BETO 2021 Peer Review
Algae Biotechnology Partnership
WBS 1.3.1.130

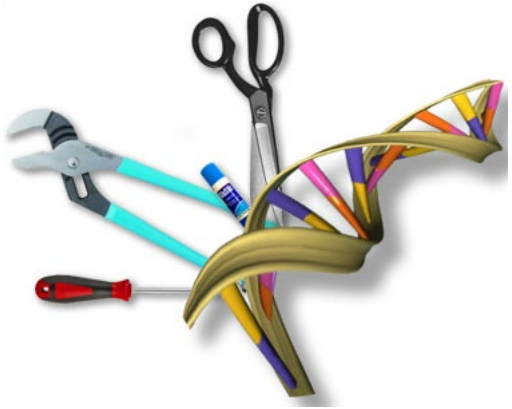
Date: March 10, 2021
Technology Area Session: Advanced Algae Systems
Principal Investigator: Mike Guarnieri
Organization: NREL

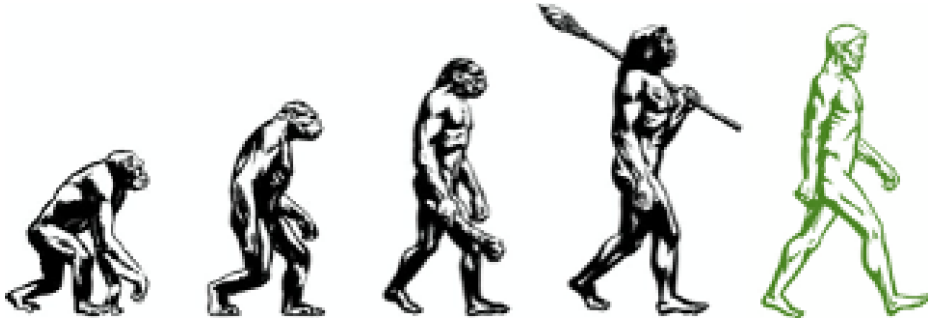
Project Overview

Big Picture: Strain engineering offers a means to target key algal biofuels cost and sustainability drivers.

- Integral to achieving BETO biomass productivity, composition, and cost targets
- Few successes in non-model algae to-date.
  - Universal tool development is hindered by strain-specific regulatory mechanisms.

Goal: Develop modular genetic editing tools, synthetic and orthogonal genetic regulatory systems, and functional genomic pipelines to enable broad host-range metabolic engineering strategies in top-candidate deployment algal strains.





1. Management

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

BIOENERGY TECHNOLOGIES OFFICE

PM, TM: Dan Fishman, Philip Lee

DISCOVR

Outdoor Deployment

PI: Michael Huesemann

NREL Transforming ENERGY

PI: Mike Guarnieri

Platform Lead: Lieve Laurens

Collaborators and Industry Engagement

EPA | UC San Diego | GWT Gross-Wen Technologies | ALGIX | Auburn | Texas A&M | Cincinnati | Synthetic Genomics

NREL Transforming ENERGY | Colorado School of Mines

Genetic Tool Development

Mike Guarnieri, Matt Posewitz

Los Alamos National Laboratory Est. 1943 | Berkeley Lab

Function Genomics

Shawn Starkenburg, Igor Grigoriev

1. Management

- **Multi-disciplinary Staffing Plan:**
  - –Molecular & Microbiologists: strain characterization and genetics
  - –Computational Biologists: multi-omics and systems biology
- **Synergistic interaction**
  - Algae Genetic Blueprint AOP, DISCOVR Consortium
  - Academic, government, and industrial partners

- **Technical Meetings:** Team (weekly), Inter-team (bi-monthly), Platform (monthly)
- **Risk I.D. and Mitigation:**
  - Annual SMART and Go/No-Go decision points define global project direction
  - Leads are empowered to make minor changes to the research plan (no milestone impact)
  - Decisions resulting in major changes require approval of the PI and Platform Lead
  - Team review is deployed for risk assessment, mitigation, and evaluation of the impact the change will have on the **S**chedule, **D**eliverables, and **B**udget.
  - Following internal review, DOE is engaged to refine/approve proposed major changes.

2. Approach

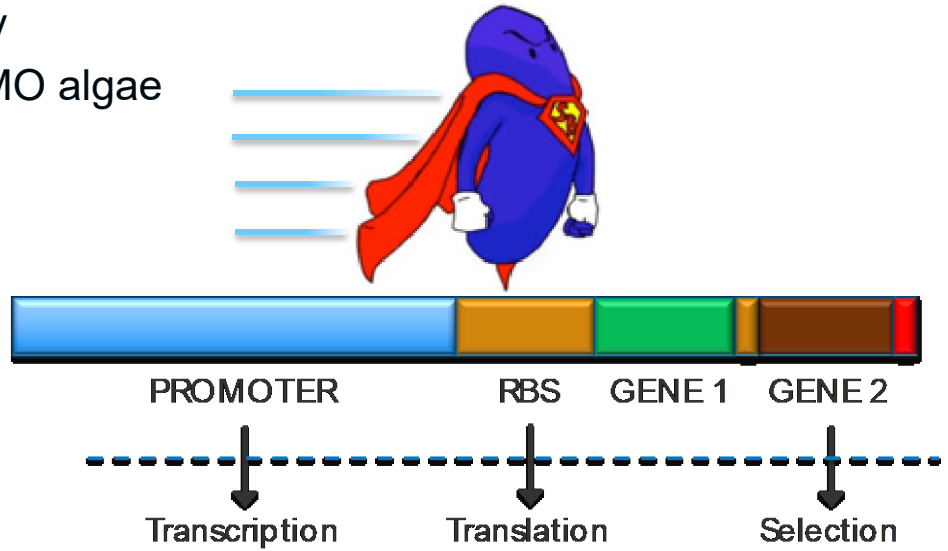
Our **Technical Approach** leverages core capabilities of partner institutes in i) strain development and ii) functional genomics, to generate broad-host range tools and deployment-viable algal production strains.

