10 Years On the Road to Awesome!

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U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review

Bioenergy Technologies Incubator 2:
Development of a Sustainable Green Chemistry Platform for Production of Acetone and downstream drop-in fuel and commodity products directly from Biomass Syngas via a Novel Energy Conserving Route in Engineered Acetogenic Bacteria

March 9, 2017
Biochemical Conversion

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LanzaTech, Inc.

This presentation does not contain any proprietary, confidential, or otherwise restricted information

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Goal Statement

Project Goal:

To develop and scale up a novel technology platform for direct conversion of biomass syngas to acetone, which can be converted into a range of fuels and commodities via existing downstream technologies

Key Project Outcomes:

- Demonstration of novel process for acetone production from biomass syngas at commercially relevant rates in a continuous process and scalable pilot reactor
- Optimization of natural and synthetic pathway for acetone production
- Validation of commercial potential through TEA and LCA

Relevance to Bioenergy Industry/Impact:

- Offers a new stand-alone route to advanced hydrocarbon fuels and bioproducts from biomass residues
- Provides environmentally friendly alternative to current petrochemical acetone production
- Produces a platform chemical that can be readily converted into a range of drop-in fuels (isooctane and jet fuel) or important chemical building blocks (isobutylene, propylene, bisphenol A and PMMA)
- Increases yields over ABE fermentation through a novel pathway that is acetate independent and energy generating

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Quad Chart Overview

Timeline

- Project start date: Oct 1, 2016
- Project end date: Sep 30, 2018
- Percent complete: ~16% *(month 4 of 24 as of end of Jan 2017)*

Budget

	FY16 Costs (Oct-Dec 2016)	FY17 Project End Date (Jan 2017-Sep 2018)	Total Project
Total Project Costs	$977,523	$913,557	$1,891,080
DOE Funded	$752,523	$688,557	$1,441,080
Project Cost Share	$225,000 (23%)	$225,000 (25%)	$450,000 (24%)

Barriers

- Ct-A: Feedstock Variability
- Ct-H: Efficient catalytic Upgrading of gaseous intermediates to fuels and chemicals
- Ft-I: Overall integration and Scale-Up
- Im-E: Cost of production
- St-E: Best practices and systems for sustainable bioenergy production

Partners

Split in total project funding

- 51% to LanzaTech
- 49% to Oak Ridge National Lab

Sequencing input from JGI (Funded separately)

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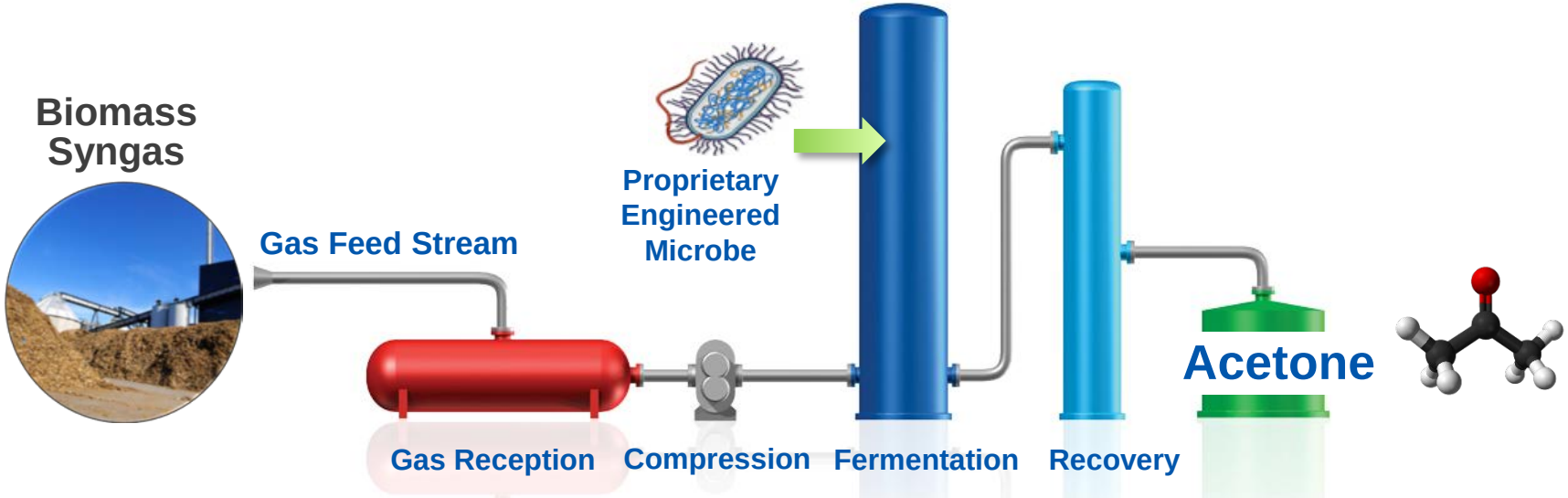
1 – Project Overview

The LanzaTech Process



- **Direct process from biomass syngas to acetone**
  - **Reduce GHG emission**
  - **Does not compete with food resources, land and water**
  - **Sustainable**
- **Current industrial acetone production is the by-product of petrochemical phenol production**
  - **Promote GHG emission**
  - **Produce chemical wastes (one mole waste produced per mole of product) and environment pollutants**

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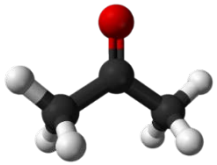
Acetone as the platform molecule for hydrocarbon fuels and bio-products

Product	Use	Market ($US Billion)
Jet-fuel	Drop-in jet fuel for aviation industry	228
Isobutylene	Fuel additives, synthetic rubber	25-29
Isooctane	Drop-in fuel for standard gasoline cars	0.6 (only US market)
Isopropanol	Solvent	2.5-3.5
Propylene	Polymer and solvent	125
Bisphenol A (BPA)	Polymer or epoxy resins	10
Polymethyl methacrylate (PMMA)	Polymer (Plexiglas)	7



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Acetone as the by-product of the petrochemical phenol production process

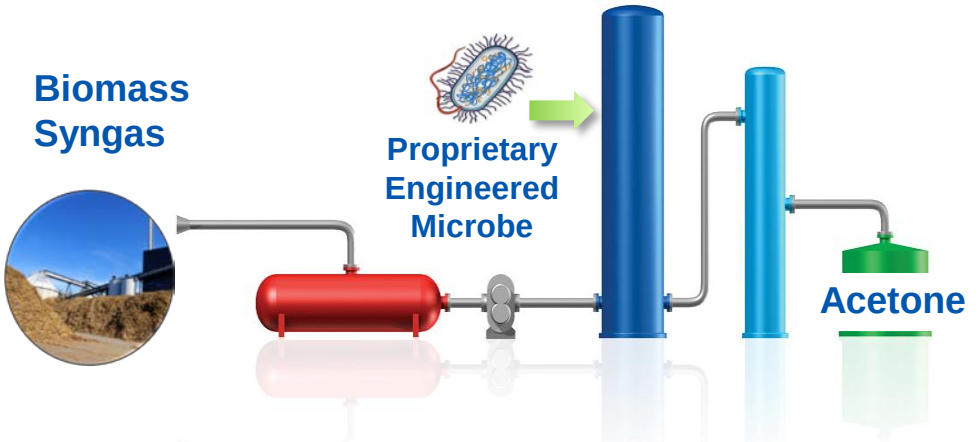
$$C_6H_6 + C_3H_6 + O_2 \rightarrow C_3H_6O + C_6H_6O$$

