DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review

Developing Advanced Genetic and Synthetic Biology Tools for Improved Algae Productivity

March 22, 2021
Technology Area Session

Stephen Mayfield
University of California, San Diego

Project Overview

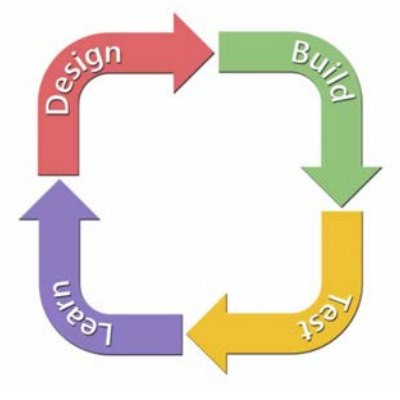
- We will develop advanced genetic tools, high-throughput screening methods, and *breeding strategies* for industrial strains of green algae and cyanobacteria
- We will develop these tools by engineering recombinant proteins and polymer precursors as high value co-products in cyanobacteria and green algae, and then transfer these tools into new industrial strains
- We will grow industrially relevant strains under field conditions to demonstrate the utility of the developed tools – PEAK Challenge
- We will perform LCA and TEA of demonstrated products at green house scale
- Key Industrial Partners - Algenesis (polymers), Triton Health & Nutrition (proteins), Global Algae Innovations (GAI - engineered systems)

1 – Management

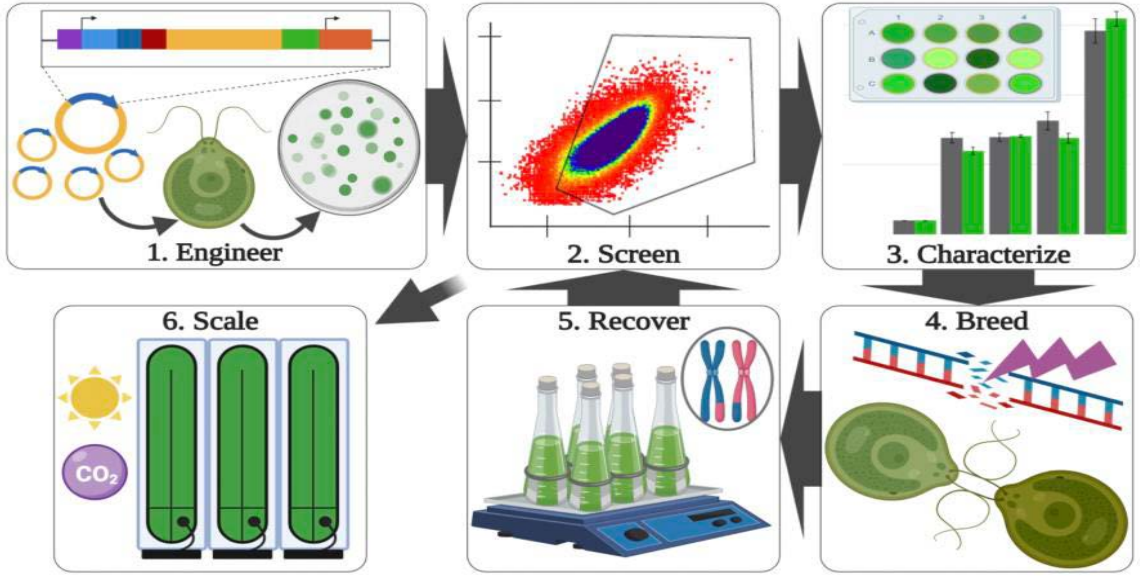
- Stephen Mayfield – (PI) UCSD
  - Develop Molecular Genetic, high throughput screening and breeding tools for Green Algae
- Michael Burkart - UCSD
  - Extraction and conversion of lipids to biofuels and polymer precursor co-products
- Ryan Simkovsky - UCSD
  - Develop Molecular Genetic tools and engineer protein and polymer precursor production in cyanobacteria
- Frank Fields - UCSD
  - Algae Biomass Production at Green House Scale – PEAK Challenge
- Alissa Kendall - UCD
  - Life Cycle Assessment and Techno Economic Modeling of biofuel and co-products
- Peter Morgan - Algenesis
  - Production of commercial polyurethane products using UCSD generate monomers
- Dave Hazlebeck - GAI
  - Integration of engineered production and harvesting system at pilot scale