Major challenges:

- **Technical:** (i) low genetic throughput, (ii) lack of broad-host selection markers, (iii) lack of genetic tunability (iv) poor genetic stability
- **Market:** TERA Permitting, adoption of GMO algae

Critical success factors:

1. Identify and select a rapidly testable genetic host
2. Systematically generate modular, orthogonal genetic parts
3. Demonstrate tools in robust, high-productivity DOE-relevant strains: reduce risk to early adopters



2. Approach

FY19-21 Overarching Project Goals

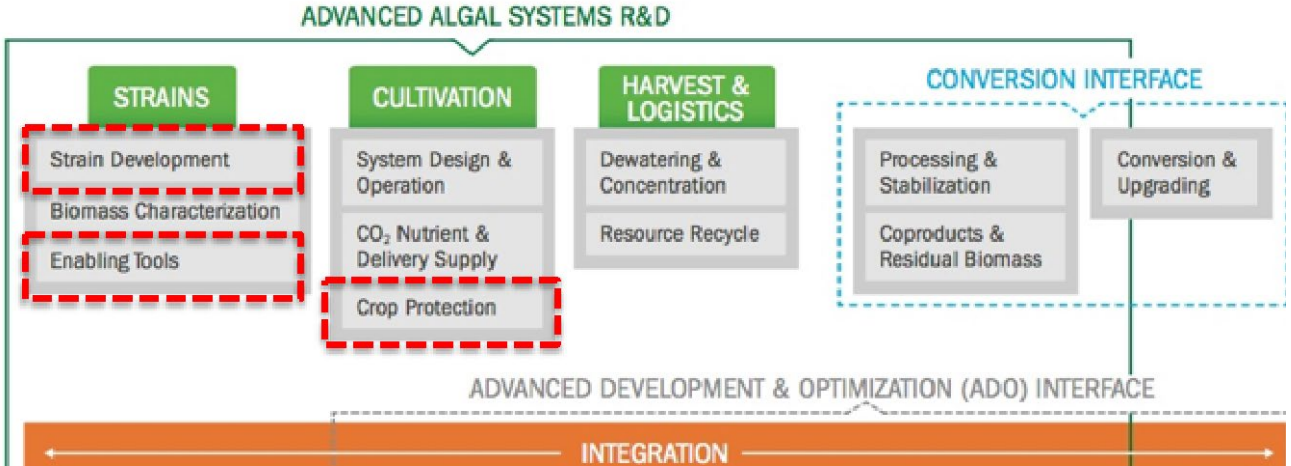
1. Identify top-candidate production chassis for high-throughput genetics
   - Screen >300 algal isolates for rapid growth, high biomass productivity, and halotolerance
2. Develop modular “orthogonal genetic parts” for bypass of host regulation
   - Synthetic promoter and RBS libraries for tunable gene expression
   - Heterologous RNAP for orthogonal transcription
3. Establish broad-host range auxotrophic selection marker(s)
   - Bypass the necessity for antibiotic resistance markers
4. Demonstrate broad-host range efficacy

Go/No-Go (FY20): Achieve orthogonal gene expression in P. renovo and one additional top-candidate strain selected from the DISCOVR AOP in order to demonstrate multi-organism applicability. Achieve transformation efficiencies >10cfu/ug

End Period-of-Performance: Demonstrate tool efficacy in 4 deployment candidates via incorporation and orthogonal regulation of fatty acid biosynthetic machinery.

3. Impact

- ABP activities enable **targeting of MYPP key cost hurdles**:
  - Biomass Genetics and Development
  - Enhanced algal biomass productivity and value (composition)
- **Addresses sustainability concerns** related to fresh vs. saltwater deployment.
- Broad-host range tools present **platform-wide impact.**
- Rapid exchange of strains and meta-data enables **rapid adoption by algal industry**.
- EPA engagement will establish and de-risk GMO deployment hurdles.



