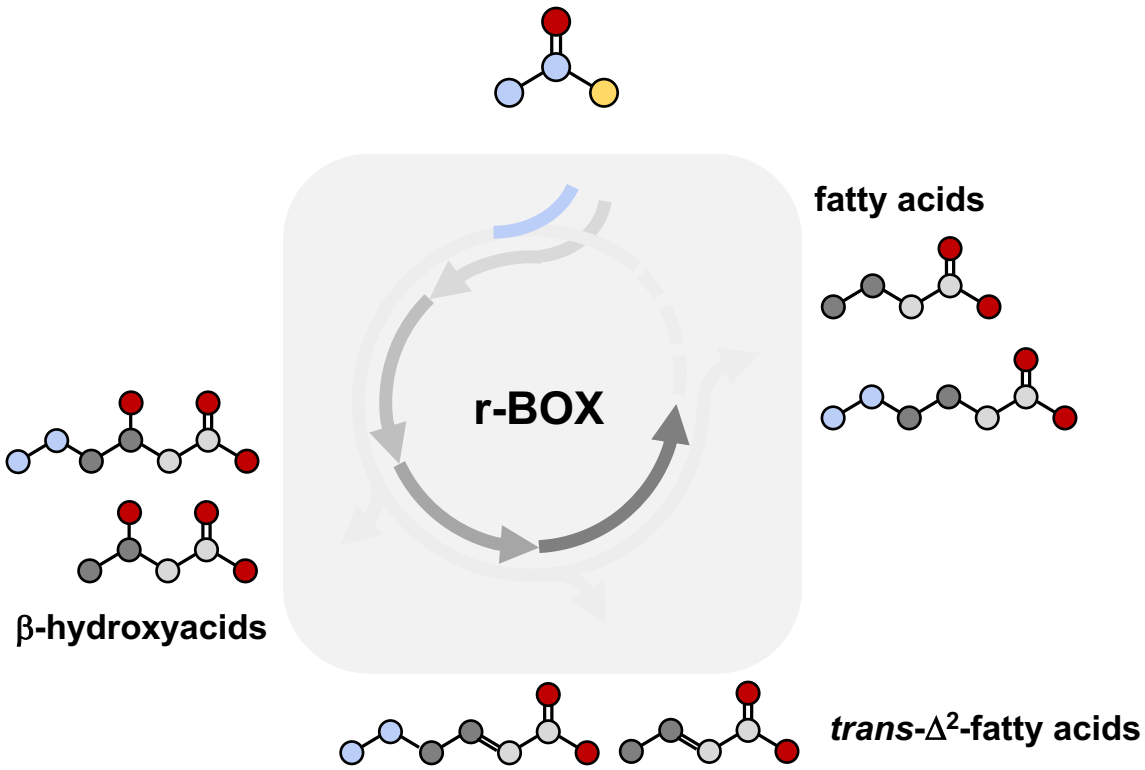
r-BOX gives access to a diverse set of products



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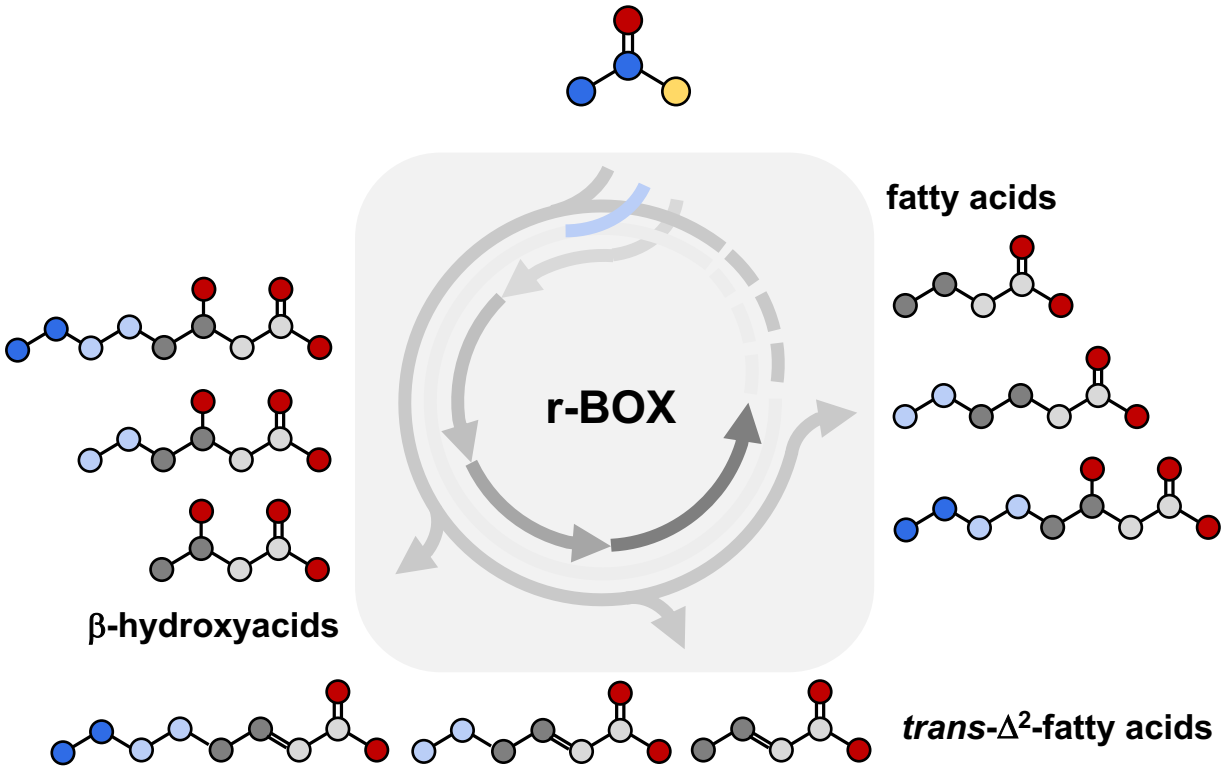

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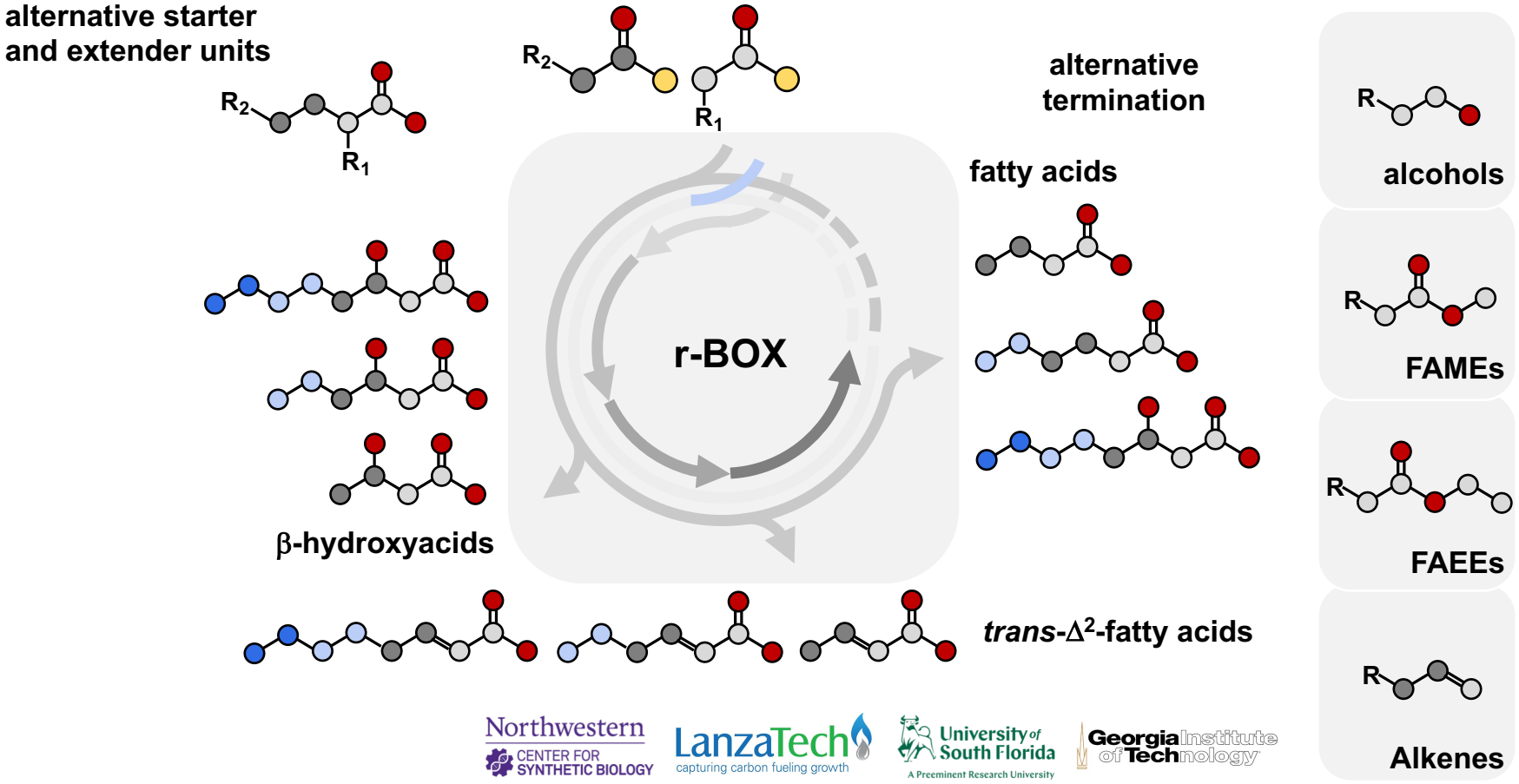
Highly modular architecture and combinatorial nature of r-BOX provides access to thousands of molecules with different chemistries and chain lengths