Acetone Phenol

Acetone demand expected to increase

Production cost of acetone is increasing due to

- Expected decline of phenol demand
- No commercially viable solution for acetone production



GHG reduction

Carbon Reduction: Fossil petroleum

90%

- **LanzaTech process for acetone production is providing an environmentally sustainable solution to current process.**

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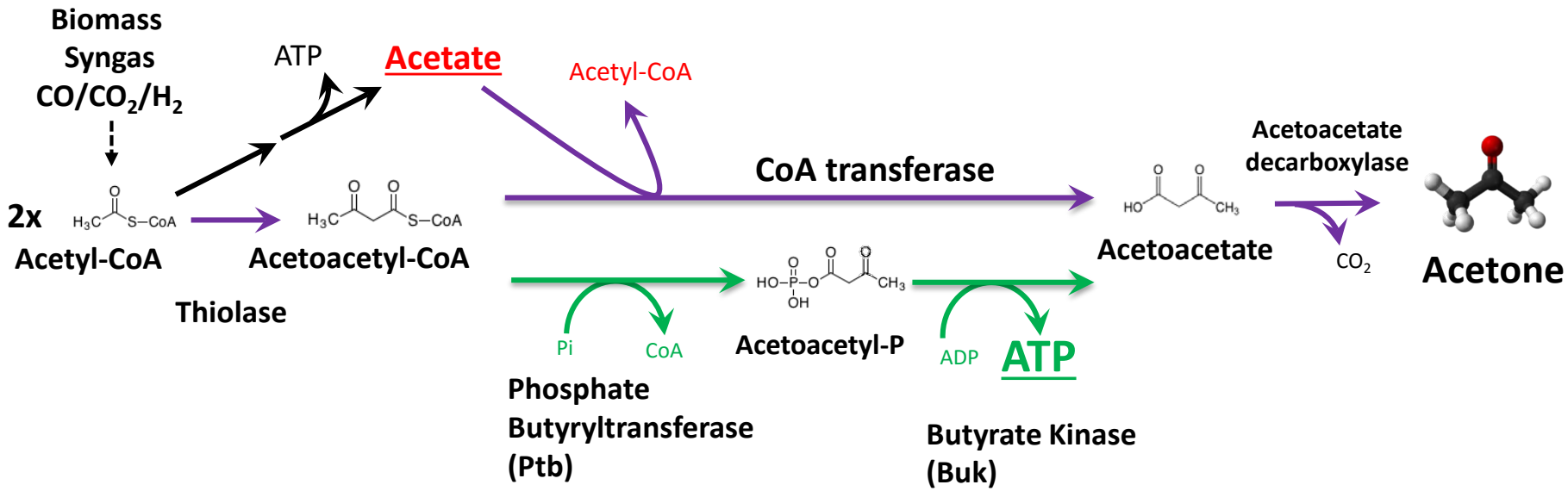
The LanzaTech Approach

Clostridial ABE pathway

- Acetate as by-product
- No net ATP generation



Novel synthetic pathway

- No by-product
- Generates ATP
- Higher selectivity to acetone

→ *C. autoethangenum* native pathway
→ heterologous Clostridial ABE pathway
→ Synthetic heterologous pathway

Optimizing native pathway by mining for improved enzymes

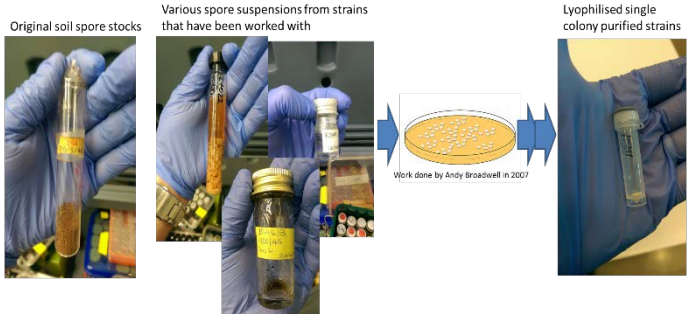
Very limited variety of acetone pathway enzymes in public databases

- Only very few native acetone producing species (**<5**) are known
- These are mainly ABE fermenting Clostridia, where acetone is a byproduct
- The few sequenced strains (**<10**) only produce very little acetone and offer little diversity of acetone pathway genes

Expanding the space by mining the world's largest collection of ABE fermentation strains

- LanzaTech owns the largest collection of industrial ABE fermentation strains - The David Jones collection
- Collection comprises of **>400** strains spanning over 4 decades of industrial production, including **some strains that make substantial higher amounts of acetone**
- Collection is currently sequenced by JGI and will be mined as part of this project to identify better variants of acetone pathway enzymes (thiolases, CoA transferases, acetoacetate decarboxylases, Ptb-Buk). This will also include phenotype-genotype modelling
- This analysis will also provide important insight about what genes/enzymes were critical for process optimization over time

Strains from collection were used in 12 x 90,000 liter production facility in South Africa



Strains spanning >4 decades of development and date back to the 1940s have been purified from soil and spore stocks and are currently sequenced at JGI

2 – Approach (Management)