3. Impact

Public dissemination of tools and data to enable algal R&D

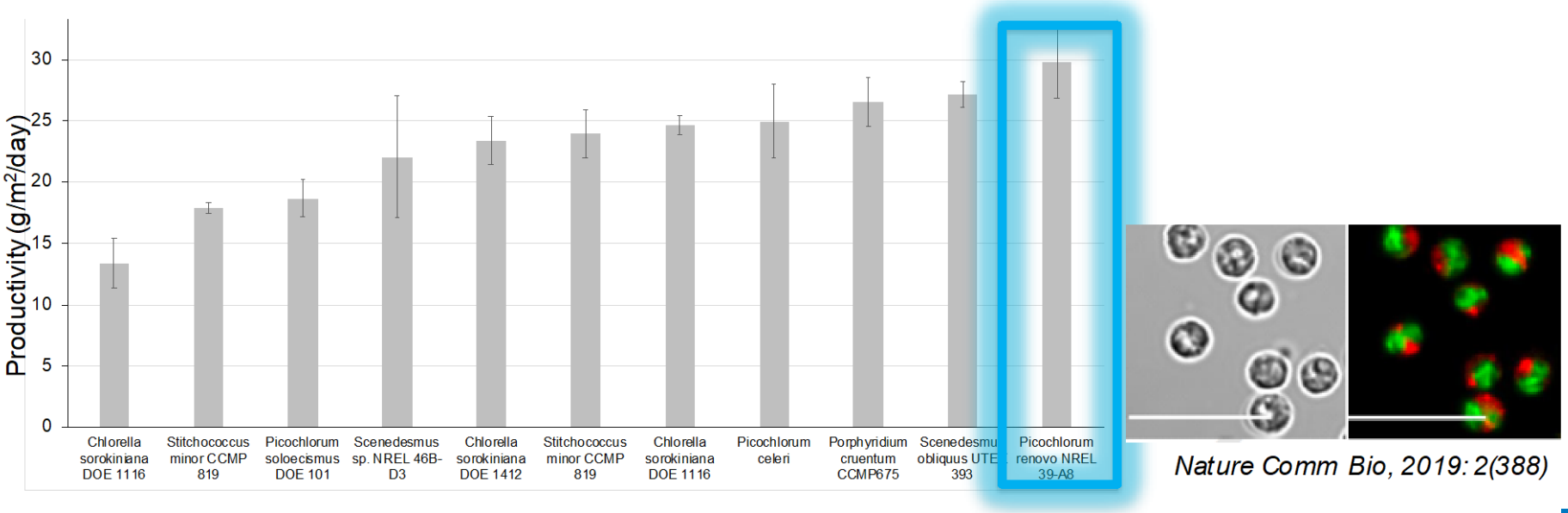
- Tool Dissemination
  - Addgene deposition of plasmids and enabling tools
- Data Dissemination
  - JGI, NCBI, LANL Greenhouse
  - Publications, Patents, Presentations
- Strain Dissemination
  - 11 active Material Transfer Agreements encompassing strains and tools with both academia and industry.
- Platform and Commercial Partnership

4. Progress: Technical Achievements

Rapid and Robust Strain Serves As Tool Development Chassis

Critical Success Factor 1: Identify and select a rapidly testable genetic host

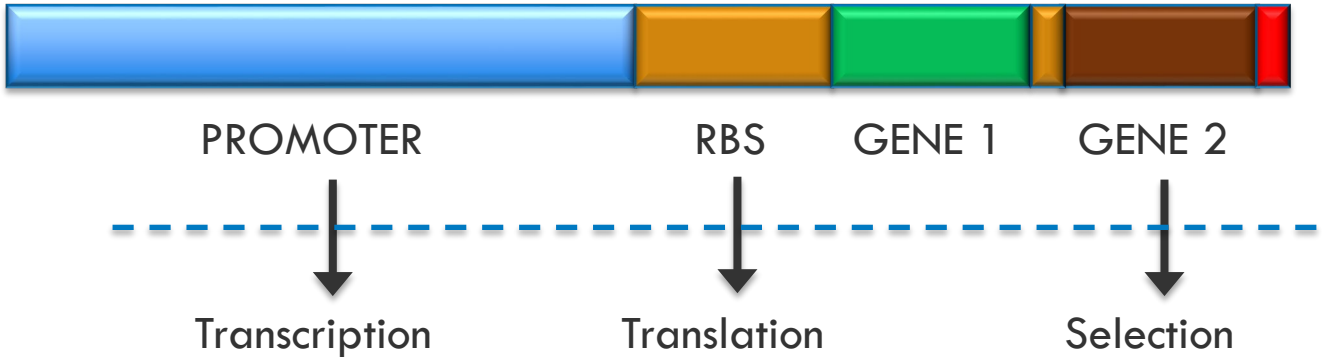
- ***Picochlorum renovo***
  - **Temperature Optima:** 35°C
  - **Salinity Tolerance:** >3X Seawater
  - Fastest doubling time in NREL screen and highest productivity in DISCOVR LEAPS



Nature Comm Bio, 2019: 2(388)

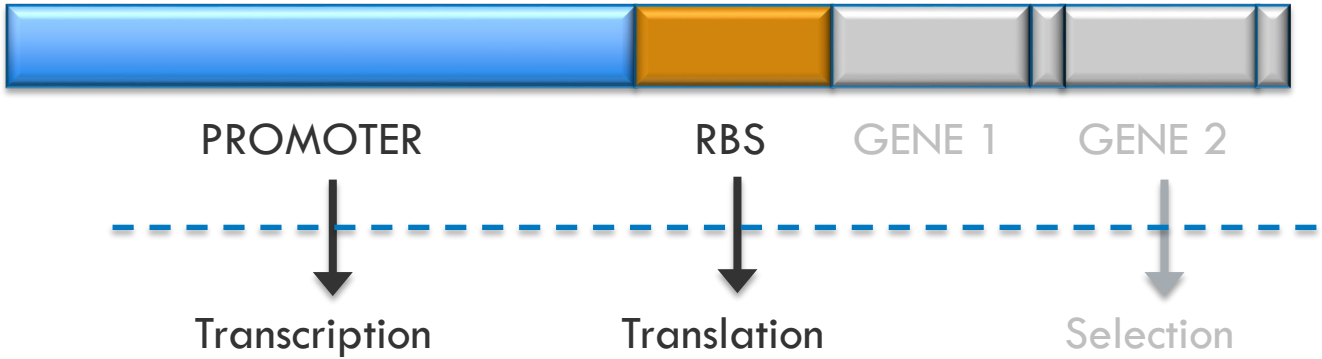
Anatomy of a Gene

Critical Success Factor 2: Systematically generate orthogonal genetic "parts"