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Quad Chart Overview

Timeline

- Project start date - 10/1/2018
- Project end date - 12/31/2021
- Project complete 60% (8/14 milestones)

	FY 20 Costed	Total Award
DOE Funding	$1,062,765	$1,600,000
Project Cost Share*	$303,507	$400,000

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

Project Goal

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

End of Project Milestone

- 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

Funding Mechanism

DE-FOA-0001637, Topic B: Biofuels and Biobased Products Development, 2018

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1 – 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

- Northwestern and LanzaTech have **collaborated 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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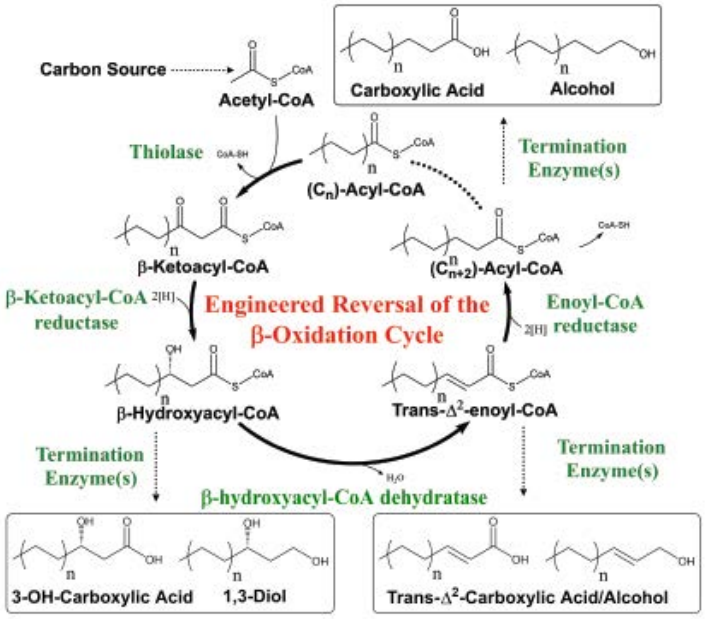
Risks and mitigation

- *Risk: r-BOX has never been demonstrated clostridia*
  - Mitigation: rBOX has been shown in *E. coli* and the enzymes are ubiquitous. Having the DJ collection resource reduces risk to find efficient enzymes.
- *Risk: E. coli cell-free pathways may not represent in vivo clostridia activity*
  - Mitigation: Preliminary data suggest a correlation between data generated in the cell-free framework and *in vivo* clostridia data (minimally for poor performing enzymes). Use clostridia extracts.
- Risk: Clostridia cells with rBOX pathways do not grow well in continuous culture
  - Mitigation: An automated strain selection process will be employed to select for strains that display improved growth performance on gas.



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

2 – Approach

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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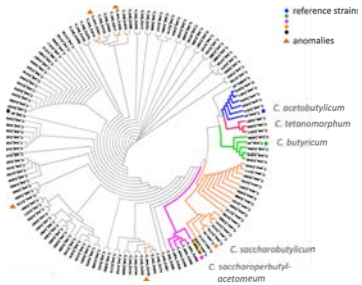
Correlation between three platforms allows for detailed understanding of the pathway

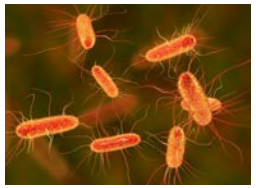
Genome Collection
of Industrial strains



<u>>200 new gene variants</u>
mined and synthesized from the the largest collection of industrially-deployed acetone-butanol-ethanol (ABE) clostridia strains

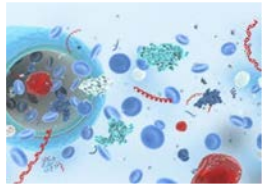
3 Platforms

E. coli
Established rBOX



- **Modelling predictions**
- **Generate & validate <u>chassis</u> optimizations**

Cell-Free
Rapid Prototyping



- **Rapid prototyping of rBOX <u>pathways</u>**

Clostridium
Industrial Production

- **<u>Production system</u>, combining optimal pathways and chassis designs**

Establish correlation between platforms

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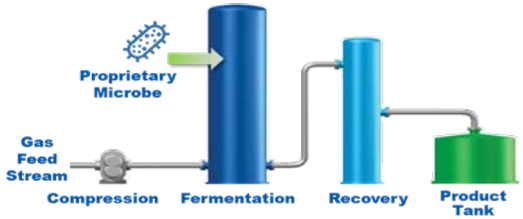
Clostridia gas fermentation allows high yield conversion of lignocellulosic feedstocks

- 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.



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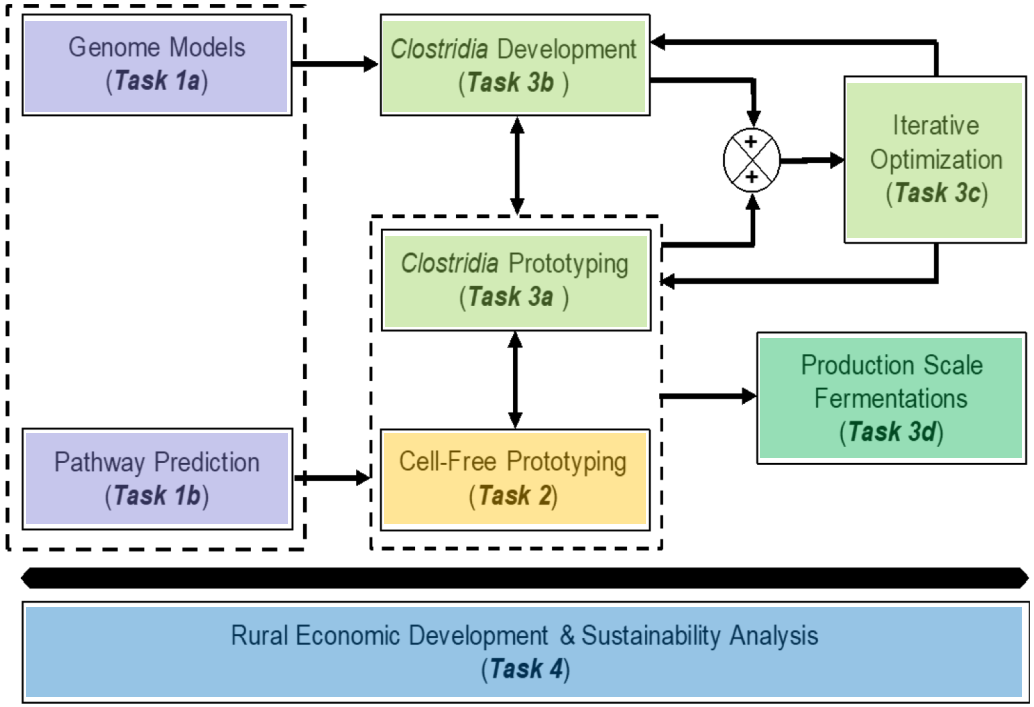
How are we going to achieve our goals?