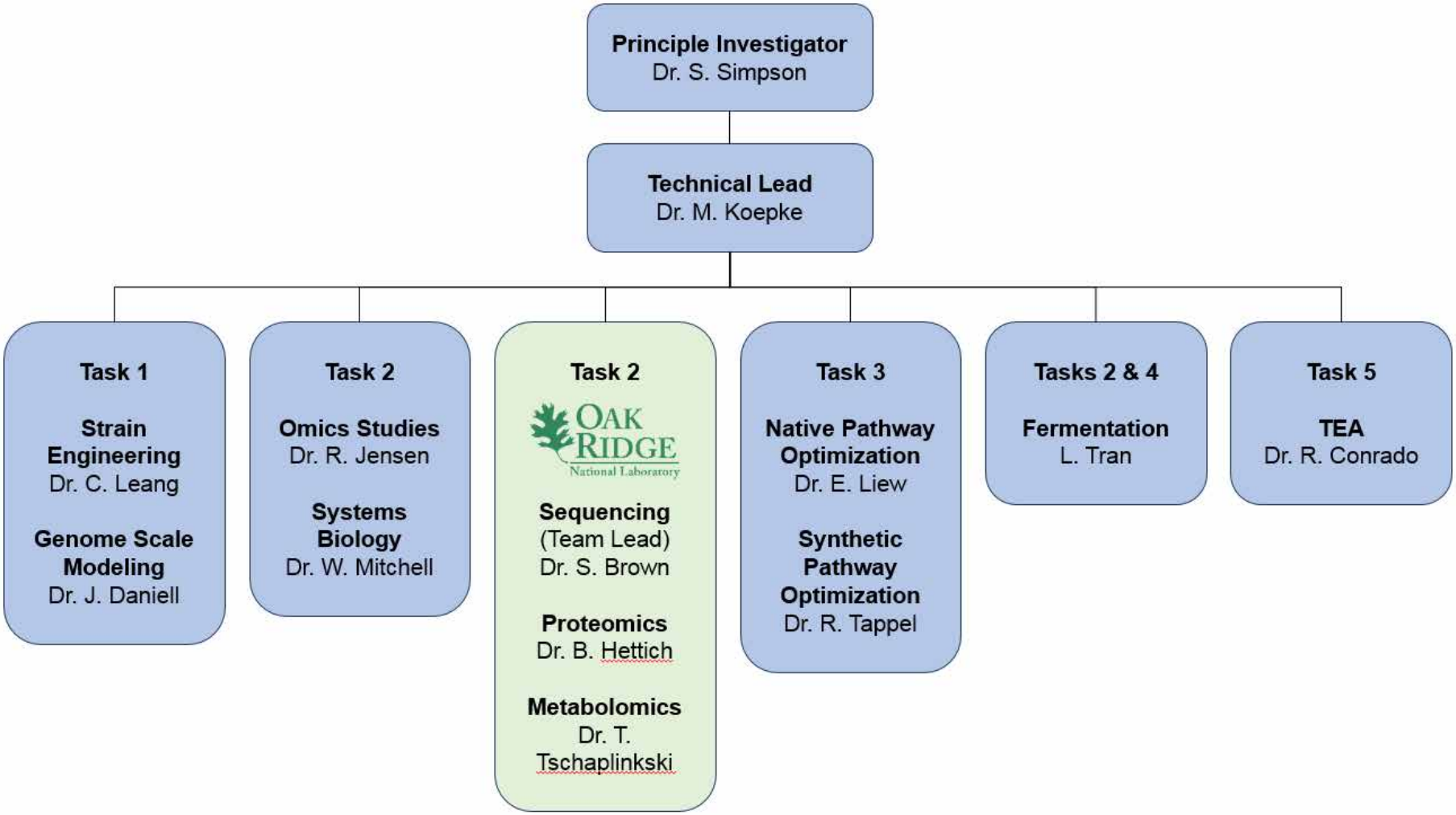
Team Management

Meeting	Frequency	Location
LanzaTech internal	Weekly	LanzaTech
Full project team (phone)	Semi-monthly	Respective offices
Task Leads (in-person)	Semi-annually*	Alternating locations
Individual team members (phone)	As needed	Respective offices

**November 2016: Project kick-off meeting held at LanzaTech*

- LanzaTech and ORNL have been working in collaboration since 2011. They have developed successful mechanisms for technical coordination, data sharing and integration.
- LanzaTech will be responsible to DOE for all technical and financial reporting and securing and filing of IP

Project Team



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3 – Approach (Technical)

Approach

Identification of bottlenecks through omic studies

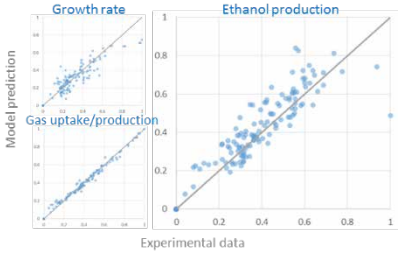
Novel synthetic pathway (Ptb-Buk)

Biomass Syngas

Proprietary Engineered Microbe

Acetone

Genome scale modeling



Elimination of competing pathways (Chassis strain optimization)

Pathway optimization (combinatorial analysis)

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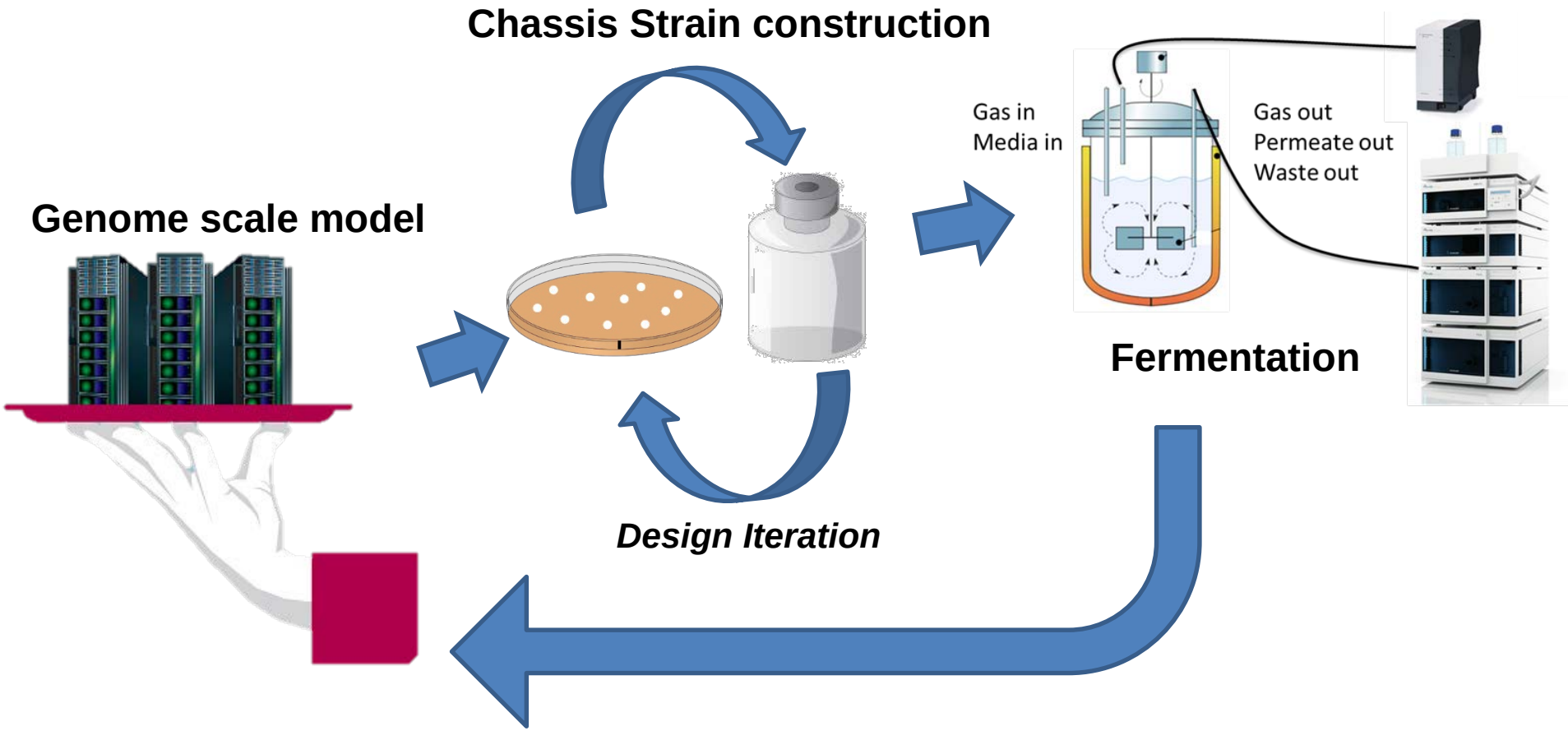
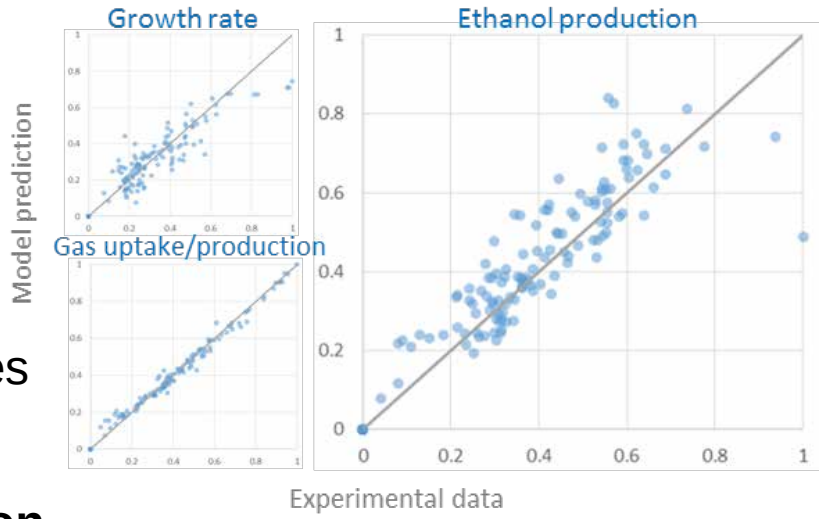
Approach