2 – Approach

Strain Development Process



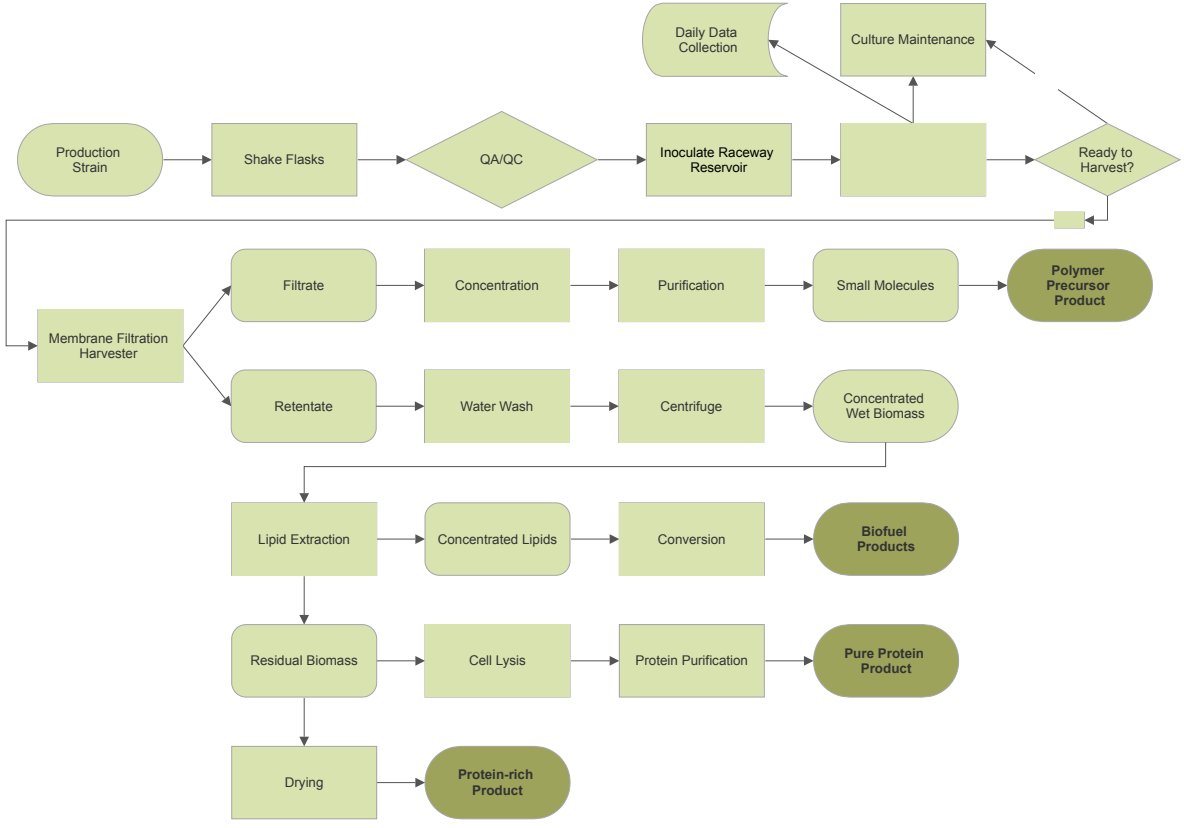
Synthetic Biology Paradigm



PEAK – Strain Development Process

2 – Approach

Production process and outcomes



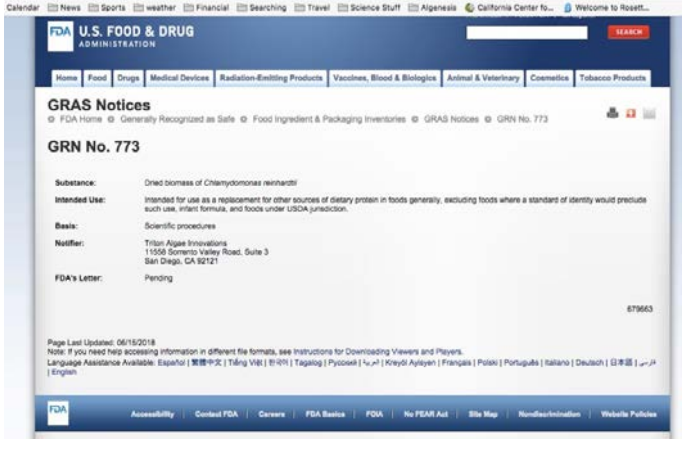
3 – Impact

Protein

Triton – OPN – PEAK Challenge

Covid Spike Protein Antigen

Hochland SE – Milk Proteins



Polyurethanes

Algenesis – Polyols – Peak Challenge

Reef – flip flops

BASF – isocyanates

Pepsi – food packaging

Callaway – foam padding …

4 – Progress and Outcomes

Project Goals:
1) The primary goals of this project are to develop the advanced genetic tools, breeding, and high-throughput technologies that will advance the entire algae biotechnology field, and enable economic viability of algal biofuels ... through production of high value co-products.

2) Apply these tools to commercial production strains of algae and cyanobacteria

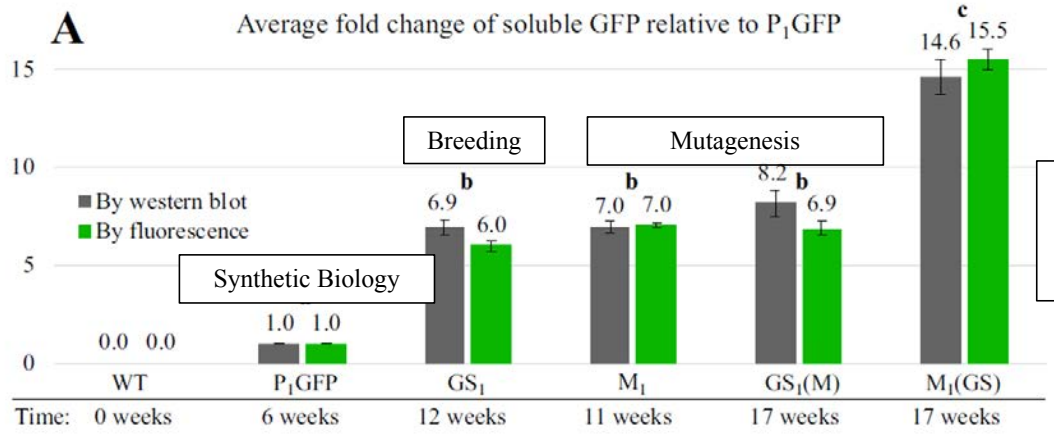
3) To advance the technology from their present baseline state (TRL3 - proof of concept), to demonstration at the pilot scale with our commercial partners (TRL6 - prototype demonstration in a relevant environment)