Highly integrated approach to ensure success



Go/No-go decision points

- Demonstrate correlation between cell-free and in vivo systems
- Achieve > 100mg/L/day rBOX product in clostridia

→ *Both completed*

→ *Initial focus on hexanol*

3 – Impact

- 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)

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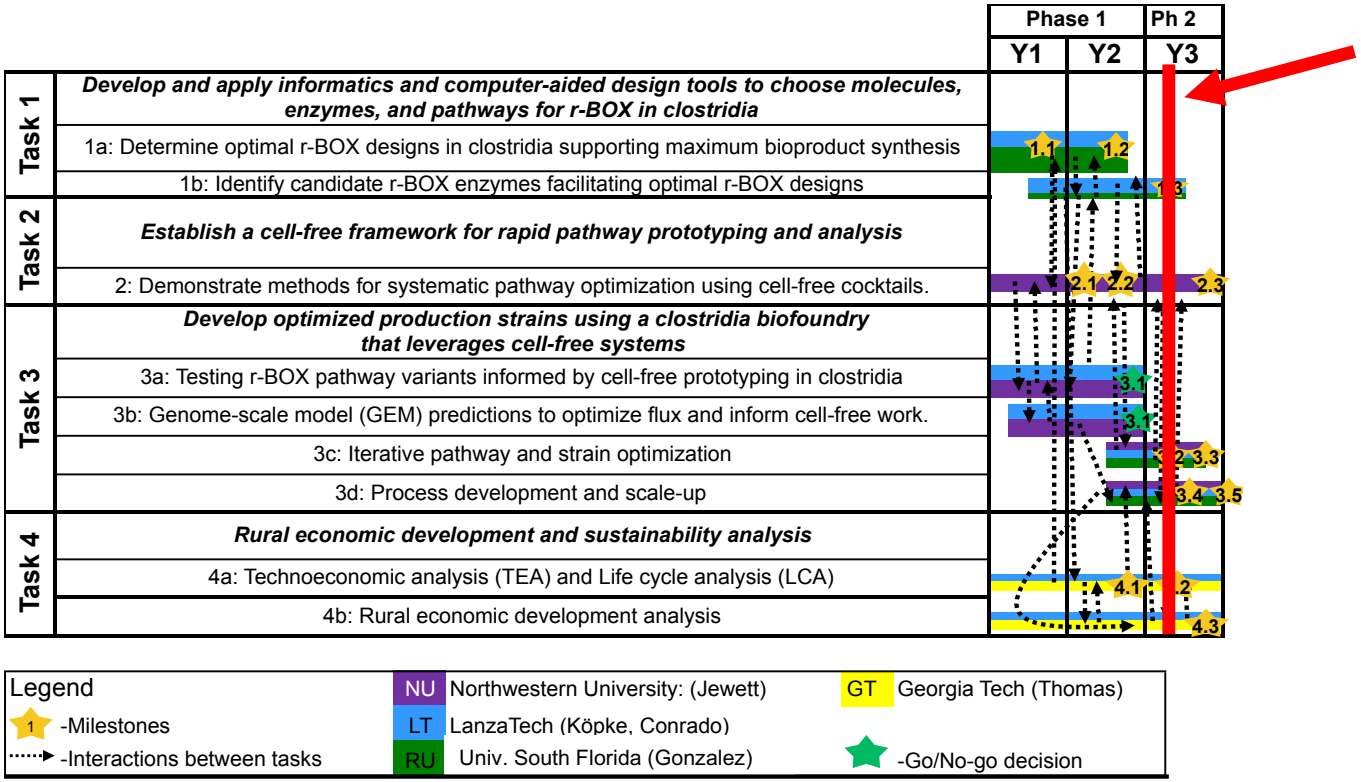
4 – Progress and Outcomes



Our current project progress is on schedule (8/14 milestones)

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Progress summary - Task 1

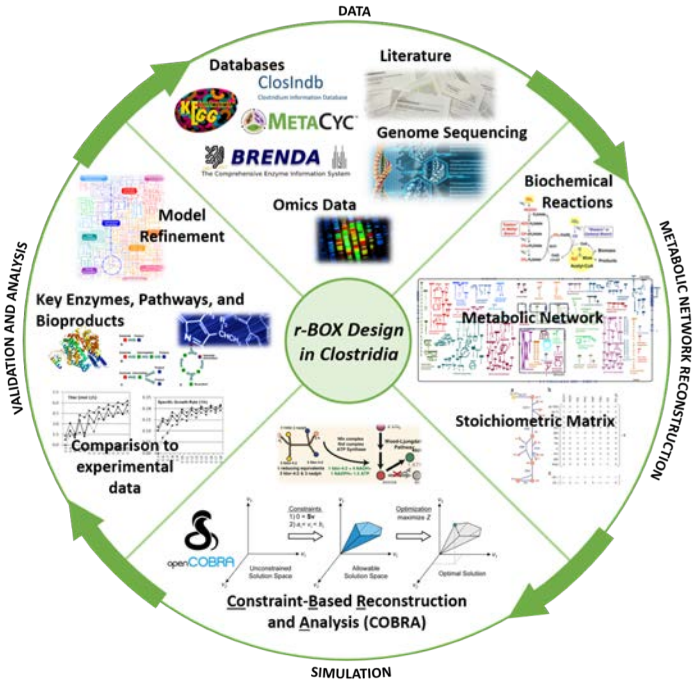
- **Milestone 1.1**. Identify and compare rBOX enzyme variants by data-mining complete *Clostridium* collection and public databases: **>100 additional unique rBOX gene variants over the public domain identified** (Y1/Q4)

  Completed (>200 gene variants synthesized)

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

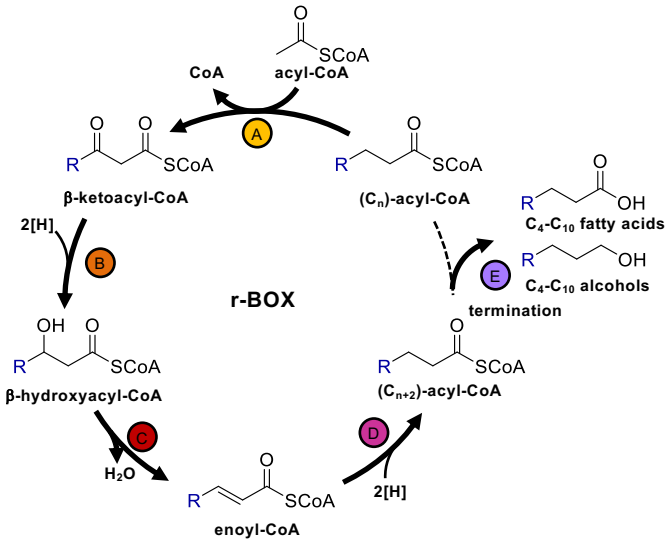
  Completed (261,000 simulations per design)

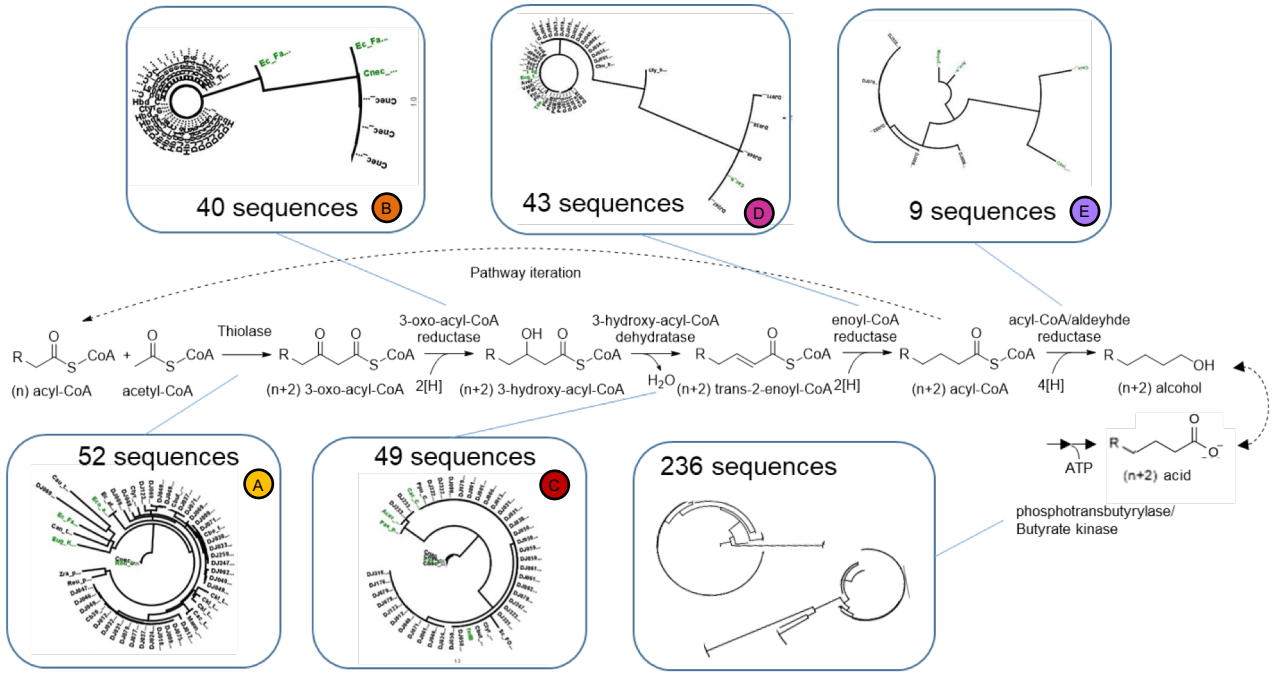
- **Milestone 1.3**. **Optimize computational framework** for generating novel pathways based on feedback from other Tasks and refine pathway design (Y3/Q2) **Ongoing**