Chassis Strain construction
Genome scale model
Gas in
Media in
Gas out
Permeate out
Waste out
Fermentation
Design Iteration

LanzaTech's Genome Scale Model

- **Model is based on our internal *Clostridium autoethanogenum* metabolic knowledgebase**
  - List of metabolic reactions, enzymes and associated genes in *C. auto*
  - Curated with enzyme data (cofactor specificities), etc
  - Consists of >1000 reactions and metabolites
- **Links *C. autoethanogenum* genotype to phenotype. Very predictive of gas fermentation across the full spectrum of gas mixes.**
  - Validated against >250 fermentation runs and >100 KO strains

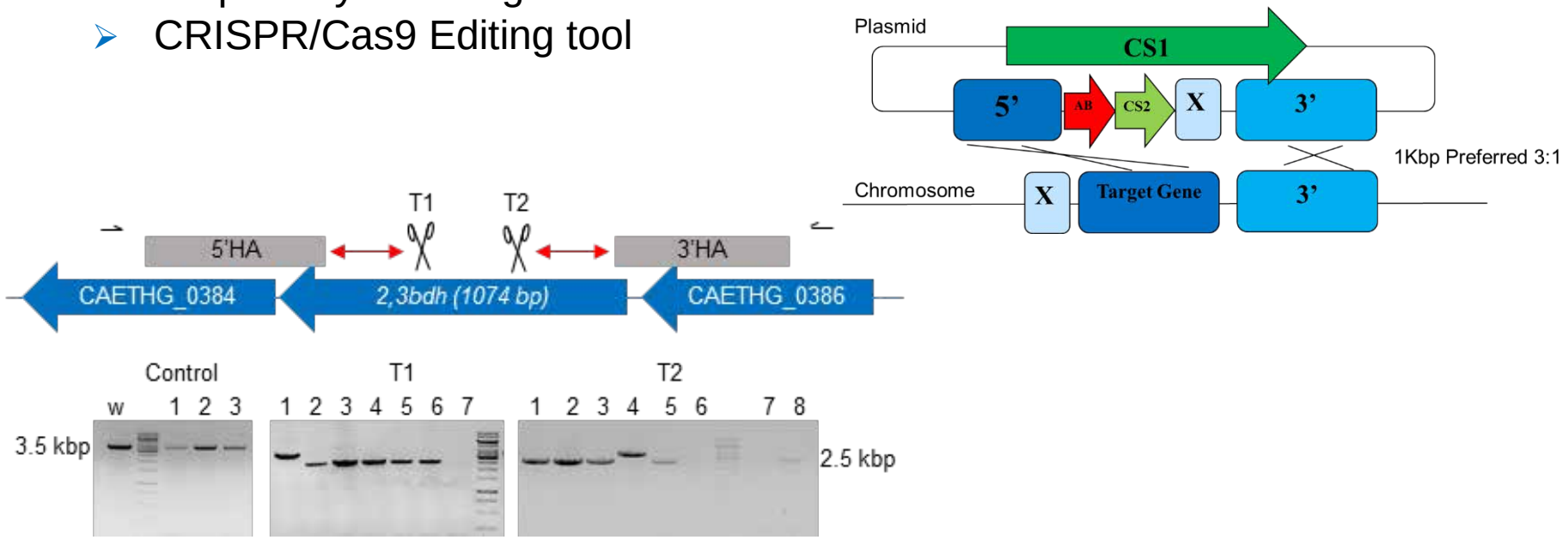


Work complete

Modelling used to identify optimal combination of gene knockouts to maximize flux to acetone through novel pathway

