Progress towards a new model chemolithoautotrophic host , CID 33729, WBS# 2.5.3.700

Steven Singer, Lawrence Berkeley National Laboratory

Agile BioFoundry

Project Type

- DFO (ABF CRADA)
- Development of novel microbial hosts and bioproducts that will advance all aspects of the biomanufacturing cycle.

Timeline

- 1/1/2019
- 12/31/2021
- 5%

	Total Funding Pre-FY17*	FY 17 Funding	FY 18 Funding	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$0	$0	$0	$900,000 LBNL-39% NREL-34% ORNL-27%
Project Cost Share (if applicable)	$0	$0	$0	Kiverdi $385,700

Management Approach

Agile BioFoundry

BERKELEY LAB, Lawrence Berkeley National Laboratory

- Project management
- 1-dodecanol production

OAK RIDGE National Laboratory

Improving transformation efficiency

NREL, National Renewable Energy Laboratory

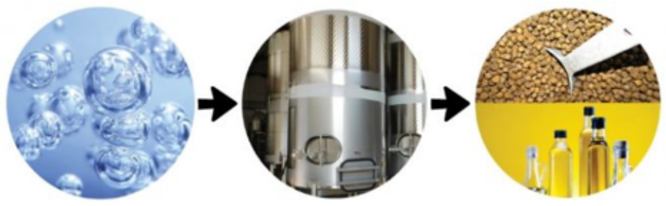
Developing gene-editing in *C. necator*

Kiverdi

- 1-dodecanol production
- Bioreactor production

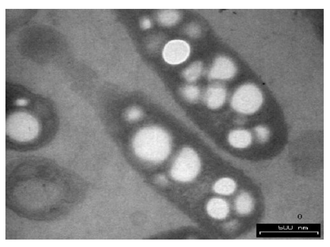
Technical Approach

Kiverdi

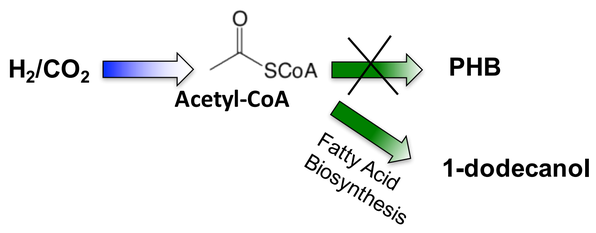


Kiverdi has partnered with ABF to develop approaches to convert CO_2 to bioproducts

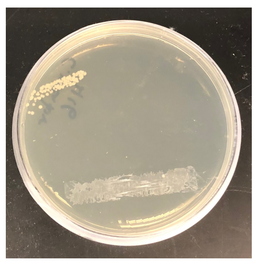
Cupriavidus necator



C. necator grows vigorously on H_2/CO_2 and is genetically tractable

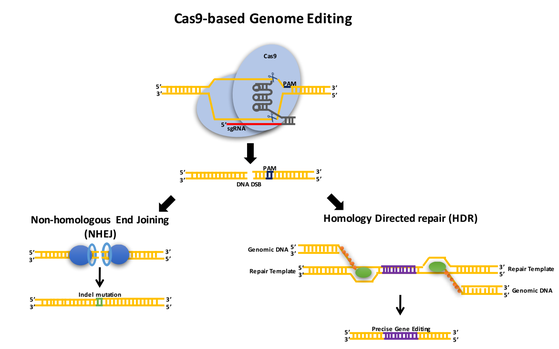


In this project, tools will be developed to optimize production of fatty alcohols from H_2/CO_2 by *C. necator*

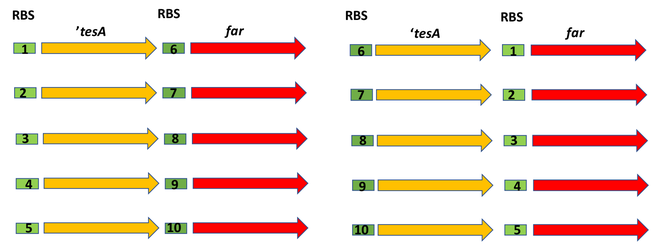


Altering *C. necator* restriction modification system

C. necator transformation efficiency and electroporation methods will be improved



Cas9-based gene editing will be established in *C. necator* to accelerate genetic modifications



Ribosomal binding sites (RBS) will be tested for expression of genes for 1-dodecanol production (thioesterase and fatty acyl-CoA reductase)

Technical Progress and Future Plans

- **Progress**
  - CRADA was executed in FY 2019 Q1; project began Q2
  - RBS selection commenced
  - Experiments to improve *C. necator* transformation initiated
- **Key FY2019 Milestones**
  - Identify 10 RBSs for testing (Q2) (LBNL/Kiverdi)
  - Increase *C. necator* transformation efficiency 10-fold (Q3) (ORNL)
  - Identify one functional Cas9-based gene editing system (Q4) (NREL)
  - Final FY2020 milestone: Optimize *C. necator* fermentation with H_2/CO_2 (Q8) (Kiverdi)

Relevance and Impact

- **Goal**
  - Develop host for scalable biosynthesis of bioproducts from H_2/CO_2
- **Relevance**
  - CO_2 is an emerging feedstock to support a bioeconomy that is centered around carbon as a resource
  - 1-dodecanol is a drop-in replacement for detergent precursors derived from petroleum
- **Impact**
  - This project will develop a microbial platform to convert CO_2 to bioproducts
  - *C. necator* host development will broaden the hosts explored by ABF