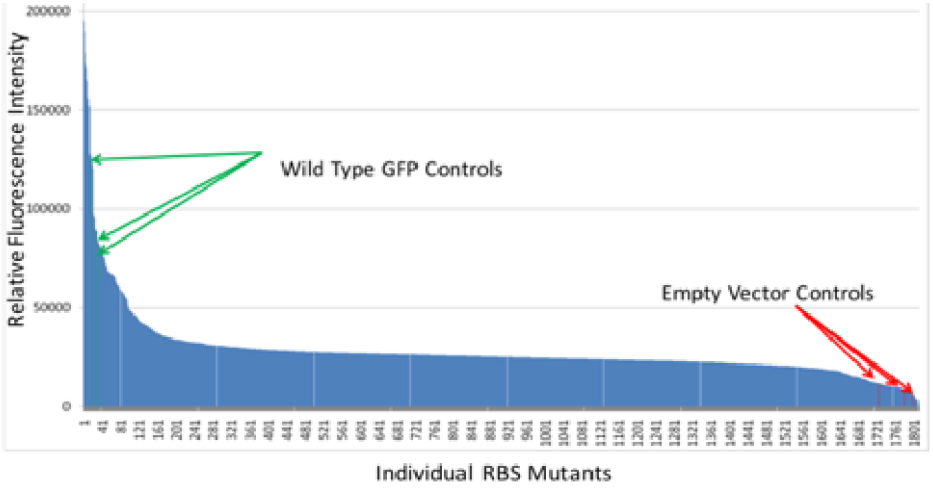


Anatomy of a Gene: Gene and Protein Expression

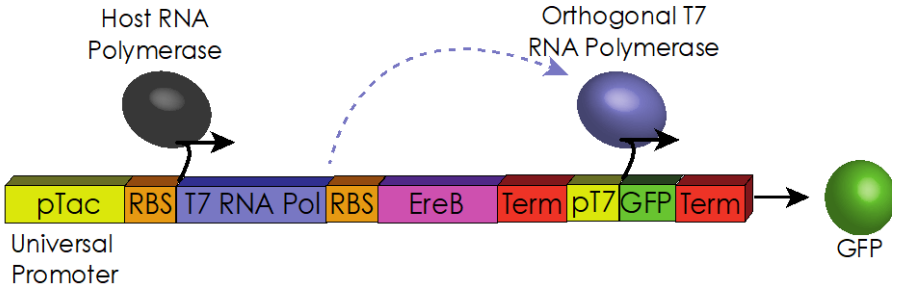
Critical Success Factor 2: Systematically generate orthogonal genetic "parts"



Achieved Tunable and Orthogonal Gene Expression



1. Tunable gene expression across three orders of magnitude via deployment of synthetic algal promoter and RBS elements.

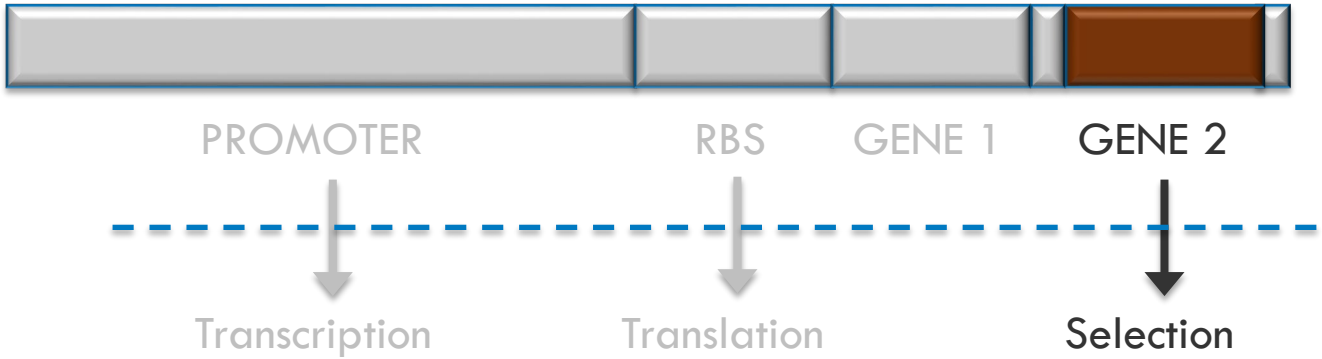


2. Heterologous RNAP plastid localization and expression to establish the foundation for orthogonal transcriptional regulation

These tools enable tunable gene expression and bypass of host regulation.