Genome Editing Tools

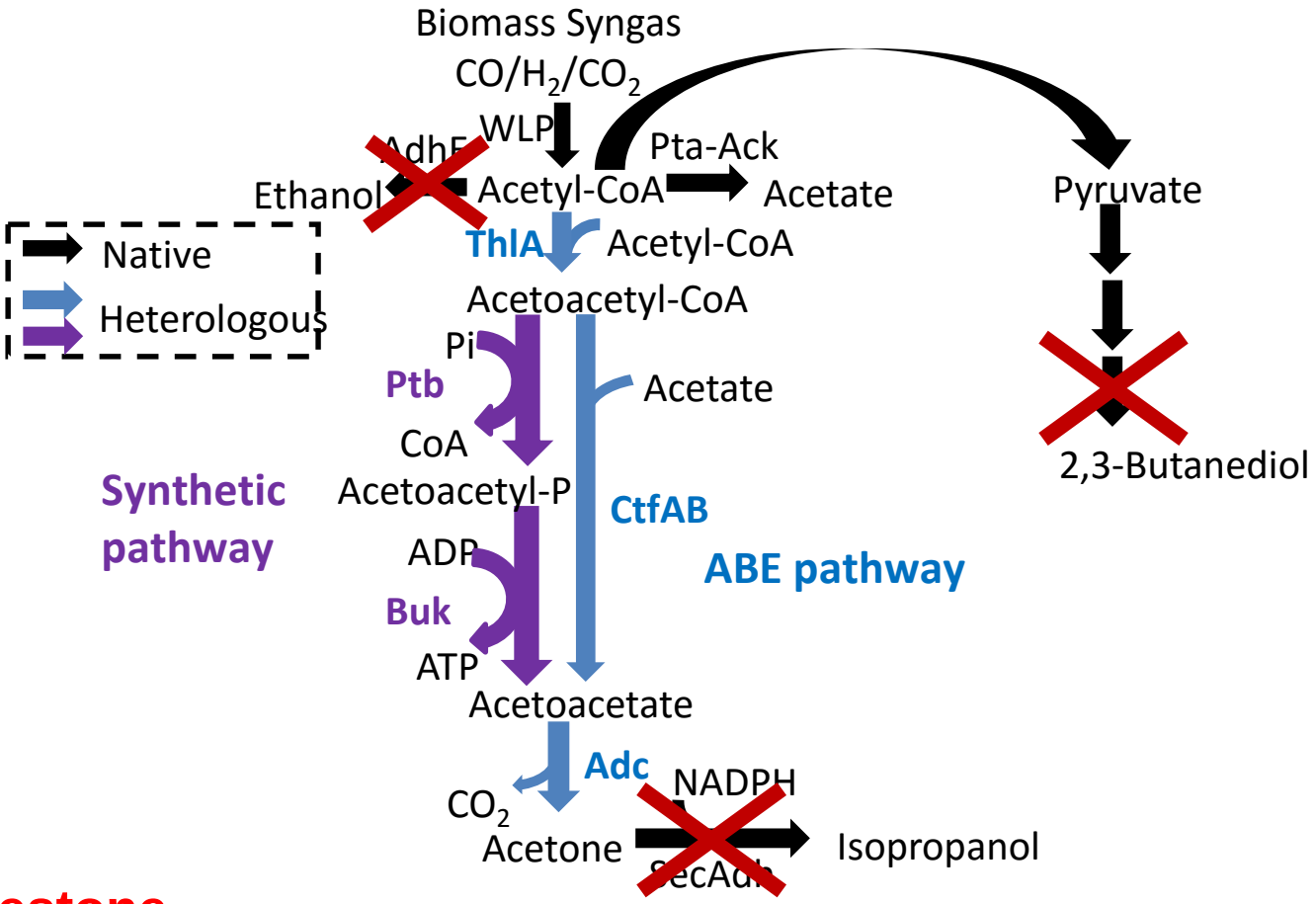
- Genome Editing Tools
  - Gene Knock-Out and /or integration
  - Proprietary homologous recombination tool
  - CRISPR/Cas9 Editing tool



On-going work

Modelling output used to guide genome editing effort to produce an optimized microbial chassis for acetone production

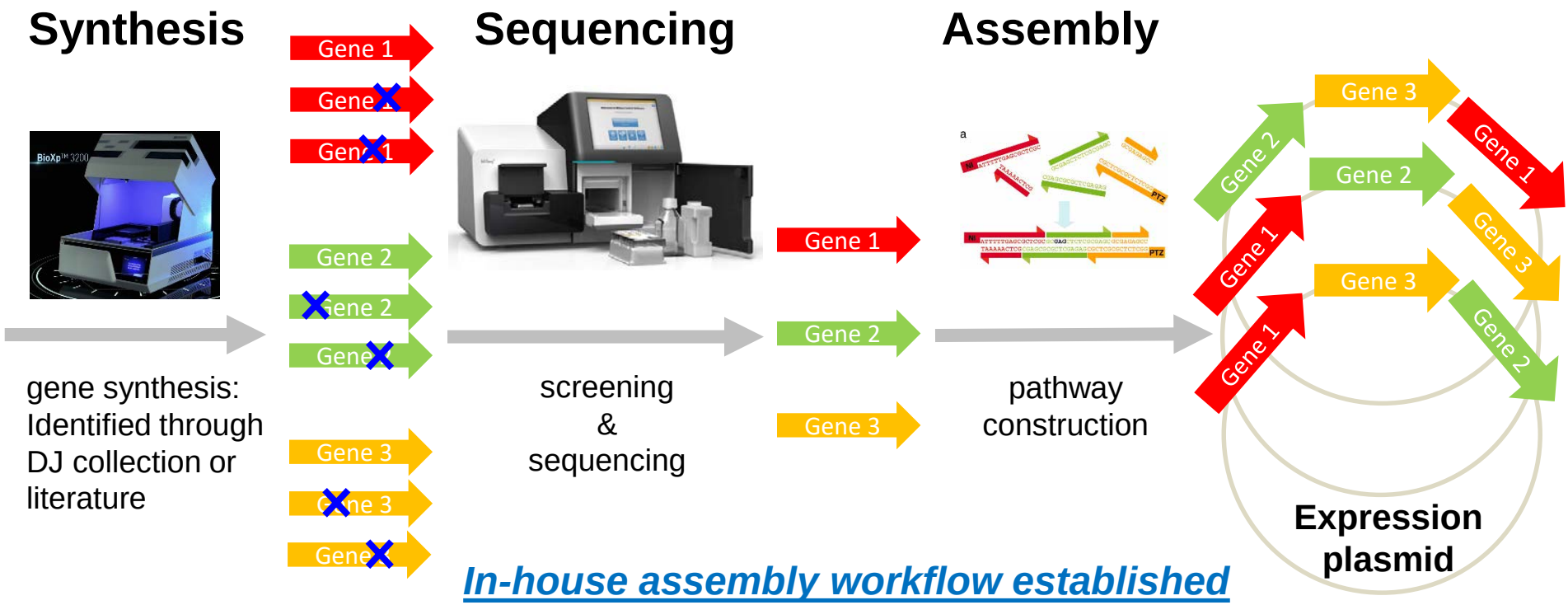
Elimination of Competing pathways



Milestone

A Microbial chassis with three competing pathways deleted by the end of March 2017

Pathway optimization-Combinatorial Analysis



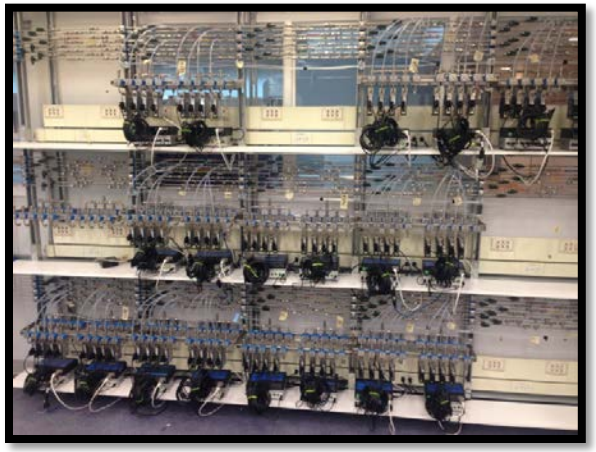
In-house assembly workflow established

Future work

In-house combinatorial gene assembly capability are used to identify optimal synthetic parts combination for novel acetone pathway.

Optimal microbial chassis combined with acetone pathway configuration output.