4 - Progress and Outcomes

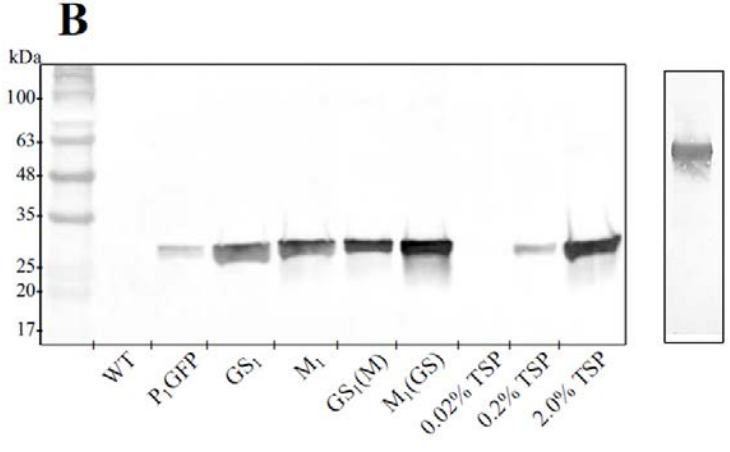
Objective	Description	Deliverable
Objective 1	Develop advanced genetic tools for improved nuclear transgene expression and advanced metabolic engineering to enable economic production of biofuels from algae.	A process for designing synthetic biology tools for industrial species of green algae. PEAK Process
Objective 2	Improve expression and secretion of recombinant proteins as co products to improve the economics of biofuel production.	Validated sequences and vector design methods to increase recombinant protein expression from industrial strains.
Objective 3	Establish a rapid high throughput screening method for strains with improved protein production and secretion abilities.	Rigorous methods for rapidly isolating production strains from genetically diverse pools generated by mating or mutagenesis.
Objective 4	Develop genetic tools and genome editing strategies to enable metabolic engineering of commercially viable species of cyanobacteria.	A process for developing synthetic biology tools for novel cyanobacteria with industrial production potential that currently lack genetic tools
Objective 5	Develop a suite of highly controlled gene expression tools for metabolic engineering of cyanobacteria to produce *diacids and diols as polyurethane precursors*.	Methods for engineering high value co-products from cyanobacteria. Demonstrate by producing and extracting diacids and diols for polyurethane production
Objective 6	Develop improved targeted genome editing and transgene delivery methods for green algae to accelerate strain engineering.	Improved methods for delivering synthetic biology tools to green algae genomes.
Objective 7	Develop non transgenic improvement strategies using *breeding, high throughput screening*, and mutagenesis to develop new production strains.	A process for production strain improvement using tradition breeding strategy.
Objective 8	Develop genetic tools for commercial strains, based on learning from previous objectives, to demonstrate rapid adaptation of genetic tools to new strains. *Covid-19 Spike protein*	Generation of new genetic tools for increasing the economic viability of biofuel production for novel strains through generation of high value co products.
Objective 9	Grow unmodified and genetically modified strains under field conditions to determine impact of genetic modifications on biomass productivity and product yield.	Key data on biomass productivity and the viability of using co products to improve the economic viability of biofuel production.
Objective 10	Conduct a life cycle assessment and techno economic assessment (TEA) on GM and non-GM strain under outdoor cultivation conditions. Task 11	TEA/LCA and Socio-economic reports comparing unmodified and genetically modified strains under field conditions

4 - Progress and Outcomes

Can we combine synthetic biology, breeding, and in vitro evolution into a single process



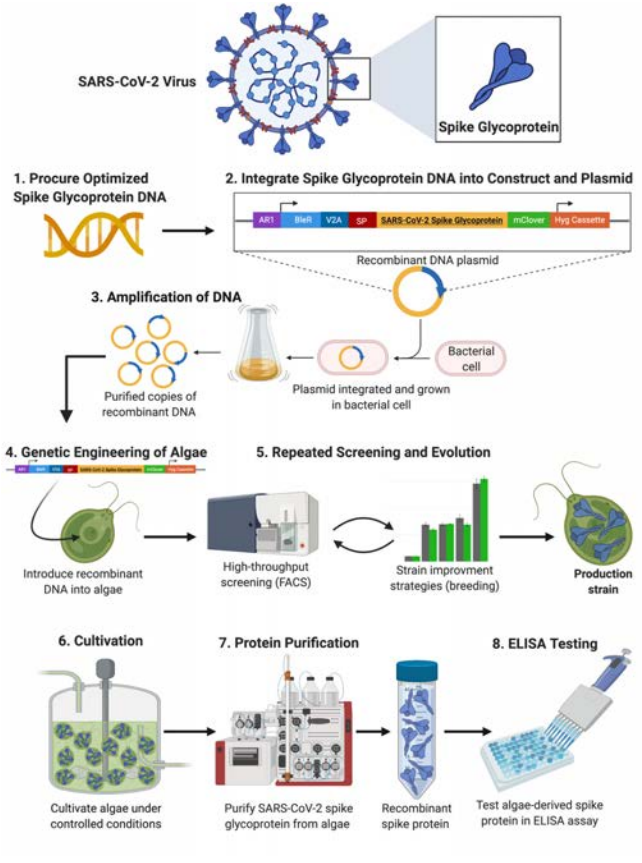
- Synthetic Biology
- Breeding
- Mutagenesis