Mined and synthesized > 200 r-BOX gene variants

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





More than 200 unique r-BOX gene variants identified and synthesized

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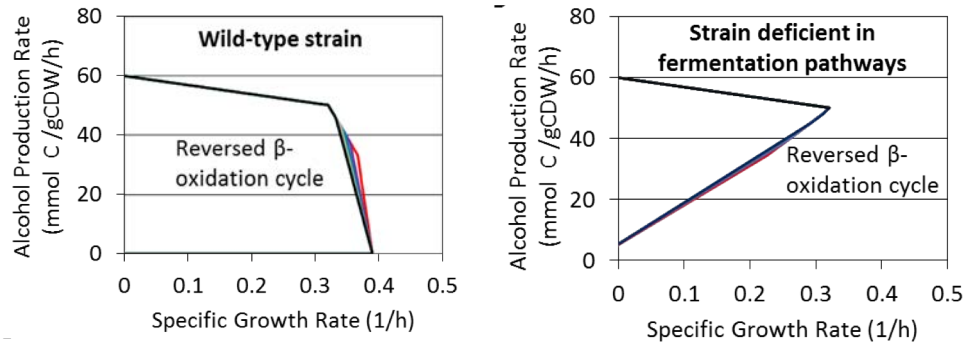
In vivo validation of GEM rBOX predictions for n-alcohol production in *E. coli*

Chassis optimized for testing rBOX modules

***E.coli* JST07**



- Deleted genes responsible for mixed-acid fermentation and endogenous acid termination pathways

- Modeling predicts knockouts that are necessary to couple product synthesis with growth

Cintolesi, A, Clomburg J., and Ramon Gonzalez. *Metabolic engineering* 23 (2014): 100-115.

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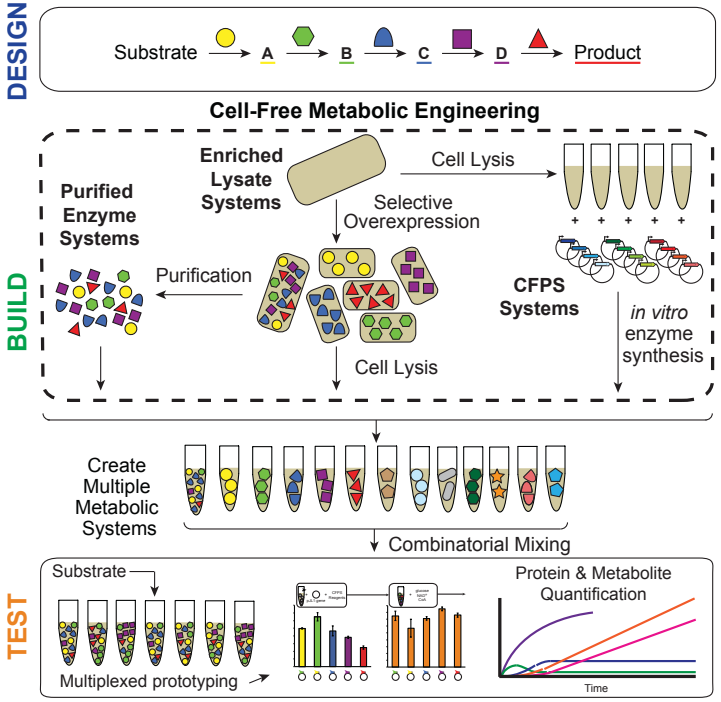
Progress summary - Task 2

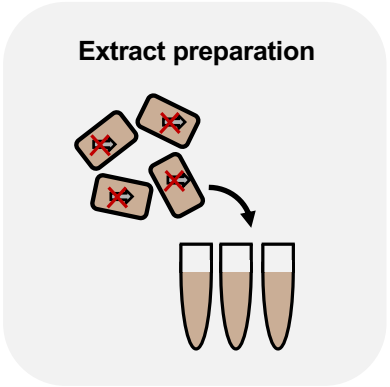
- **Milestone 2.1**. Develop, implement, and demonstrate methods for the **cell-free mix-and-match approach to optimize biosynthetic pathways** that are ***2x faster than the state of the art*** *in vivo* approach (Y2/Q2). **Completed (>10x faster)**

- **Milestone 2.2.** 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). **Completed (>80 enzymes at > 50μg/mL)**

- **Milestone 2.3.** Study and **optimize pathways using our cell-free framework**, and refine and optimize pathways with ***at least 2-fold improvement*** (Y3/Q2). **Completed (>100x improvement of 1-hexanol and hexanoic acid)**



CFPS-ME platform workflow for r-BOX



- Strain selection was crucial for CFPS-ME of r-BOX
- **Fermentation pathways** knocked out – less side products, more acetyl-CoA
- **Acetate assimilation** knocked out – all carbon flux comes from glucose
- **Thioesterases** knocked out – prevents premature termination

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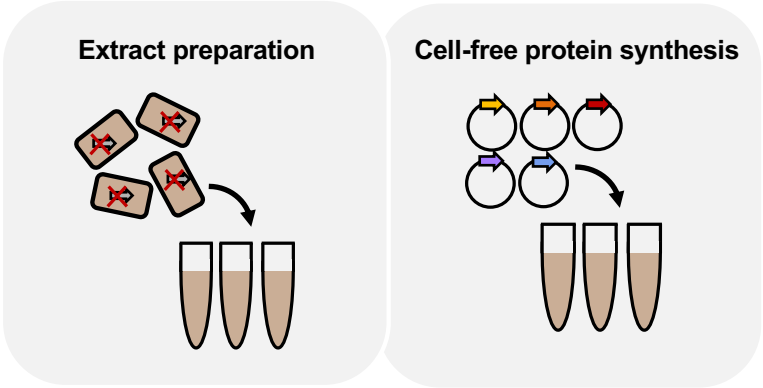

- Codon-optimized for ***C. autoethanogenum*** in Golden-Gate Vectors **compatible** for quick assembly of *in vivo* constructs.
- Solubly expressed >100 r-BOX enzymes in JST07 extract
- GamS allows for **LET expression**, **CSLT-tag** and *E. coli* optimization help with solubility

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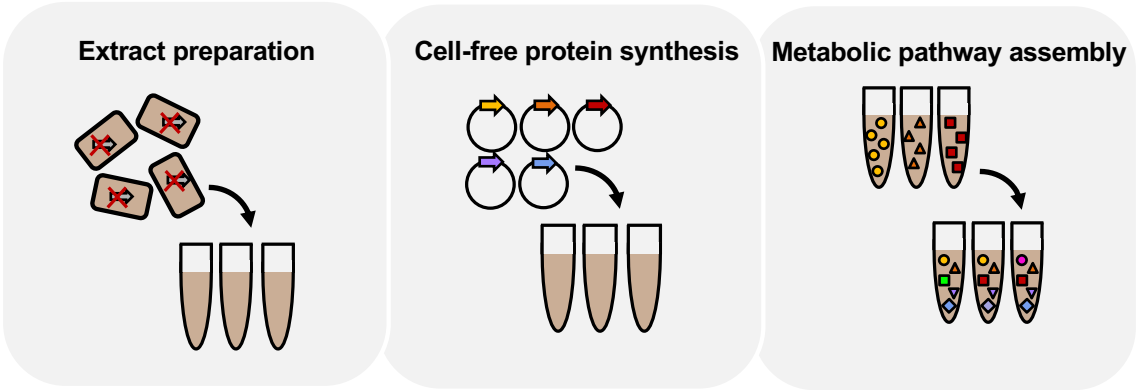

- Glucose used as the carbon substrate, glycolysis produces acetyl-CoA and regenerates cofactors
- Assemble **4μL reactions** in 96-well plates or HPLC compatible PCR plates
- Combinatorially assembled more than **500 different enzyme combinations** and 800 different buffer (ie. pH, glutamate, acetate salts), cofactors (NAD, NADP, ATP, CoA) and enzyme concentrations.