State-of-the-art gas fermentation facilities



Over 40 dedicated gas fermentation reactors

- **Total gas composition flexibility**
- **Online analytics and control for all reactors (gas, biomass, metabolites)**
- **Multiple reactor configurations**

Future work

Stability, selectivity, and productivity of optimized microbial chassis containing novel acetone production pathway characterized in multiple, parallel gas fermentation operations using start-of-art facilities.

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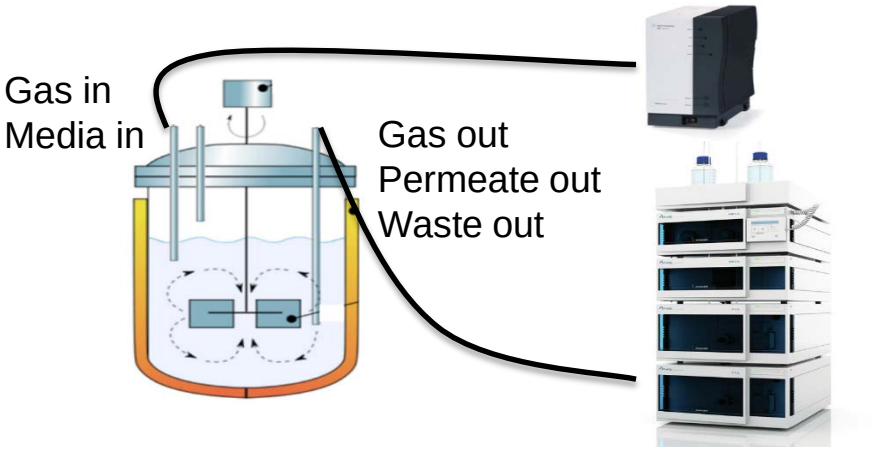
Identification of bottlenecks through omic studies



Triple Quadropole LC/MS/MS

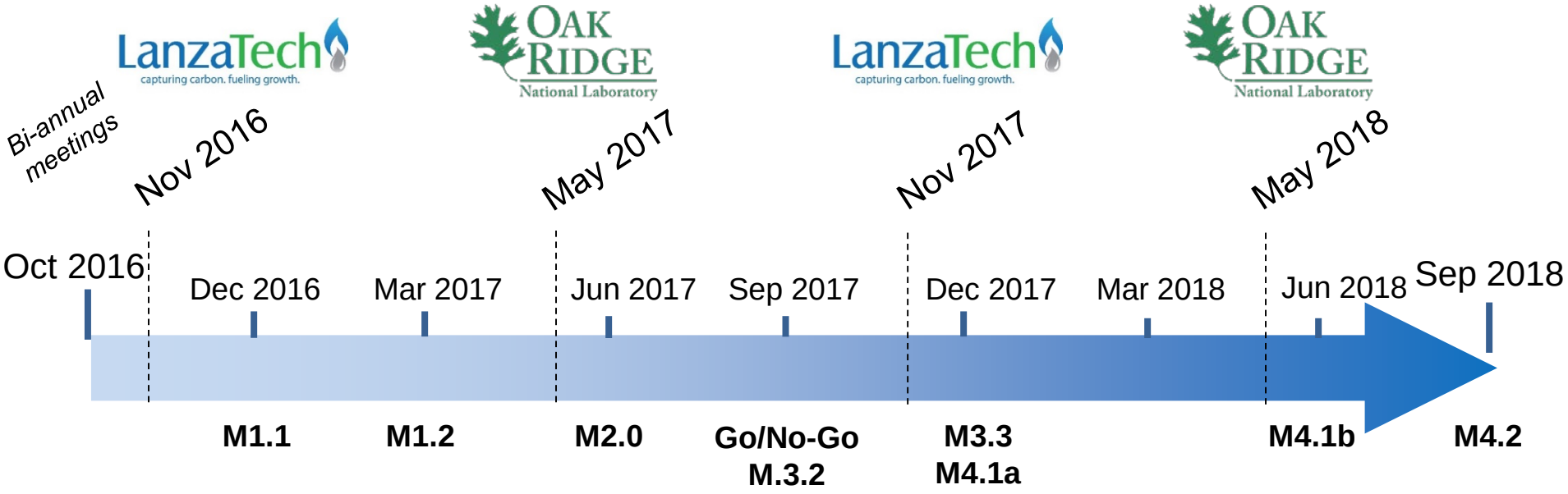
- **4 fermenters in parallel**
  - Biological triplicates and a backup
- **Continuous CSTR fermentation**
- **Measurements**
  - Metabolites
  - Biomass
- **Samples**
  - RNAseq
  - Metabolomics
  - Proteomics

Future work

Detailed multi-omic studies used to precisely identify bottlenecks to carbon flux in syngas to acetone pathway. This will create a prioritized work flow of chassis engineering activity.