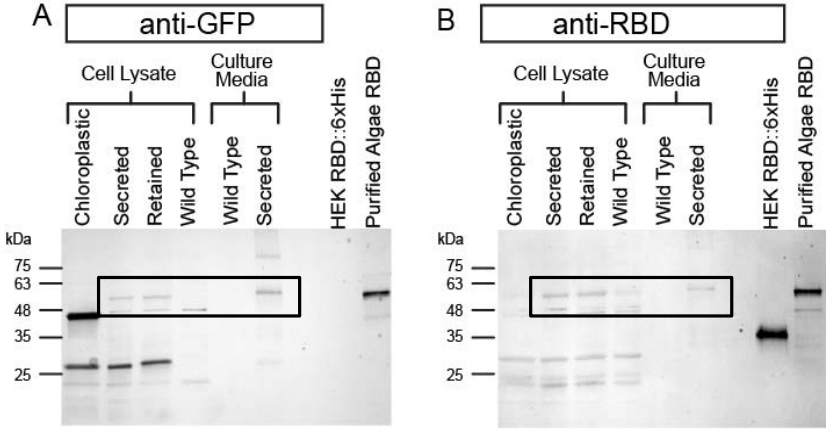


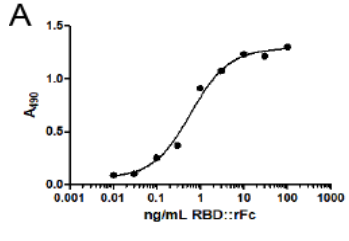
OPN final strain
(15x improvement from
primary transformant)

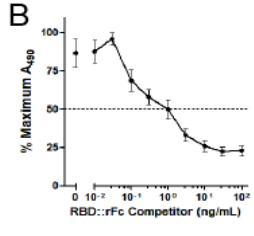
4 - Progress and Outcomes

Can we translate these tools to develop new strain - rapidly







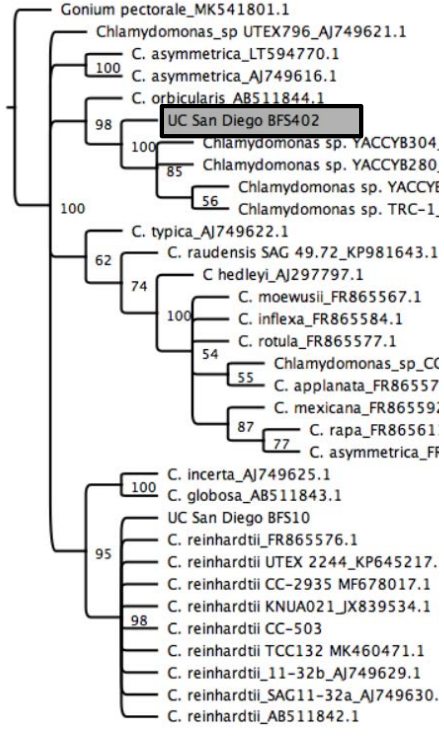


Binding to human ACE2 protein

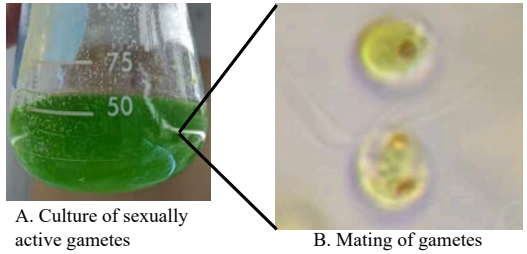
4 - Progress and Outcomes

New commercial candidate strain

1. Within *Chlamydomonas* genus

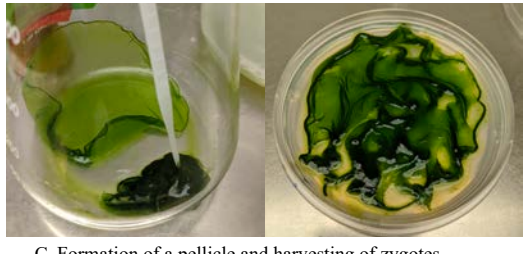


2. Capable of mating/breeding



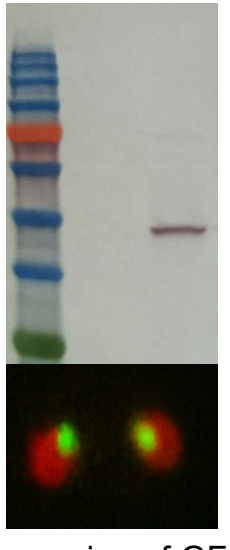
A. Culture of sexually active gametes

B. Mating of gametes



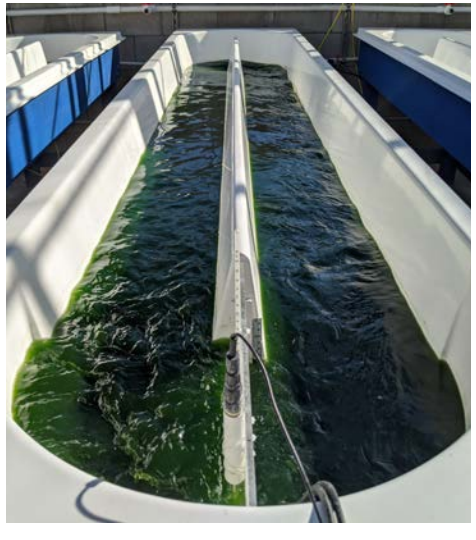
C. Formation of a pellicle and harvesting of zygotes