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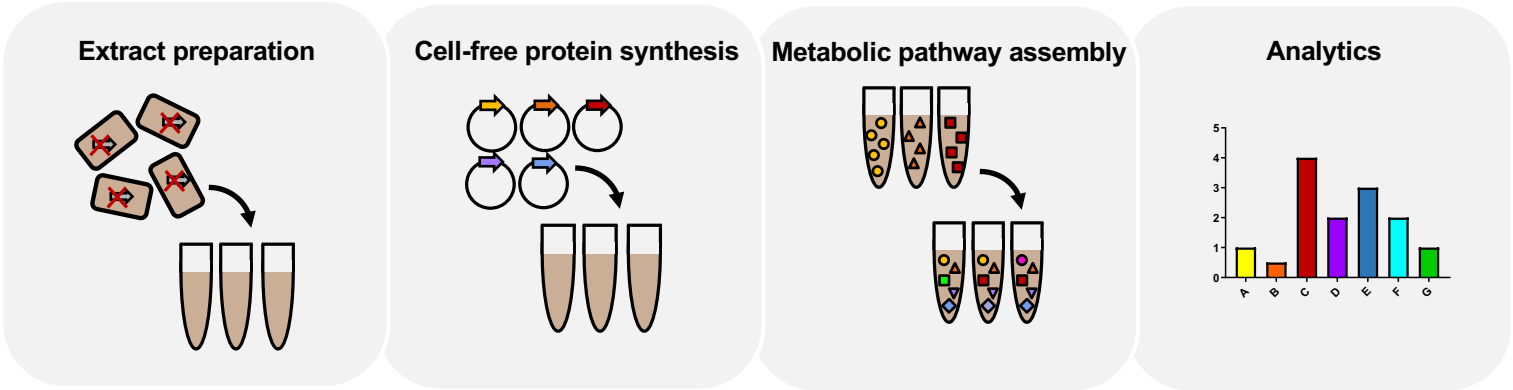

- **GC-MS**: extraction into hexane, derivatization in glass vials with BSTFA, 10 min. run to detect >C4 compounds.
- **HPLC**: direct injection from 96 well plate – very quick and easy work up, but 30min runs for detection of glucose, side products and butanoic acid
- **SAMDI-CoA**: very fast detection of all CoA-ester intermediates, not very quantitative.

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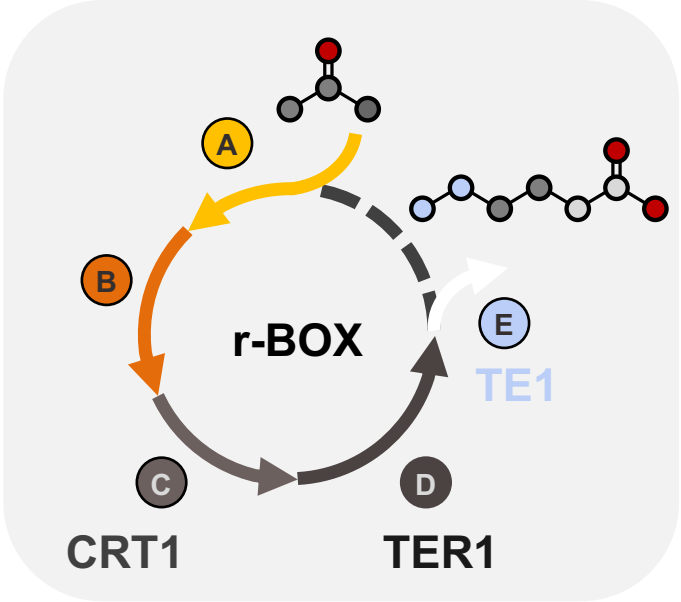
The cell-free system rapidly identifies best sets of enzymes for product synthesis



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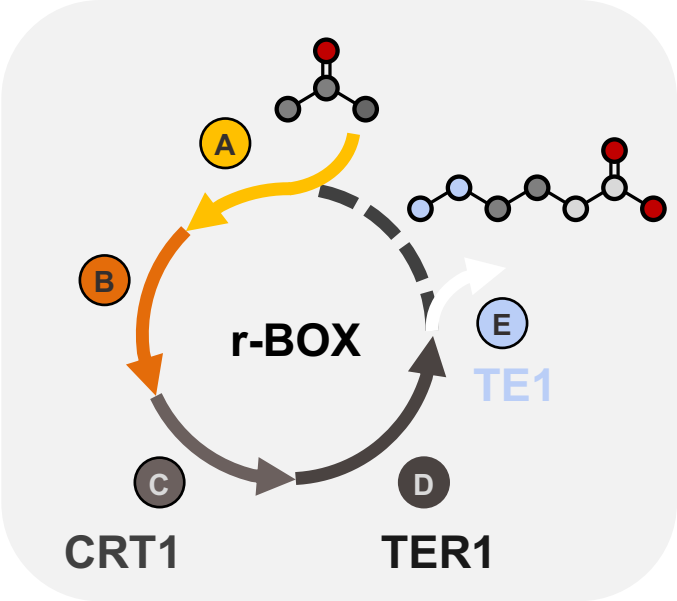

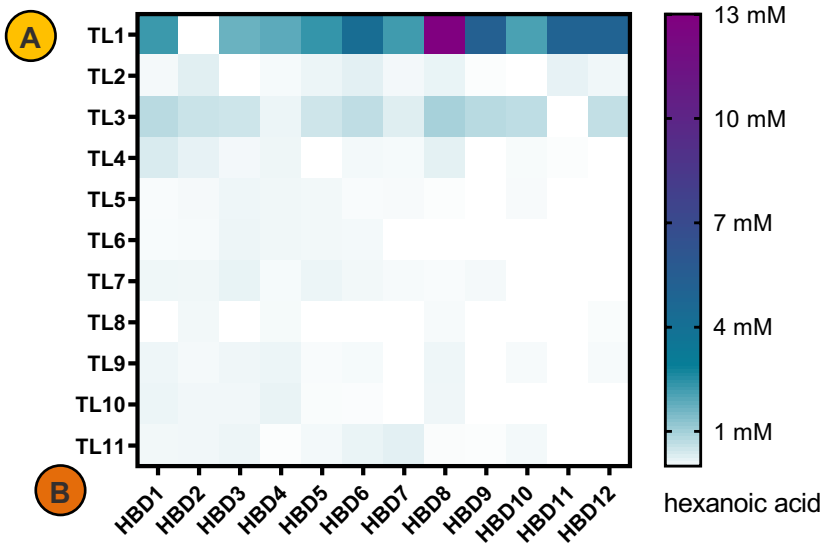

- TL1 and HBD8 work very well together – synergistic effect?
- Good at C6 production – can we control products using termination enzymes?

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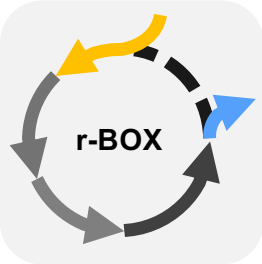
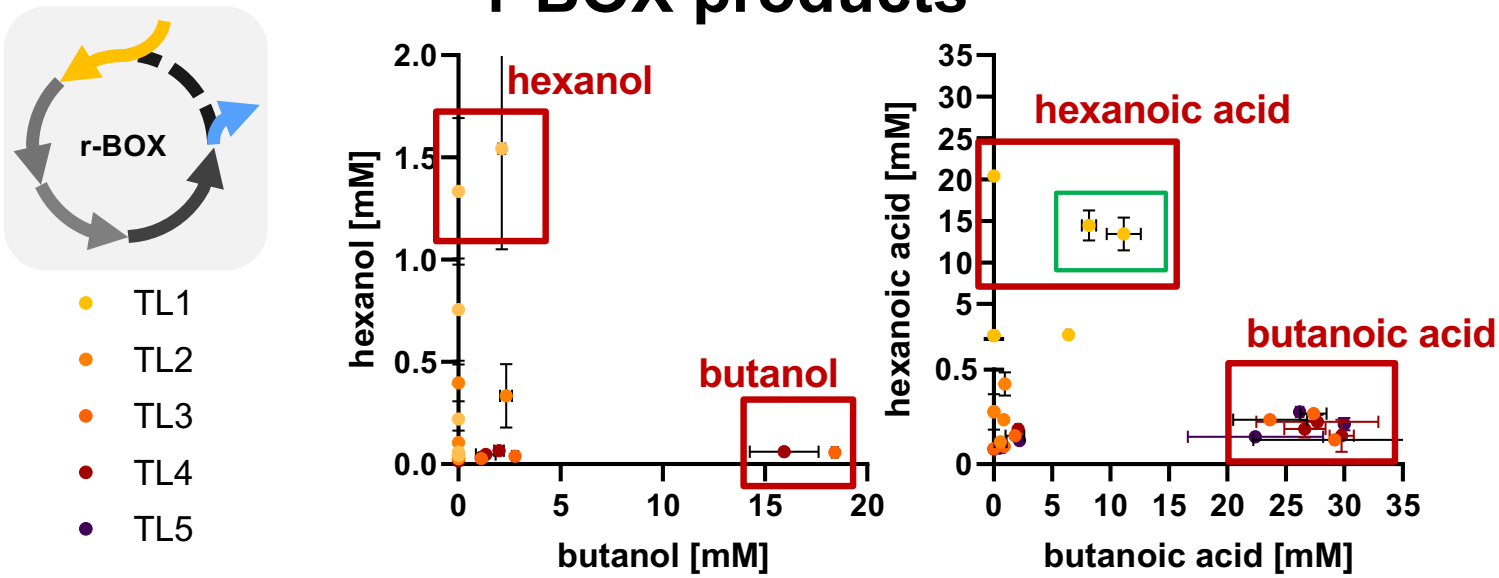
Thiolases and termination enzymes determine r-BOX products