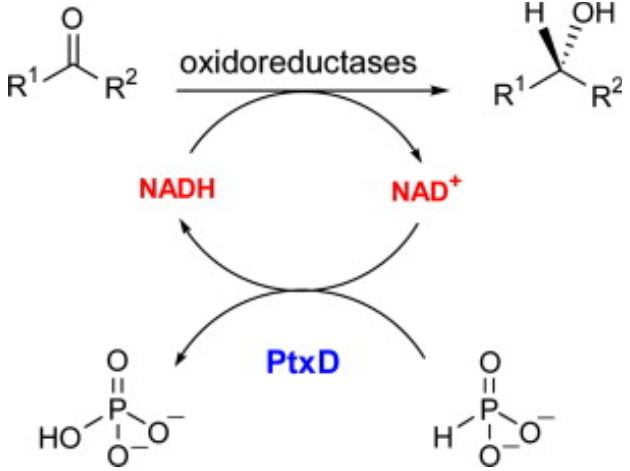
Anatomy of a Gene: Selection Marker

Critical Success Factor 2: Systematically generate orthogonal genetic “parts”

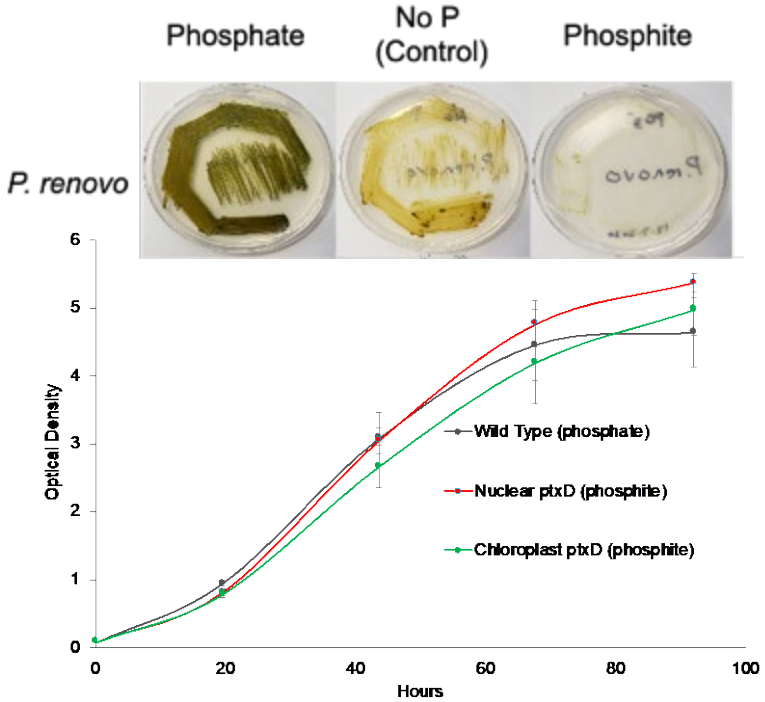


Successful Generation of Broad-Host Genetic Selection Marker

- Engineered phosphite auxotrophy **expands available selection markers** for top-candidate deployment strains AND presents a potential **crop protection strategy**
- Enables non-antibiotic-mediated genetics: no false positives detected to date
- Effective in top DISCOVR strains (including *Picochlorum* and *Scenedesmus* spp.)



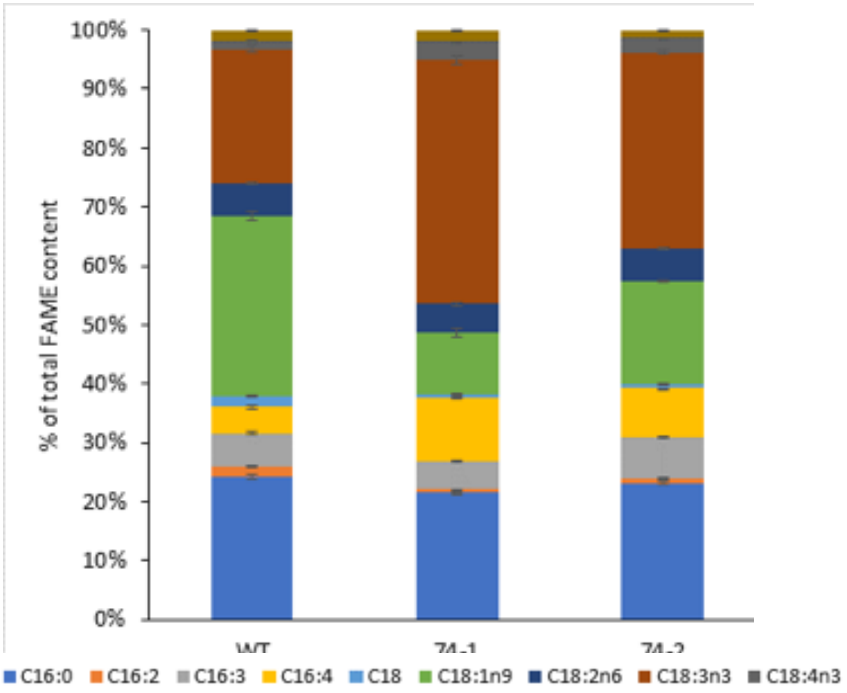
doi.org/10.1016/j.bioorg.2005.01.003



Reduction to Practice: Omic-Informed Strain Engineering

Critical Success Factor 3: Demonstrate tools in robust, high-productivity DOE-relevant strains: reduce risk to early adopters