3. Transformable with Recombinant protein accumulation



Expression of GFP

4. More productive than *C. reinhardtii* in alkaline ponds

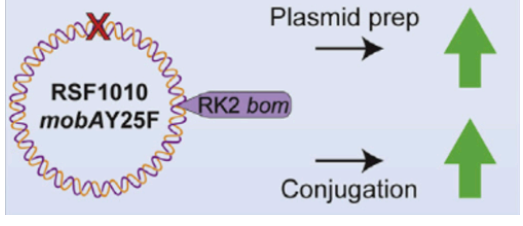


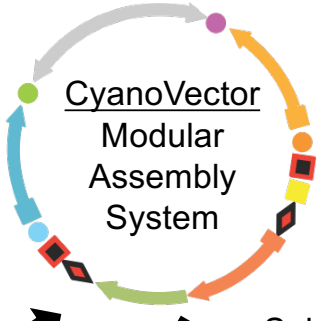
Cultured outdoors in both San Diego and Hawaii, ranging from 10-15 g/m2/d
High Salt / pH 10.5

4 - Progress and Outcomes

Development of modular synthetic biology tools for commercial cyanobacteria

Broad Host Range Vector



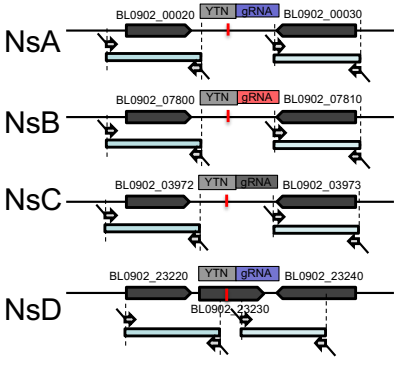


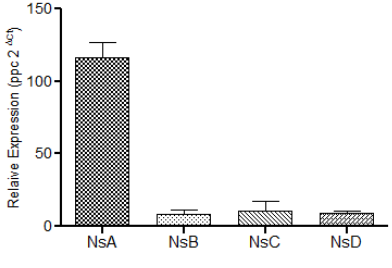
Rapidly-generated cloning and expression vectors for diverse cyanobacteria strains

- RBD
- sfGFP-tagged proteins
- Pimelic acid pathway
- Succinic acid pathway
- etc.