- TL1
- TL2
- TL3
- TL4
- TL5

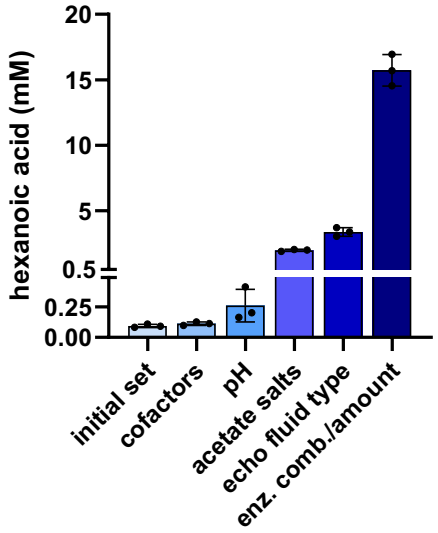
Thiolases and termination enzymes determine r-BOX products



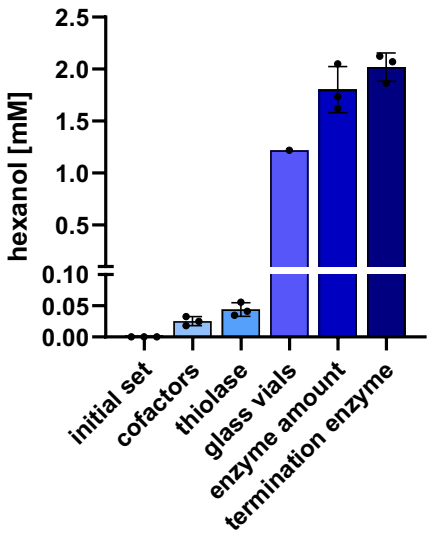
- Specific r-BOX variants characterized for butanol, butanoic acid, hexanol, hexanoic acid.
- High hexanoic acid production for two **Ptb-Buk** termination enzyme combinations. Great candidates for *C. autoethanogenum* implementation due to ATP conservation.

Cumulative improvements so far, enabled by cell-free prototyping system on production

in vitro hexanoic acid production improved to produce 1.7 g/L/d



in vitro hexanol production improved to produce 0.2 g/L/d



The cell-free environment is leading to selection of better enzyme sets to increase product synthesis

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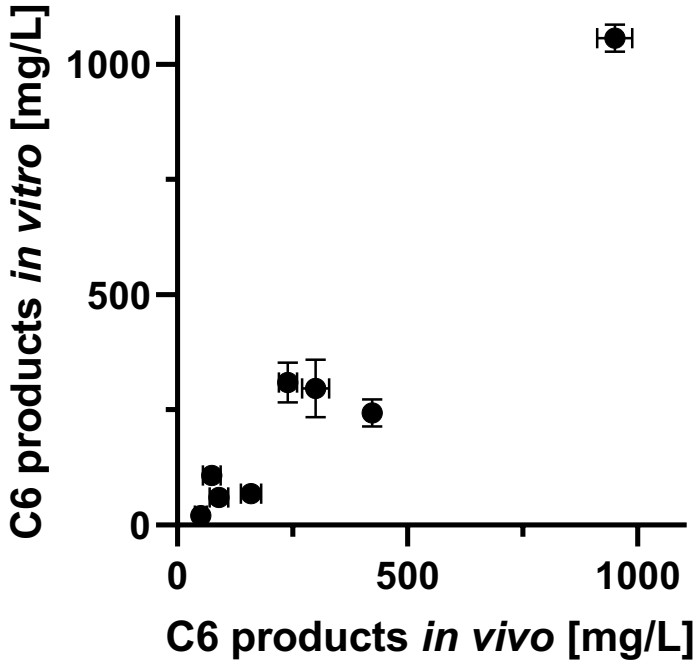
The best hits from the hexanoic acid optimization were tested in *E.coli*

Our screen identified a previously uncharacterized TL – HBD pair that showed strong synergy with each other for the production of C6 r-BOX products.

- Optimized *in vitro* C6 r-BOX combinations correlate well with *E.coli in vivo* data
- TL1–HBD8 combination shows high selectivity for C6 products (93 ± 6 %)
- Identification of optimal termination enzyme could further improve specificity



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Progress summary - Task 3

- **Milestone 3.1. (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) **Completed (for 1-hexanol)**
- **Milestone 3.2.** Proof of concept for **additional rBOX target products** on syngas. (Y3/Q2) **Completed**
- **Milestone 3.3.** Construct and evaluate an **additional 150 unique pathway designs** for target molecules *in vivo* and *in vitro*. (Y3/Q3) **Ongoing**
- **Milestone 3.4.** 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) **Ongoing**
- **Milestone 3.5.** One selected r-BOX product at a target ***metric of >0.1g/l/h*** in **>80L scalable pilot reactor**. (Y3/Q4)

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Generated and screened >50 unique design pathways *in vivo* in *clostridia*

Strain	Butanol (mg/L)	Hexanol (mg/)	Octanol (mg/L)
B8.S4	0	0	0
B8.S6	0	0	0
dB19	0	0	0
dB28	0	0	0
B8.S5	0	0	0
B8.S6	0	0	0
B8.S8	0	0	0
B8.S9	0	0	0
S6	1	0	0
S12	1	0	0
S39	1	1	0
S21	2	0	0
S22	2	0	0
S20	2	0	0
S24	2	2	0
S34	2	2	0
S41	2	2	0
B8.S1	3	0	0
B31	3	0	0
S23	3	3	0
S2	4	2	0
S3	4	2	0
B30	4	0	0
S17	5	2	0
B25	5	0	0

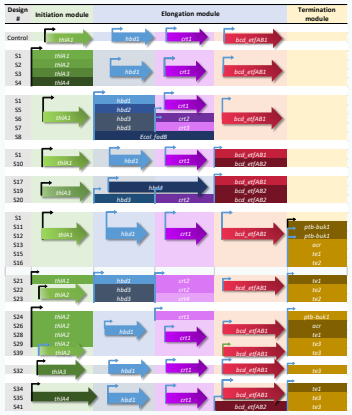
rBOX strains with 5 mg/L or less C4-C8 alcohols

Strain	Butanol (mg/L)	Hexanol (mg/)	Octanol (mg/L)
B7	6	0	0
B4	7	0	0
B5	9	0	0
B6	9	0	0
B5	9	0	0
B8.S11	6	12	0.2
S7	10	0	0
B7	10	0	0
B8	12	0	0
S1	13	0	0
S5	15	0	0
B8.S2	15	0	0
S29	15	12	0
B8.S7	16	82	3
S4	16	12	0
S35	20	16	0
B8.S10	24	0	0
S32	25	30	0
S19	26	14	4
S26	30	60	0
S28	70	47	2
B8.S3	85	0	0
S8	96	0	0
S15	105	0	0
S13	115	0	0
S10	115	0	0
S16	127	0	0
S11	152	0	0

rBOX strains making up to 150 mg/L of C4-C8 alcohols

Key achievements

- Constructed and tested > 50 unique rBOX pathways in *C. autoethanogenum* in 3 design cycles, **surpassing Go/No-Go target**



- Data from bottle screening
- Identified gene candidates that enable hexanol production
- Improved selectivity towards hexanol (S26, S32)

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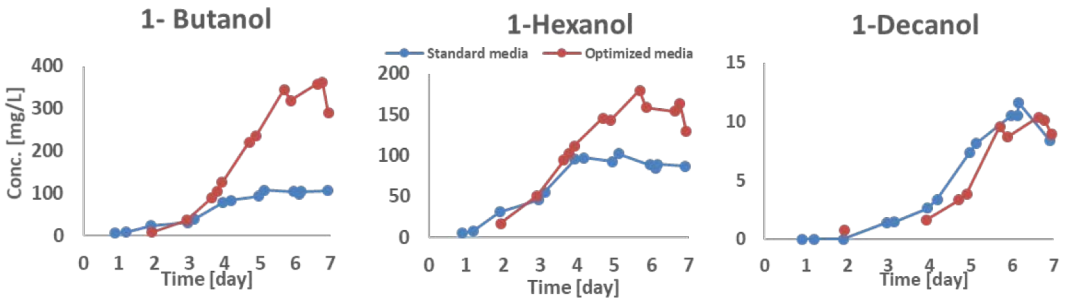
Fermentation process developed and exceeded > 100mg/L/d 1-hexanol *in vivo* in *clostridia*