3 – Technical Accomplishments / Progress / Results / Milestones

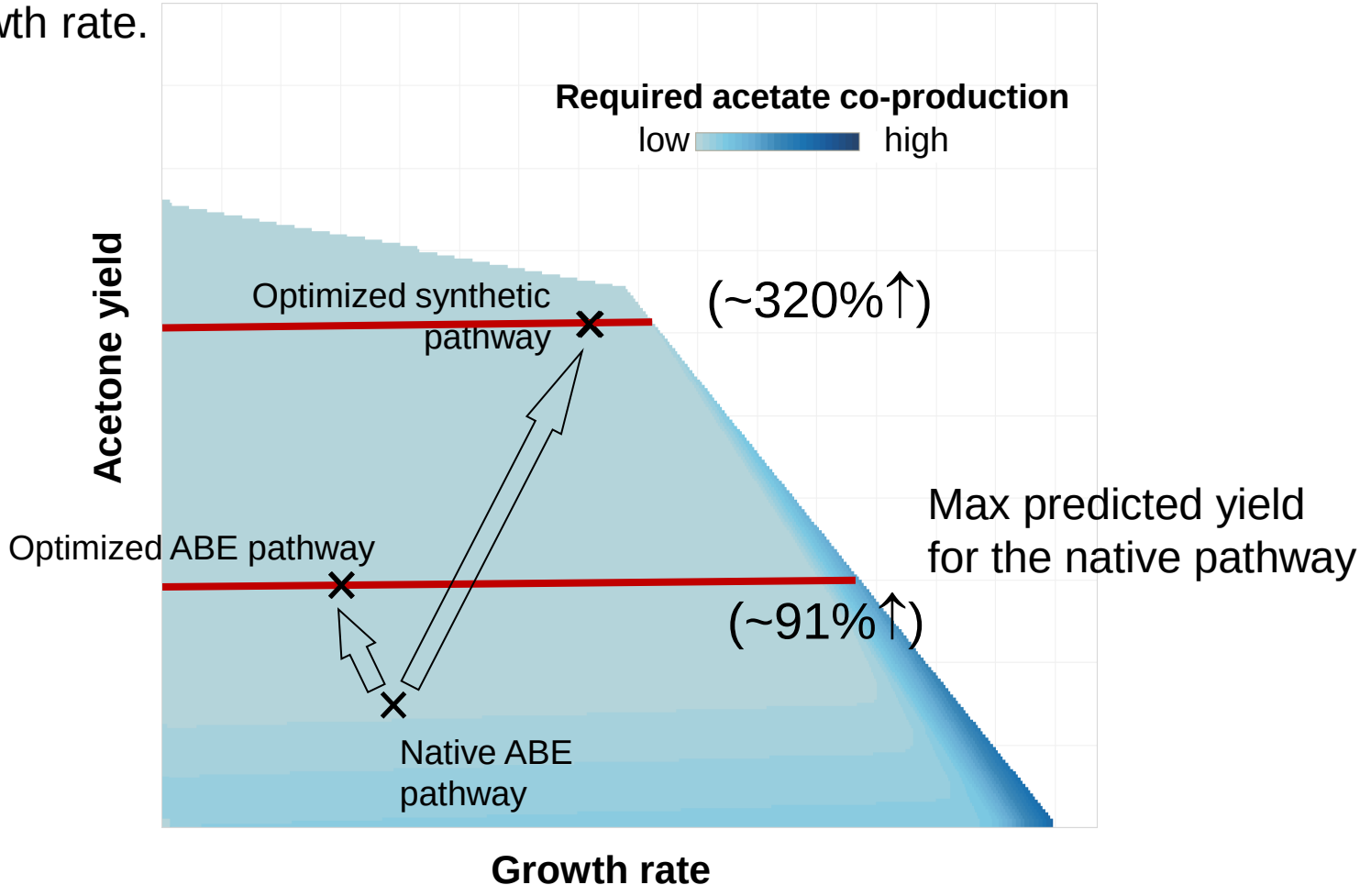
Milestones



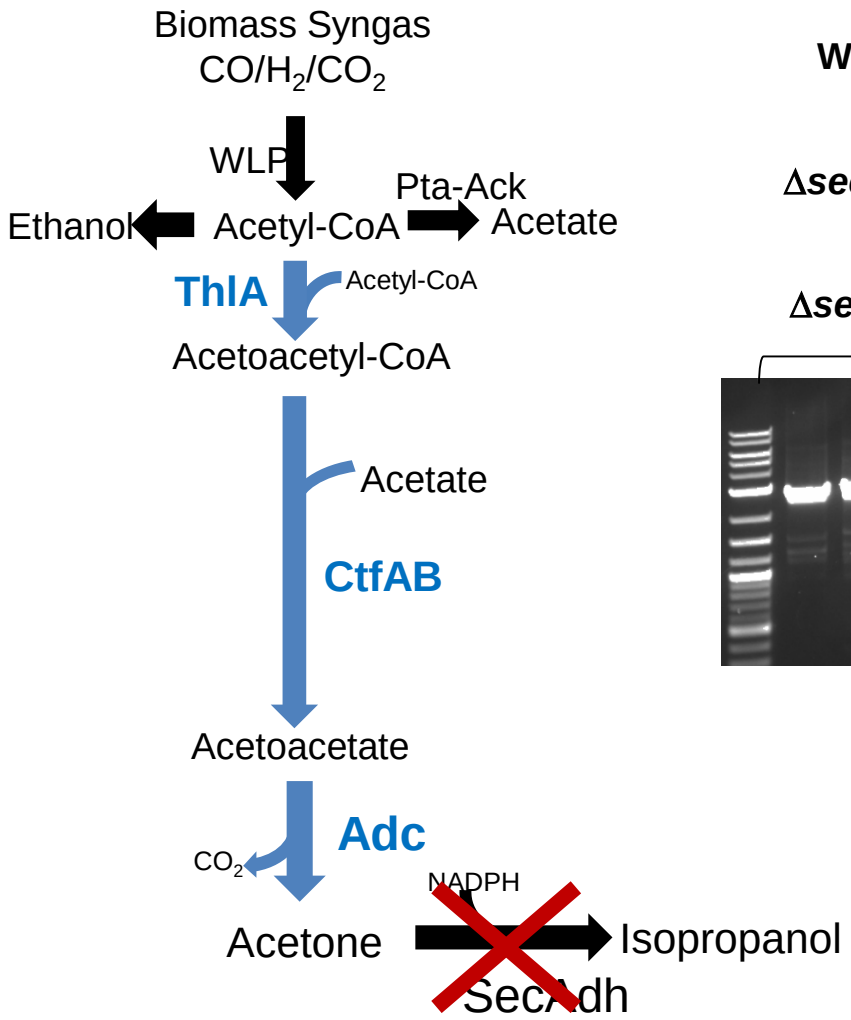
M1.1: Prioritized list of KO targets that are predicted to increase acetone yield provided
M1.2: Chassis strain with at least 3 KOs predicted by GEM constructed
Go/No-Go: Acetone production at 50% commercial rate and 35% commercial titer for 7 days stable production in continuous lab-scale fermentation
M2.0: ABE genes from >300 strains sequenced by JGI identified and sequence aligned
M3.2: Ptb-Buk pair with 50% greater selectivity compared to current acetoacetyl-CoA vs. acetyl-CoA
M3.3: Acetone production data from >50 combinatorial strains using genes identified in the David Jones collection
M4.1a: Omics profiles for the three best performing strains from Task 3
M4.1b: Stable acetone production at commercial viable rate and titer for 7 days in fermentation
M4.2: Stable acetone production at commercial viable rate and titer for 4 days stable production in scalable reactor

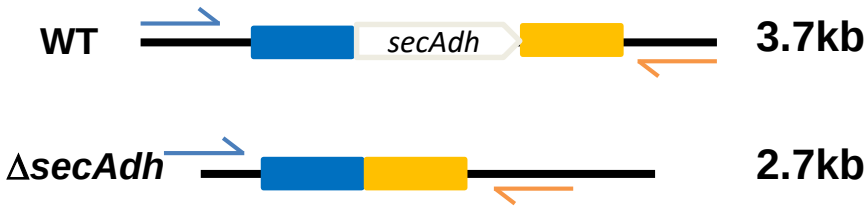
Genome scale model prediction

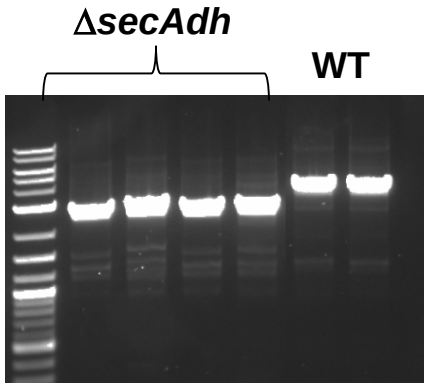
- About 30 million unique strain variants were modeled using an algorithm.
- Modeling confirmed that the synthetic pathway will produce higher acetone yield at a better growth rate.