\+ Suicide Gene-Based Cloning Device

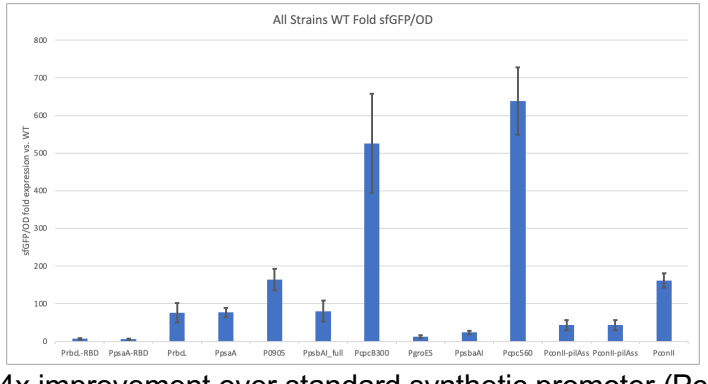
Novel *Leptolyngbya* Neutral Sites





Natural NS expression, lower is better

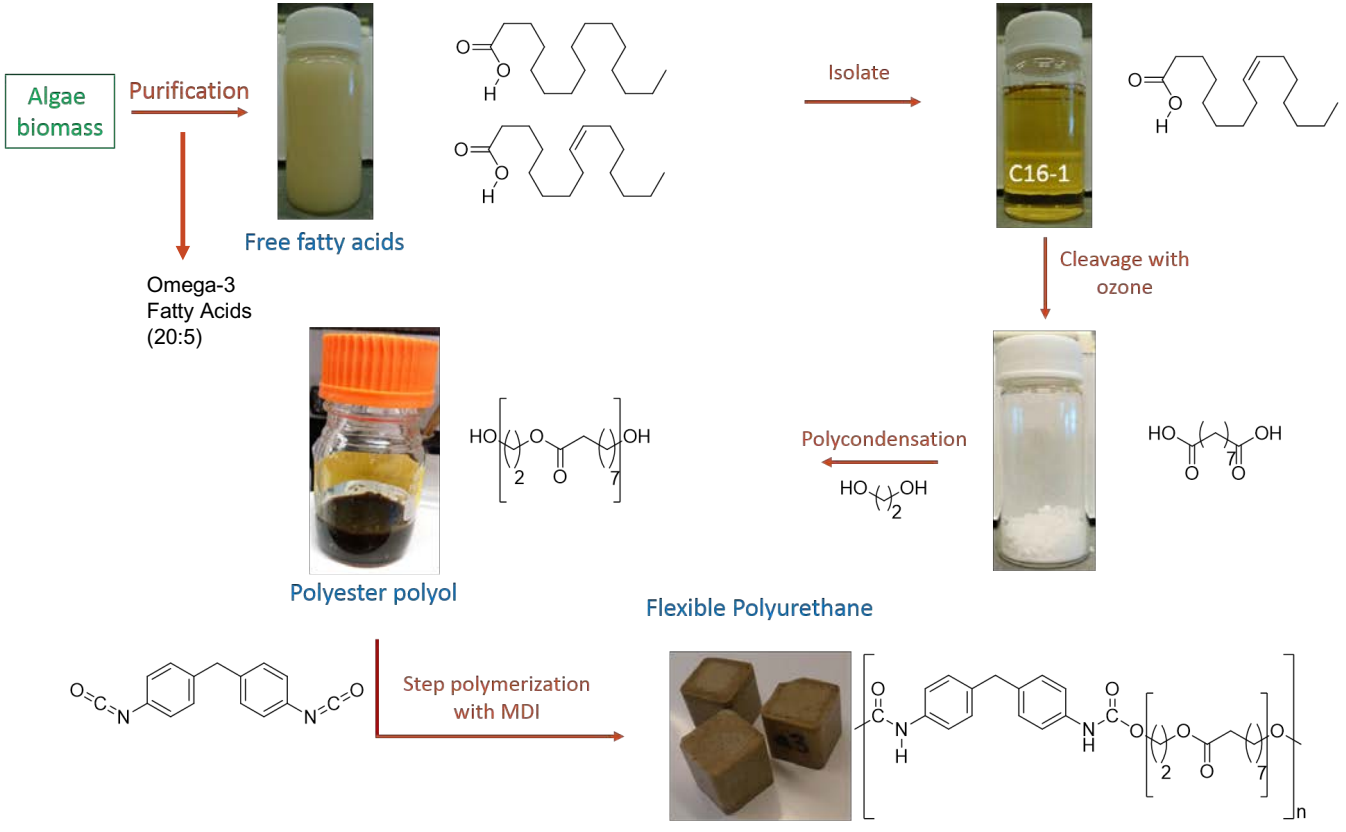
Novel Promoters & RBSs



>4x improvement over standard synthetic promoter (PconII)

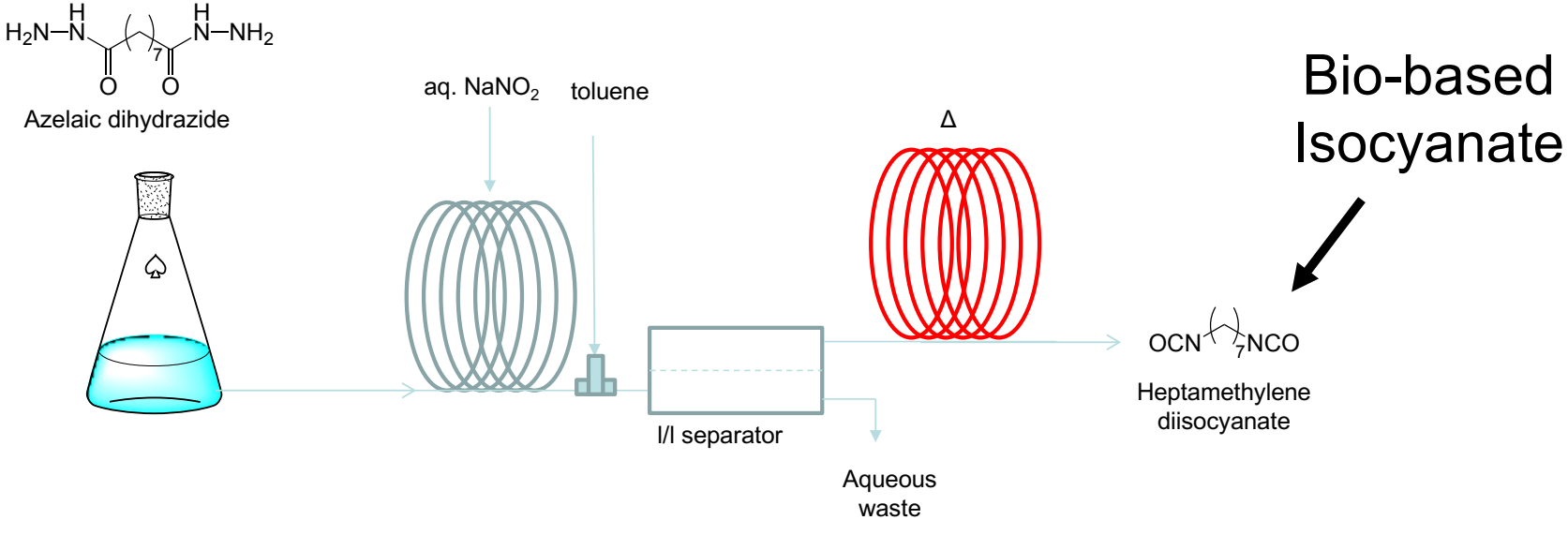
4 - Progress and Outcomes

Developing high value polymer co-products



4 - Progress and Outcomes

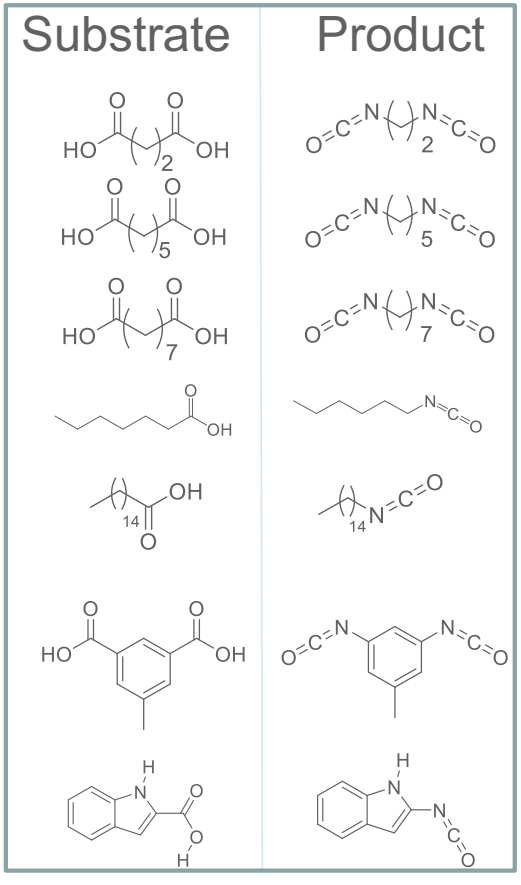
Renewable Isocyanates using Flow Chemistry