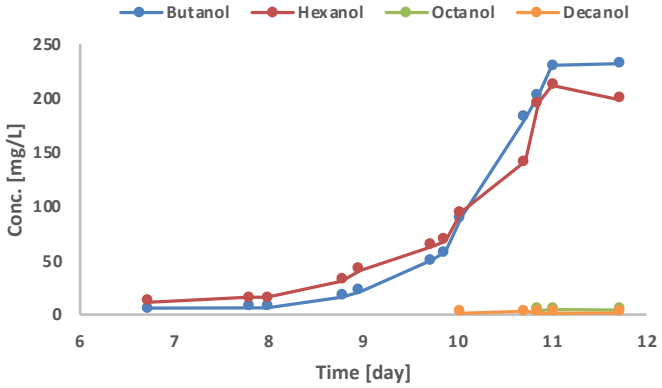
B1.strain 28: Optimize C4-C10 alcohol production regime



Key results

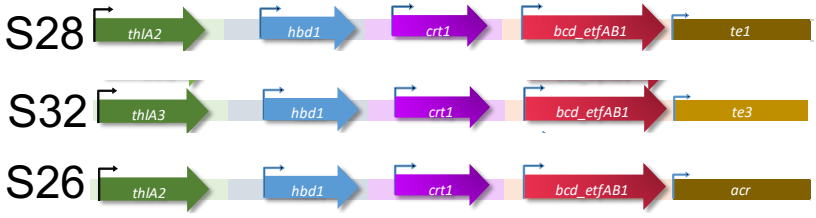
- Optimized media to extended production period and achieve higher maximum titer
- Data shown is concentration in the broth, expecting higher actual production (stripping)

B1.strain 32: Achieved >100 mg/L/d hexanol



Key results

- 129 mg/L/d productivity achieved for hexanol between days 9.8 and 10.8 (in broth), **<u>surpassing Go/No-Go target</u>**
- Best hexanol strain from bottle screening yet to test



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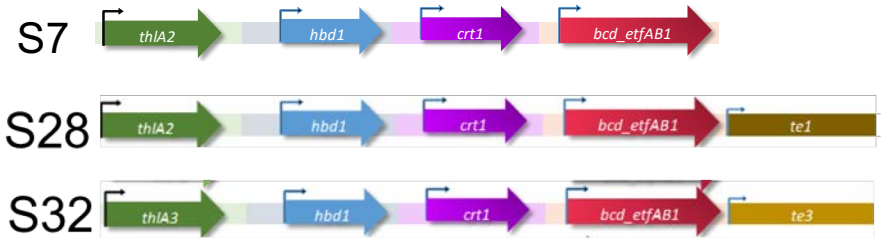
Overview of fermentation results across strains

	B8.strain 7	B1.strain 28	B1.strain 32	B1.strain 28 (opt)	B1.strain 32 (opt)
Butanol	18.98 (day 4.6-5.6)	47.9 (day 2.9-3.9)	70.6 (day 2.2-2.9)	117.9 (day 3.6-4.9)	147.8 (day 9.8-10.8)
Hexanol	46.2 (day 4.6-5.6)	50.5 (day 2.9-3.9)	43.5 (day 2.2-2.9)	60.5 (day2.9-3.9)	**129.4** (day 9.8-10.8)
Octanol	1.75 (day 4.6-5.6)	1.75 (day 4.17-4.9)	1.76 (day 2.9-3.9)	N/A (below detection limit)	N/A (below detection limit)
Decanol	N/A (below detection limit)	5.07 (day 4.2-5.1)	4.27 (day 2.9-3.9)	5.09 (day4.9-5.7)	N/A (below detection limit)
Total C6-C10	**47.96**	**57.32**	**49.53**	**65.59**	**129.4**
Total C4-C10	**66.94**	**105.22**	**120.13**	**183.49**	**277.2**



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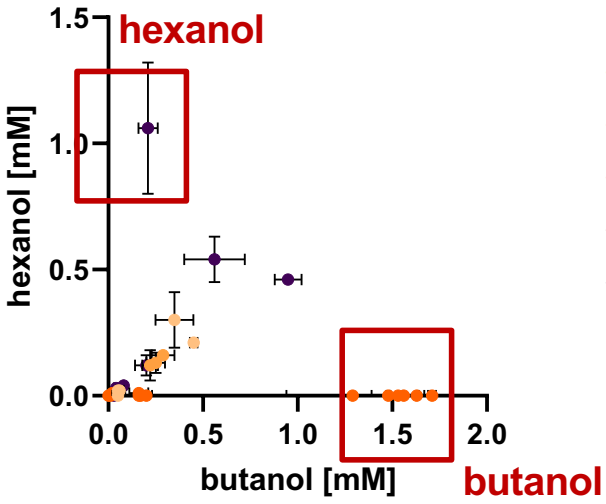
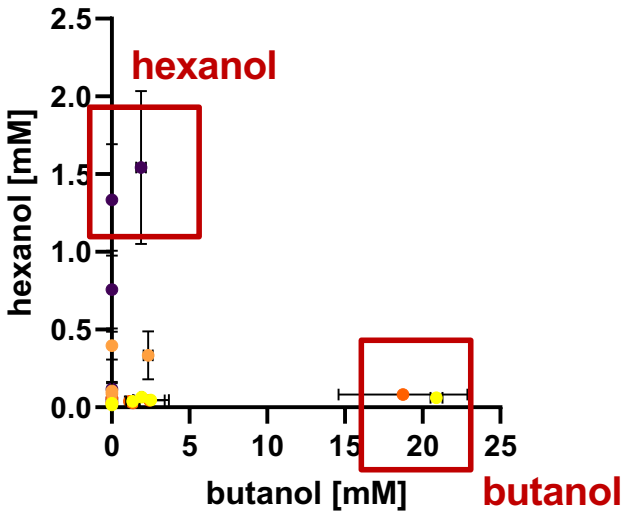
Product specificity of r-BOX variants in *C. autoethanogenum*

C4/C6 product analysis in *C. auto* strains



in vitro variants for comparison



The first two rounds of *C. autoethanogenum* strain engineering led to the identification of specific butanol and hexanol producers that correlate well with the set identified *in vitro*.

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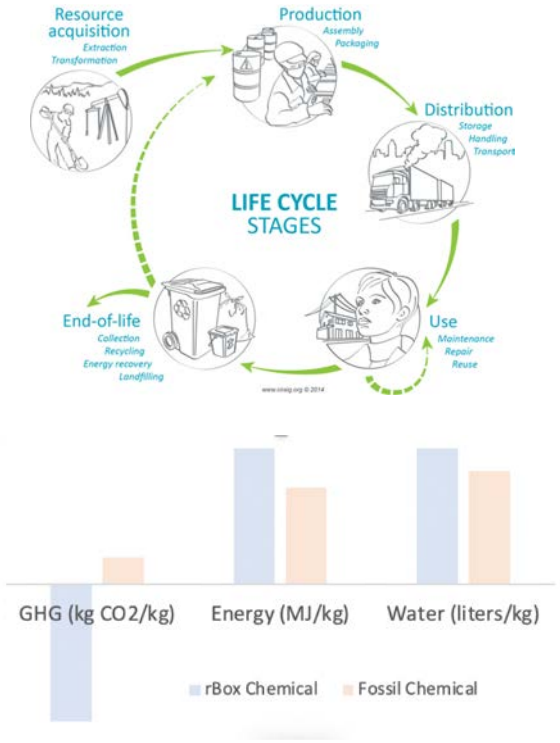
Progress summary - Task 4

- **Milestone 4.1**. Complete **2 workshops to inform environmental community and rural economic development analysis**. 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) **Completed**

- **Milestone 4.2**. LCA for two rBOX molecules. (Y3/Q2) **Ongoing**

- **Milestone 4.3**. Completed assessment of infrastructure and supply chains for biomass feedstock supply of two rBOX molecules in the US southeast. (Y3/Q4) **Ongoing**



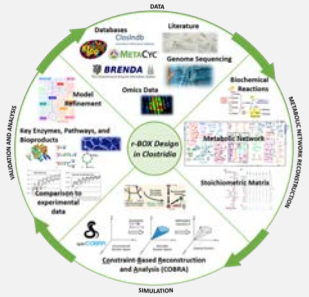
Key Activity: Plan two workshops to guide the project's strategic direction and product emphasis

- *Workshop 1.* Atlanta, March 3, 2020.
  - Invitees included Georgia Rural Economic Development Authority representatives, industry representatives, environmental NGO representatives, experts on community impacts, ethanol manufacturers