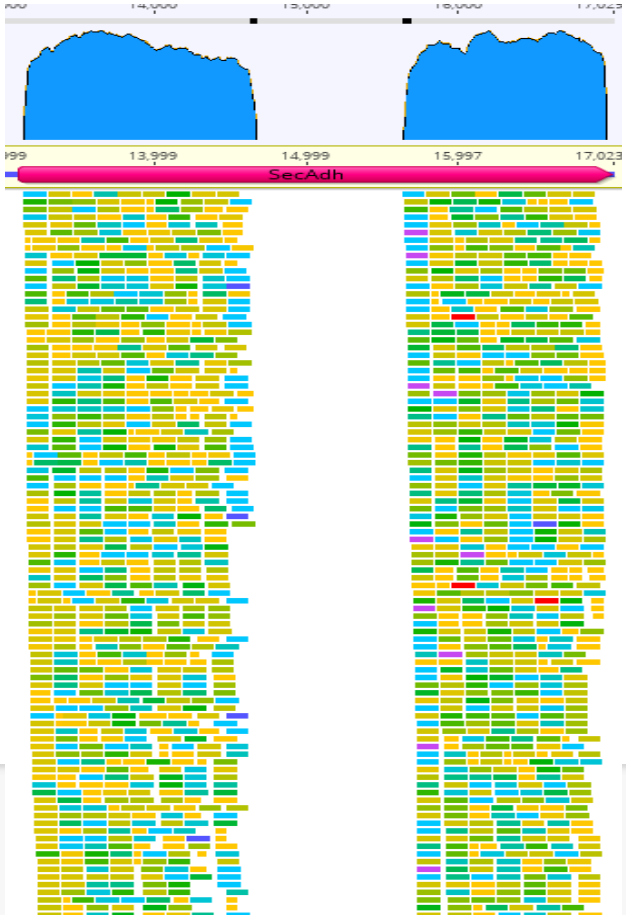


Early Results: Knock-out secondary alcohol dehydrogenase leads to acetone production



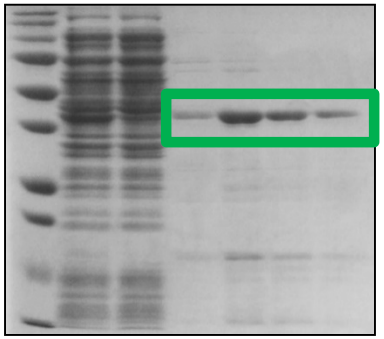




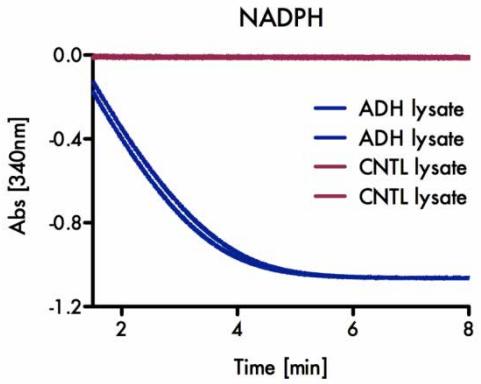


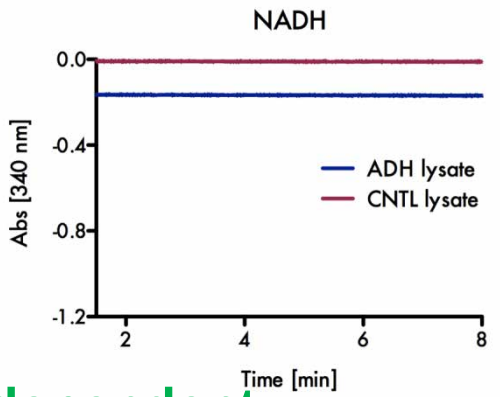
Early Results: Primary:secondary alcohol dehydrogenase identified

- **Identified a novel NADPH dependent primary:secondary alcohol dehydrogenase in *Clostridium autoethanogenum***

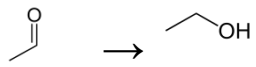
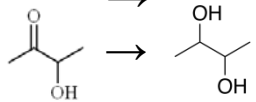


~ 38 kDa





NADPH dependent

	K_{cat} [sec^{-1}]	K_M [mM]	K_{cat}/K_M [sec^{-1} mM^{-1}]
Primary Adh			
Acetaldehyde to Ethanol 	93 ± 6	5.5 ± 1.4	**1.7×10^4**
Secondary Adh			
Acetone to Isopropanol 	51.4 ± 0.8	0.60 ± 0.02	**8.6×10^4**
Acetoin to 2,3-Butanediol 	41.8 ± 0.7	5.7 ± 1.4	**7.4×10^3**

Köpke et al., *Appl Environ Microbiol* 80: 3394-403 (2014)

4 – Relevance

Project Goal:

Develop and scale up a sustainable alternative to current acetone production routes, with an improved safety and environmental footprint, enabling cost-competitive production of acetone as a fuel intermediate or co-product.

Relevance to BETO Mission:

- *The process contributes to the Conversion R&D goal of commercially viable technology to convert biomass to finished liquid transportation fuels via a biological route*
- *Coproduction of acetone and hydrocarbon fuels will enable production of biofuels at or below BETO's target of $3/gge target.with > 50% GHG reductions*

Industry Relevance:

- *The acetone market value is heavily dependent on crude oil prices (~$3B in 2015, ~$6B in 2014). Production of acetone from biomass and residues will reduce price volatility.*
- *This project offers a sustainable, cost-effective alternative to meet the needs of end users in the chemical and consumer products industries seeking to "green" their supply chains.*

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5 – Future Work

- **Highlighted in Slides 18-20:**
  - In-house combinatorial gene assembly capability are used to identify optimal synthetic parts combination for novel acetone pathway.
  - Optimal microbial chassis combined with acetone pathway configuration output.
  - Stability, selectivity, and productivity of optimized microbial chassis containing novel acetone production pathway characterized in multiple, parallel gas fermentation operations using start-of-art facilities.
  - Detailed multi-omic studies used to precisely identify bottlenecks to carbon flux in syngas to acetone pathway. This will create a prioritized work flow of chassis engineering activity.
- **By month 12: Demonstration of fermentation stability for acetone production for 7 days at 50% commercial rate, 35% of commercial titer.**
- **By month 15: Combinatorial library strains constructed with at least 50 variant genes identified in the David Jones collection.**

Summary

- Overview: Project to develop a novel microbial process for the production of acetone directly from biomass syngas at high yields and efficiencies.
- Approach: Introduce a novel, biosynthetic pathway for acetone production into a proven gas fermentation microbial chassis and eliminate side product formation. Use Model and system biology-guided fermentation process optimization. Demonstrate production of acetone at commercially relevant rates and yields from biomass syngas in a scalable system.
- Technical Accomplishments/Progress/Results: Modelling complete and initial gene knock out targets defined. Initial knock out strain successfully generated.
- Relevance: Acetone is a high value chemical with a growing market demand, the developed process would provide a cost competitive route to the production of this product from sustainable resources and will enable low-cost biofuels at or below DOE's $3/gge target.
- Future work:
  - *By month 12: Demonstration of fermentation stability for acetone production for 7 days at 50% commercial rate, 35% of commercial titer.*
  - *By month 15: Combinatorial library strains constructed with at least 50 variant genes identified in the David Jones collection.*

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