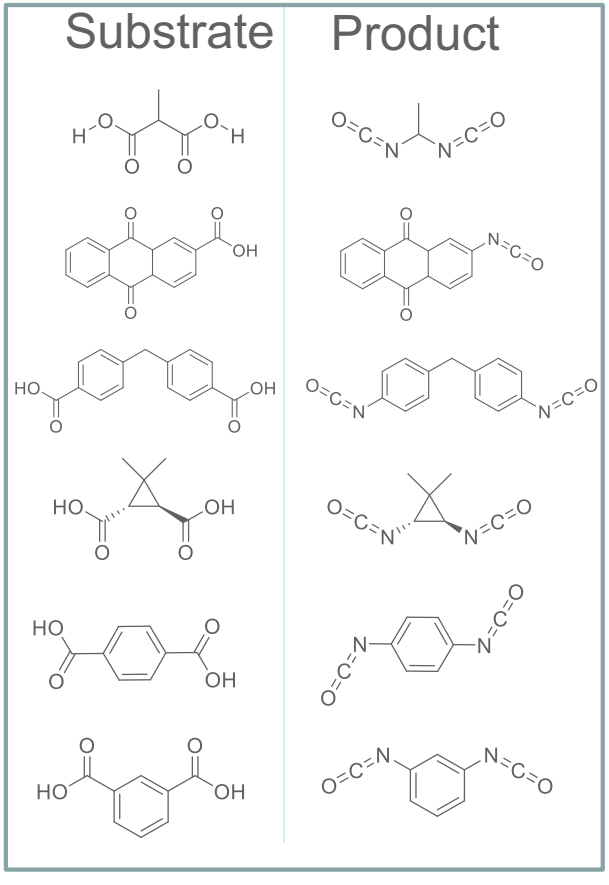


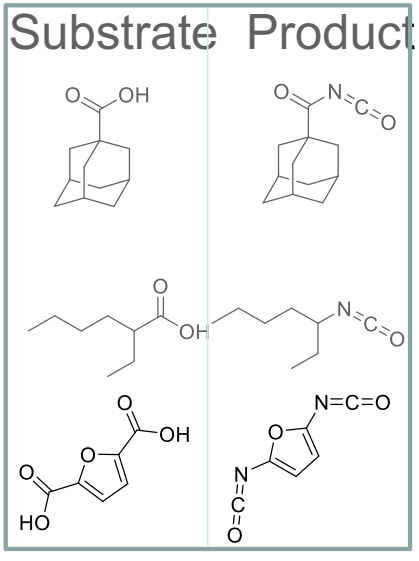
Phung, et al. *Organic Process Research & Development,* **2020**, 24(10):2342-6..

4 - Progress and Outcomes

Bio-based Isocyanate Scope







Bio-sourced

⇩

Carboxylic acids

⇩

Bio-Isocyanates

4 - Progress and Outcomes

Can we take this to a pilot commercial setting – PEAK challenge

Original wildtype:

- Nitrate –
- Salt tolerance –
- pH tolerance –
- OPN expression –
- Growth in ponds -

Improved wildtype:

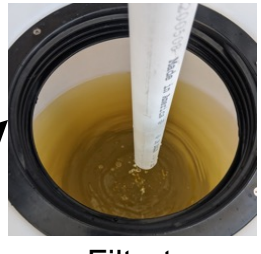
- Nitrate +
- Salt tolerance +
- pH tolerance +
- OPN expression -
- Growth in ponds +

Improved recombinant:

- Nitrate +
- Salt tolerance +
- pH tolerance +
- OPN expression +
- Growth in ponds +

Cultivation in alkaline media in GAI open ponds

Harvest via GAI membrane filtration



Filtrate

Retentate

Summary

- Our objectives were to develop a suite of synthetic biology, breeding, and HT screening tools for green algae and cyanobacteria, and to develop these as a "process" that could be transferred to any commercial strain – **We have accomplished a significant portion of that goal**

- We demonstrate this by:
  1) Rapidly producing the SARS covid spike protein in algae
  2) Obtaining high levels of protein expression in commercially relevant algae and cyanobacteria
  3) Used our developed process to achieve sustained growth in open pond setting
  4) Producing novel high value bio-based isocyanates!

- With Algenesis we have turned the polyols into commercial products – Footbeds and Outsole of Flip Flops and Shoes, that will be commercially available in 2021

Quad Chart Overview

Timeline

- Project start date 9/30/17
- Project end date 6/30/21

	FY20 Costed	Total Award
DOE Funding	(10/01/2019 – 9/30/2020) $1,001,336	(negotiated total federal share) $3,000,000
Project Cost Share	$65,040	$422,257

Project Goal

- The primary goals are to develop advanced genetic tools, breeding, and HT screening that will advance the algae industry, and enable economic viability of algal biofuels through production of high value co-products

End of Project Milestone

- Complete comparative environmental and economic impact of 1) A strain producing a high value protein product and 2) A strain producing a high value polymer product, under pilot scale production in out door ponds

Project Partners*

- Partner 1 UC Davis
- Partner 2 Global Algae Innovations

Funding Mechanism

DE-FOA-0001628
Topic Area 1 – Strain Improvement

*Only fill out if applicable.