  Completed
- *Workshop 2.* Online 2020.
  - Two workshops were held online and are made available to the public on our website on Sustainable Production of Fuels and Chemicals from Biomass in the Southeast (https://sites.gatech.edu/rbox/).
  - Speakers included Prof. Rajan Parajuli from NC State University, Dr. William Frey from the DOE/USDA Biomass R&D Technical Advisory Committee, Professor Jackie Mohan from Univ. Georgia, and Mr. Stuart Hale from The Nature Conservancy, **Completed**

Planned future work

Task 1: Genome scale modeling

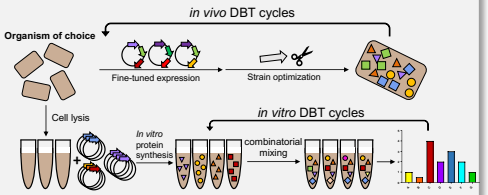


- Optimize computational framework for additional pathways

Task 4: Life-cycle assessment

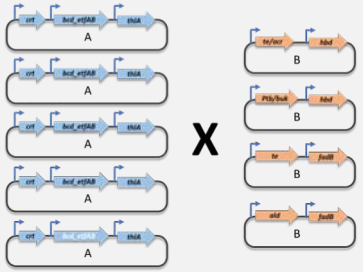
- Life-cycle assessment for two rBOX molecules

Task 2: Cell-free assays

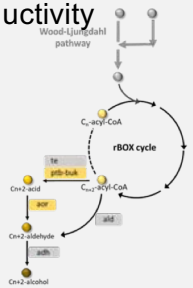


- High-throughput screening of termination enzymes using linear expression template platform.
- Leveraging SAMDI-CoA throughput to find enzyme combinations for the synthesis of alternative rBOX products
- Optimizing platform for in vitro biomanufacturing of small molecules.

Task 3: Clostridial engineering



- High-throughput strain construction to meet target of 150 new strains
- Chassis strain optimization to improve hexanol productivity



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Summary

Summary

Task 1:

- >300 new gene variants mined and synthesized
- >250,000 GEM simulations per design, confirmed growth coupling being possible
- Optimized *E. coli* chassis strain generated as basis for cell-free prototyping and blueprint for *Clostridium*

Task 2:

- Cell-Free platform for rBOX testing established, specific variants for 4 targets
- ~500 designs tested; 8000 assays run
- SAMDI-CoA method established

Task 3:

- rBOX implemented into *Clostridium*, hexanol + octanol demonstrated
- Improved initial hexanol titer by over 20-fold
- ~ 50 Designs tested, streamlined strain generation workflow established

Task 4:

- Both workshops completed, online platform available to the public, network with stakeholders established

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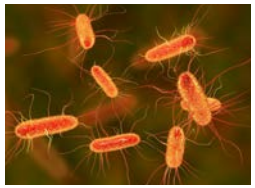
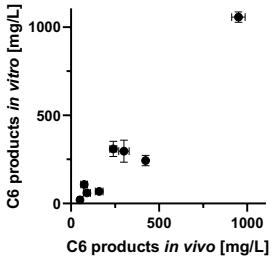
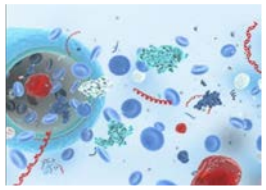
Summary

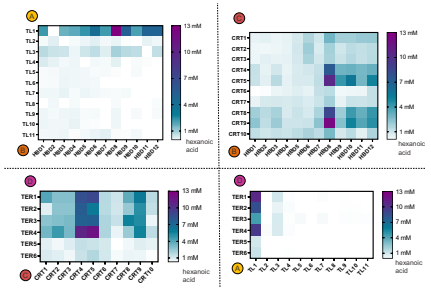
E. coli
Established rBOX



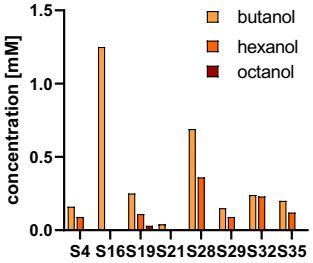


Cell-Free
Rapid Prototyping





Clostridium
Industrial Production



Across platforms found specific priming and terminating options being important

Goal → 100 mg/L/day of rBOX product

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Thank you!

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

BIOENERGY TECHNOLOGIES OFFICE

Technology Manager: Ian Rowe, Jay Fitzgerald
Project Monitor: Ben Simon, Clayton Rohman
Grants Management Specialist: Nicholas Oscarsson

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Quad Chart Overview

Timeline

- Project start date - 10/1/2018
- Project end date - 12/31/2021
- Project complete 60% (8/14 milestones)

	FY 20 Costed	Total Award
DOE Funding	$1,062,765	$1,600,000
Project Cost Share*	$303,507	$400,000

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

Project Goal

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

End of Project Milestone

- 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

Funding Mechanism

DE-FOA-0001637, Topic B: Biofuels and Biobased Products Development, 2018

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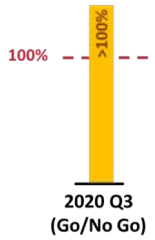
Responses to Previous Reviewers' Comments

Reviewers' comments	Response
1. Not clear if reversing the beta-oxidation pathway will work well in an anaerobic bacterium.	• We demonstrated production of hexanol showing rBOX pathway is turning at least two cycles in *C. autoethanogenum*. • By using rBOX gene variants from a large collection of anaerobic *Clostridium* species, we identified genes that enable rBOX pathway in our anaerobic host.
2. Enzymes may need to be engineered to achieve the necessary rate. This may be beyond the scope of this project period, but the team should still think about it.	• Established a high-throughput cell-free mix screening approach. The combinatorial setup also allows us to balance enzyme activities by simply adding more or less of each pathway enzyme depending on observed build up of pathway intermediates.
3. Even with specific enzymes, there will likely be a build up with different carbon length, has the team looked at final separation?	• As part of the technoeconomic analysis, we will evaluate different separation options for the selected target molecules. Technoeconomic analysis performed throughout the process will also guide decision making on when to pilot the process that will be made in coordination with DOE. • Separation trials are beyond the scope of the project, but LanzaTech has experience with different separation systems and this is an active area of parallel work. The pilot facilities are equipped with a range of separation equipment.

Highlights from Go/No-Go Review

Surpassed the Go/No Go milestone by 30% (achieved 139 mg/L/d productivity for hexanol).



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Publications, Patents, Presentations, Awards, and Commercialization

Publications/Patents:

- Silverman, A.D., Karim, A.S., and Jewett, M.C. Cell-free gene expression systems: An expanding repertoire of applications. ***Nature Reviews Genetics***. 2019; DOI: 10.1038/s41576-019-0186-3
- Karim, A. S., F. (Eric) Liew, S. Garg, B. Vögeli, B. J. Rasor, A. Gonnot, M. Pavan, A. Juminaga, S. D. Simpson, M. Köpke, M. C. Jewett. Modular cell-free expression plasmids to accelerate biological design in cells. ***Synthetic Biology***, Volume 5, Issue 1, 2020; https://doi.org/10.1093/synbio/ysaa019
- B.J. Rasor, B. Vögeli, G. M. Landwehr, J. W. Bogart, A. S. Karim, M.C. Jewett. Toward sustainable, cell-free biomanufacturing. ***Curr Opin Biotechnol***. 2021 Jan 13;69:136-144. DOI: 10.1016/j.copbio.2020.12.012
- Fackler N. et al. Stepping on the Gas to a Circular Economy: Accelerating Development of Carbon-Negative Chemical Production from Gas Fermentation. ***Annu. Rev. Chem. Biomol. Eng.*** 2021. 12:X–X 10.1146/annurev-chembioeng-120120-021122
- 1 manuscript under review, 3 manuscripts in preparation, 1 invention disclosure form submitted

Selected Presentations:

- Köpke, M. Pollution To Products. **Hello Tomorrow Global Summit**, Virtual. November 2020 ***(Keynote Talk)***
- Köpke, M. Commercial Scale Production of Low Carbon Fuels and Chemicals from Waste Gases. **ACS Fall 2020**, Virtual. August 2020
- Köpke, M. Commercial Scale Production of Low Carbon Fuels and Chemicals from Waste Gases. **SIMB Annual Meeting**, Washington, DC. July 2019 ***(Invited Talk)***
- Köpke, M. Enabling the Circular Economy through Recycling Feedstocks **BIO World Congress**, Ames, Iowa. July 2019 ***(Invited Talk)***
- Köpke, M. Commercial Scale Production of Low Carbon Fuels and Chemicals from CO2/CO Waste Gases by the Acetogen *Clostridium autoethanogenum*. **Biological Solutions for the Global CO2 Challenge, EMBL Heidelberg**, Germany. June 2019 ***(Keynoye Talk)***