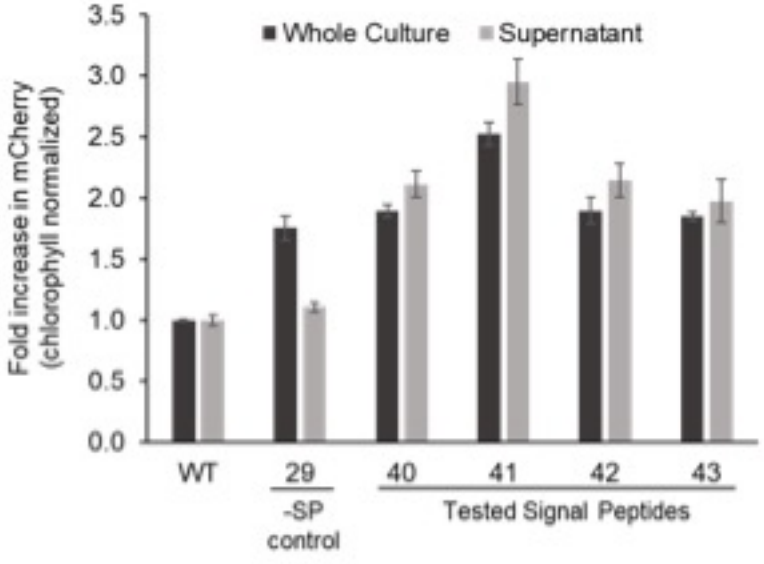
- Achieved altered FA speciation in engineered strains:
  - Shift from C18:1→C18:3
  - Increase in C16:4
- This work underscores the power of integrating multi-omics (Algae Genetic Blueprint AOP) with genetic engineering (Algae Biotech Partnership AOP)

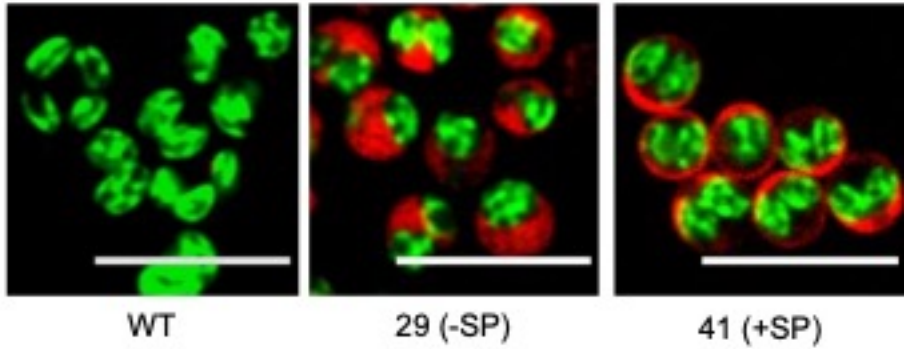


Nature Comm Bio, In Press

Reduction to Practice: Functional Protein Secretion in *Picochlorum*

Critical Success Factor 3: Demonstrate tools in robust, high-productivity DOE-relevant strains: reduce risk to early adopters





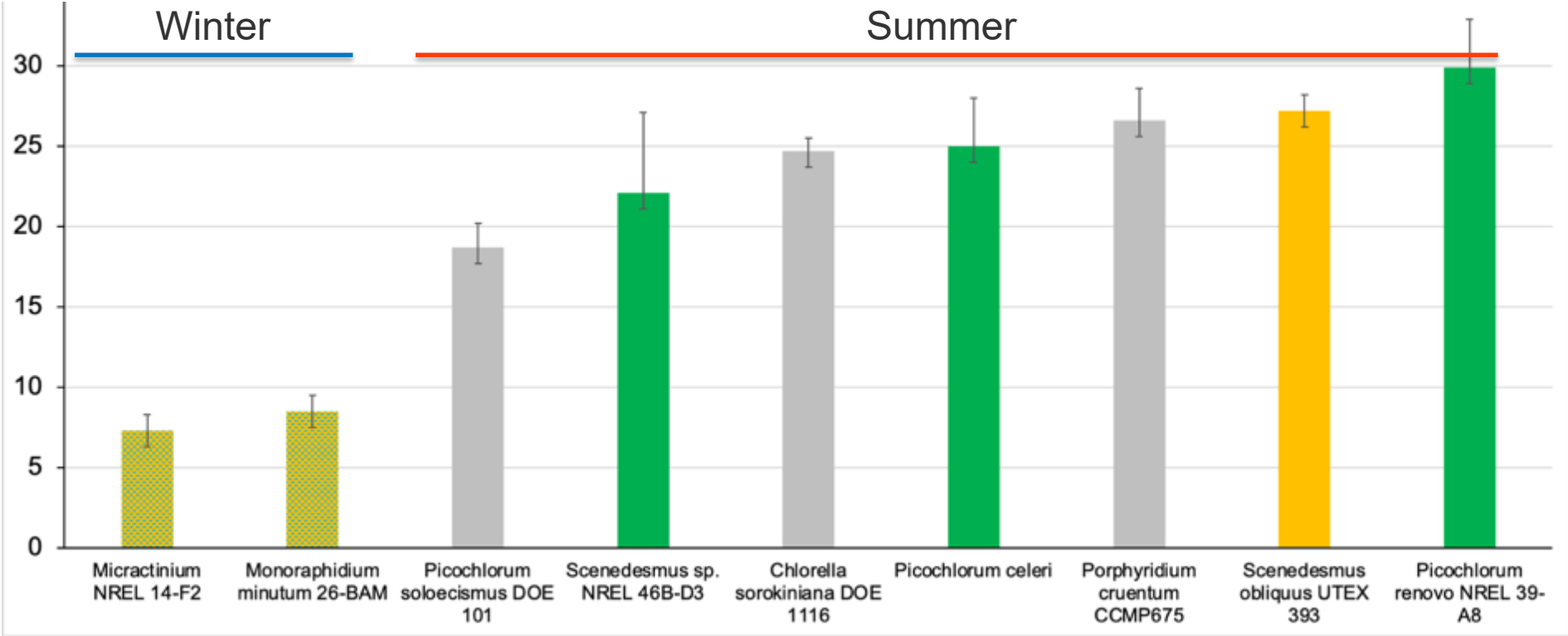
Algal Res, In Press

- Genomics–enabled identification of functional protein secretion signal peptides.
- Protein secretion expands the potential utility of algal biocatalysts and presents additional refinery integration and biomass valorization options.