Additional Slides

Responses to Previous Reviewers' Comments

Comments: No in depth discussion of the FOA technical target to achieve biomass productivities exceeding 18g/m-2/day or 80 GGE per ton biomass potential. The proposed development of co-product (secreted proteins) and specialty branched chain wax esters may facilitate achieving these targets and a final TEA and LCA analysis following biomass composition evaluation is proposed. However, an upfront assessment of the feasibility of hitting these key targets, through this workplan, would strengthen the technical merit of the proposal

Response: The proposed plan was to build the synthetic biology, breeding, and HT screening tools, first in lab strains, and then transfer those tools to potential commercial strains in this project. The biomass productivity of the commercial strains was the true baseline to address.

- We have now started that process and have good baseline biomass data for both a green algae and cyanobacteria commercial strain.
- We have deployed several of our developed tools in these commercial strains, and will analyze the entire system during the PEAK challenge

Publications, Patents, Presentations, Awards, and Commercialization

- Engineering Polyketide Synthase Machinery in Cyanobacteria
  Patent Application Number: 62643370
- Roulet, J., Taton, A., Golden, J. W., Arabolaza, A., Burkart, M. D., & Gramajo, H. (2018). Development of a cyanobacterial heterologous polyketide production platform. *Metabolic engineering*, 49, 94-104.
- Mayfield, Stephen. (2019, January). "Use of Breeding, Mutagenesis and High Throughput Screening for Enhanced Recombinant Protein Production in Algae." Presented at The 2019 Gordon Research Conference on Chloroplast Biotechnology, Ventura, CA.
- Fields, Francis, Ostrand, Joseph, Tran, Miller, & Mayfield, Stephen. (2019). Nuclear genome shuffling significantly increases production of chloroplast-based recombinant protein in *Chlamydomonas reinhardtii*. *Algal Research*. 41.10.1016/j.algal.2019.101523.
- Bishé, B, Taton, A, Golden, JW. (2019). Modification of RSF1010-Based Broad-Host-Range Plasmids for Improved Conjugation and Cyanobacterial Bioprospecting. *iScience* 20, 216-228.
- Fields, F.J., Lejzerowicz, F., Schroeder, D., Ngoi, S.M., Tran, M., McDonald, D., Jiang, L., Chang, J.T., Knight, R. and Mayfield, S., (2019). Effects of the microalgae *Chlamydomonas* on gastrointestinal health. *Journal of Functional Foods*, p.103738.
- Hai, T.A.P., Neelakantan, N., Tessman, M., Sherman, S.D., Griffin, G., Pomeroy, R., Mayfield, S.P. and Burkart, M.D., (2020). Flexible polyurethanes, renewable fuels, and flavorings from a microalgae oil waste stream. *Green Chemistry*, 22(10), pp.3088-3094.
- Phung Hai, T. A., De Backer, L. J., Cosford, N. D., & Burkart, M. D. (2020). Preparation of Mono-and Diisocyanates in Flow from Renewable Carboxylic Acids. *Organic Process Research & Development*, 24(10), 2342-2346.
- Taton, A., Ecker, A., Diaz, B., Moss, N.A., Anderson, B., Reher, R., Leão, T.F., Simkovsky, R., Dorrestein, P.C., Gerwick, L., Gerwick, W.H., & Golden, J.W. (2020). Heterologous expression of cryptomaldamide in a cyanobacterial host. *ACS Synthetic Biology*, 9(12), 3364-3376.
- Sproles, A. E., Fields, F. J., Smalley, T. N., Le, C. H., Badary, A., & Mayfield, S. P. (2020). Recent advancements in the genetic engineering of microalgae. *Algal Research*, 53, 102158.
- A. Berndt, T. Smalley, B. Ren, A. Badary, A. Sproles, F. Fields, Y. Torres-Tiji, V. Heredia, S. Mayfield. (2021). Recombinant production of a functional SARS-CoV-2 spike receptor binding domain in the green algae *Chlamydomonas reinhardtii.* bioRxiv 2021.01.29.428890; doi: https://doi.org/10.1101/2021.01.29.428890

Publications, Patents, Presentations, Awards, and Commercialization

- Torres, Y., Fields, F., Mayfield, S. (2020) Microalgae as a future food source. *Biotechnology Advances*, 41, 107536. DOI: 10.1016/j.biotechadv.2020.107536
- Fields, F. (2019) *Enhancing algal phenotypes through genome shuffling*. The 9th International Conference on Algal Biomass, Biofuels, and Bioproducts. Oral presentation, June 17th, Boulder, Colorado, USA

- Algae Biomass Summit 2020 Presentations
  - Frank Fields, “Using Genome Shuffling to Enhance Product Productivity” September 22, 2020.
  - Ryan Simkovsky, “Engineering Cyanobacteria for Production and Secretion of Diacids and Diols” September 22, 2020.
  - Frank Fields, “Effects of the Microalgae *Chlamydomonas* on Gastrointestinal Health” September 23, 2020.
  - Anthony Berndt, “Production of a Recombinant Proteins in Different Subcellular Compartments in Green Algae can Alter Post-translational Modifications and Hence Protein Biological Activity” September 30, 2020.
  - Ashley Sproles, “Improved High-throughput Screening Technique to Rapidly Isolate *Chlamydomonas* Transformants with Recombinant Protein Expression” September 30, 2020.