Broad Host Deployment

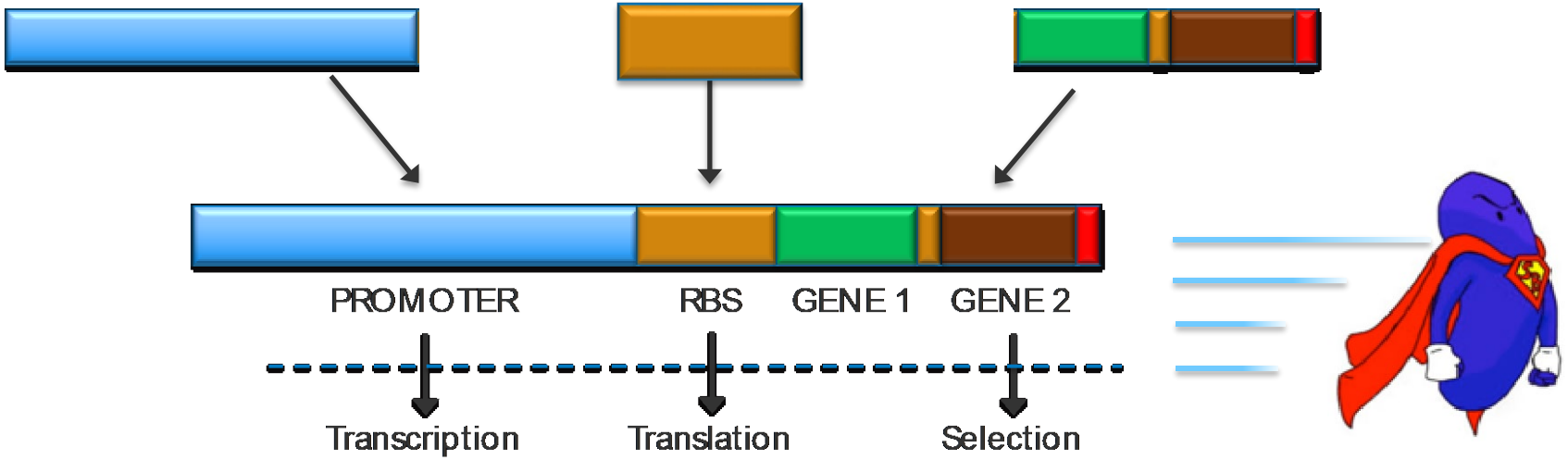
Critical Success Factor 3: Demonstrate tools in robust, high-productivity DOE-relevant strains: reduce risk to early adopters

- *Modular tool design enables rapid transfer to other strains*



Next Steps: **Integrated** Tool Deployment

- Combinatorial genetic tool designs will be pursued in the remainder FY21 to demonstrate integrated efficacy in diverse hosts.
- **End Period-of-Performance Target:** Demonstrate tool efficacy in 4 top-candidate BETO deployment candidates via incorporation and orthogonal regulation of fatty acid biosynthetic machinery.



Summary

Management
- Multi-disciplinary staffing plan and inter-project synergies
- Frequent and iterative Team, Industry, and Government Engagement
- Comprehensive risk management plans ensures agile execution

Approach
- Use of a rapid throughput genetic host for systematic development of orthogonal genetic parts
- Genome-informed tool development and strain engineering
- Demonstration of broad host-range efficacy

Impact
- Directly targets key MYPP Barriers, including Biomass Genetics and Development
- De-risks GMO deployment and responds to EPA regulatory considerations
- Potential for platform-wide impact via “universal” toolbox development
- Emphasis on public dissemination of strains, tools, and data

Progress
- Established a rapid throughput, top-candidate deployment strain, *Picochlorum renovo*
- Developed a suite of orthogonal and applied tools: promoter/RBS libraries, selection markers, secretion peptides, and rewired strains
- Demonstrated efficacy in a series of top-candidate hosts

Acknowledgements

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Igor Grigoriev (LBNL/JGI)
Sarah Calhoun (LBNL)
John McGowan (ASU)

Additional Slides

Quad Chart Overview (for AOP Projects)

Timeline

- Project start date: 10/1/18
- Project end date: 9/30/21

	FY20	Active Project
DOE Funding	(10/01/2019 – 9/30/2020)	$430,000

Project Partners

- Partner 1: Colorado School of Mines
- Partner 2: Los Alamos National Lab
- Partner 3: Lawrence Berkeley National Lab

Barriers addressed

Aft-C. Biomass Genetics and Development
Aft-B. Sustainable Algae Production
Aft-A: Biomass Availability and Cost:

Project Goal

Enable broad host-range metabolic engineering strategies in top-candidate deployment algal strains.

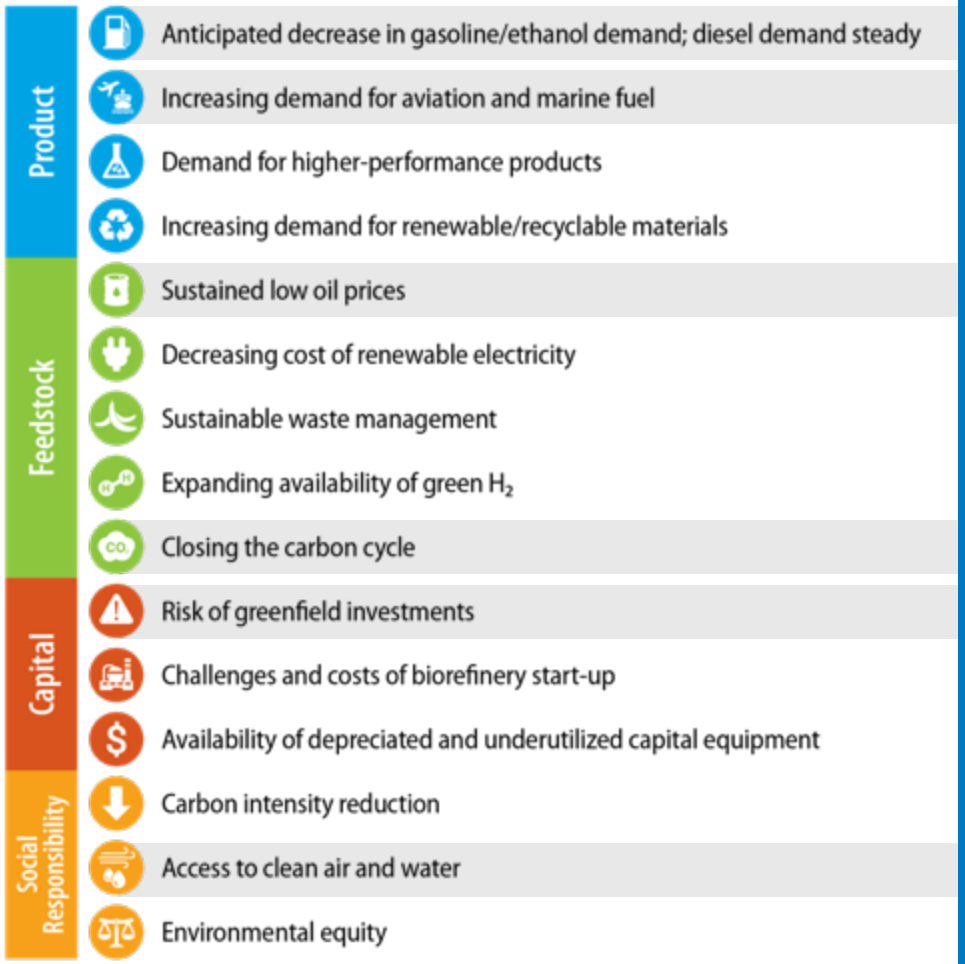
End of Project Milestone

Demonstrate system “universality” via orthogonally-regulated expression of fatty acid biosynthetic machinery in four representative deployment hosts.

Funding Mechanism

Direct Lab AOP.

Market Trends



NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- Successful development will **enable broad-host range metabolic engineering capabilities in diverse microalgae.**
- These tools will **enable improvements to biomass production, composition, and strain robustness.**

Key Differentiators

- Tool development is enabled by a **rapid throughput, top-candidate strain**, *Picochlorum renovo*.
- Multi-disciplinary tool development strategy.
- **Implementation in non-model, mass cultivation-relevant microbes**.

Publications, Presentations, Patents, MTA FY19-Present

Publications (FY19-21 Only)

- S. Calhoun, et al. 2021. *Nat. Comm Bio, In Press*
- LR Dahlin, et al. 2020. *Algal Research*, 54, 102197.
- EP Knoshaug, et al. 2020. *Metabolic Pathway Eng.* 51-59.
- LR Dahlin, et al. 2019. *Comm Bio*, 2(1), 1-9.
- C Zuniga, et al. 2019. *Nature Microbiology* 4 (12), 2184-2191
- N Arora, et al. 2018. *Biotechnology Advances,* 36(4), 1274-1292.
- LR Dahlin, et al. 2018. *Frontiers in Plant Science* 9, 1513

Presentations (NREL Invited, FY19-21)

- Guarnieri MT, et al. ABBB Conference, 2018-20.
- Guarnieri MT, et al. UNT Departmental Seminar 2020
- Guarnieri MT, et al. CU-Boulder CBE Dept. Seminar 2020
- Guarnieri MT, et al. Gordon Research Conference: Molecular Basis of Microbial C1 Metabolism. 2018.

Patents

- ROI 20-66 (Provisional Application in prep)
- ROI-21-22 (Provisional Application in prep)
- US62/884,918 (U.S. Provisional PA)

MTA

- 11 active Materials Transfer Agreements with academic, government, and industry encompassing strains, tools, and sequence data.

Responses to Previous Reviewers' Comments

- We thank the Reviewers for their positive and encouraging assessment.
- Following Reviewer guidance, we have:
  - Received feedback data from commercial deployment, confirming high productivity (>20g/m^2/day) for *Picochlorum renovo*.
  - Expanded strain evaluation beyond Chlorophyte organisms to broaden host range potential
  - Expanded engagement with industry partners to increase likelihood of commercial impact
  - Refined project scope to focus exclusively on tool development (no further strain/culture collection screening) to decrease breadth of pursuits.