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DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

1.3.2.130 Algae Biotechnology Partnership

Advanced Algal Systems
March 5, 2019

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Goal Statement

Goals

The Algae Biotechnology Partnership aims to develop:

- Advanced algal genome editing tools
- Synthetic and orthogonal genetic regulatory systems with broad-host range applicability
- Functional genomic pipelines in top-candidate deployment algal strains

Outcome

- Successful development will enable ***publicly available, broad-host range metabolic engineering capabilities in diverse microalgae***, aimed at maximizing algal outdoor biomass production, composition, and strain robustness.

Relevance to Bioenergy Industry

- Addresses an industry-wide need for genetic tools in non-model systems
- Tools, strains, and meta-data will be publicly disseminated to enable rapid adoption by algal industry for targeted enhancement of deployment strains.
- Addresses sustainability concerns related to fresh vs. saltwater deployment.

Quad Chart Overview

Timeline

- Start: FY2016
- Merit review cycle: FY2019-2021
- 15% complete of review cycle

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$700K	$300K	$350K	$1.3M

- **CSM (12%):** Genetic tool development
- **LANL/LBNL:** Functional genomics
- **DISCOVR:** Outdoor deployment

Barriers addressed

- Aft-C: Biomass Genetics & Development
  - Genomics, CRISPR and orthogonal tool development
- Aft-A: Biomass Availability & Cost
  - Identification and engineering of strains with enhanced biomass productivity
- Aft-B: Sustainable Algae Production
  - Halotolerant strains, saltwater cultivation

Objective

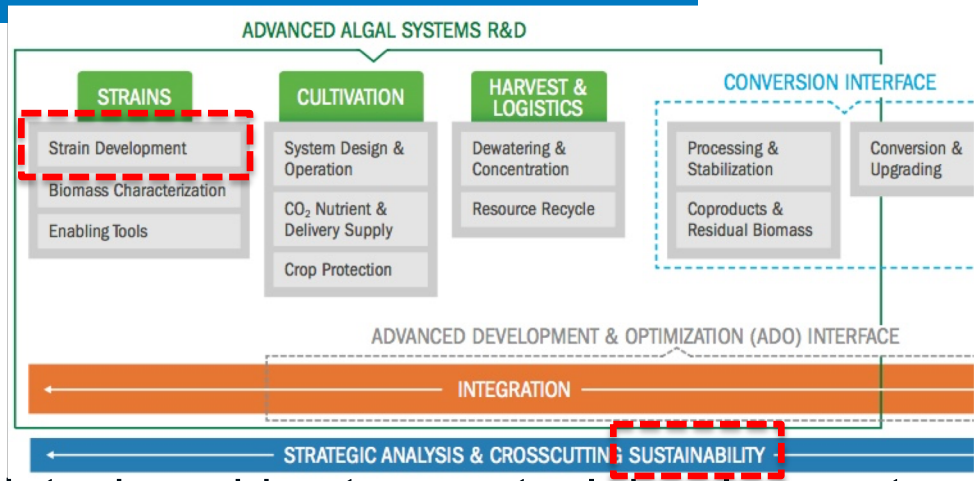
The ABP seeks to develop broad-host range algal genetic and genomic tools and demonstrate efficacy in top-candidate deployment strains.

End of Project Goal: Demonstrate integrated system "universality" via targeted integration and orthogonally-regulated gene expression of native and heterologous fatty acid biosynthetic pathway genes in 5 candidate deployment organisms.

Project Overview

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

- Development of advanced genetic and genomic tools will be ***integral to achieving BETO 2022 biomass productivity and cost targets.***



- Few successes in non-model algae to-date; broad-host range tool development hindered by strain-specific negative regulatory mechanisms.
- Synthetic, systems biology approaches present a means to construct novel genetic regulatory networks and rewire natural biological systems to ***establish an orthogonal control elements for bypass of host control***.
- FY16-18 efforts led to successful down-selection of top-candidate strains to demonstrate tool efficacy in non-model, deployment-viable hosts.

Specific Project Goals:

- Heterologously express orthogonal transcriptional & translational regulatory units
- Establish CRISPR-mediated genome editing capacity
- Demonstrate bypass of host control via deregulated fatty acid biosynth control
- Develop broad-host range control elements (RBS/promoter libraries) and ”universal” plasmids/cassetes for rapid transferability into various algal hosts

Approach - Management

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- Strain Selection and Genetic Tool Development

Los Alamos NATIONAL LABORATORY

BERKELEY LAB

- Functional Genomic Analyses

DISCOVR

- Strain Validation/Outdoor Deployment

- **Extensive Team Interaction**
  - Regular PI-technical staff and PI-partner interactivity
  - Monthly NREL team, platform, and external ABP meetings
  - Quarterly BETO meetings and reporting
- **Diverse Staffing Plan**
  - Molecular and Microbiologists, Computational Biologists, Analytical chemists
- **Consistent Industry and Regulatory (EPA) Engagement**
  - Strain and tool dissemination, safe-operation assessment

Approach - Technical

- **Approach:** Leverage core capabilities of NREL and partner institutes in i) strain development, ii) functional genomics, and iii) algal cultivation, in order to generate broad-host range tools and deployment-viable algal production strains.
  - Halotolerant strain evaluation and down-selection
  - Orthogonal tool development
  - Targeted genome editing
  - Functional genomics data integration
  - Iterative strain validation and outdoor deployment

- **Major challenges:**
  - **Technical:** (i) generation of reproducible, stable, and high-efficiency genetic tools, (ii) establishment of broad-host range control elements
  - **Market:** Conduct laboratory testing and outdoor field demonstrations to reduce risk to early adopters.
    - TERA Permitting – active engagement with EPA and AzCATI testbed to identify and address deployment requirements and hurdles.

- **Critical success factors:**
  - Achieve routine, facile genetic transformation and functional orthogonal tool suites in diverse microalgae strains (targeting five non-model deployment candidates in MR cycle).
    - Establishment of comprehensive strain knowledgebases.
  - Widespread adoption of strains and associated tools.
    - Demonstrate robust, high-productivity saltwater deployment.

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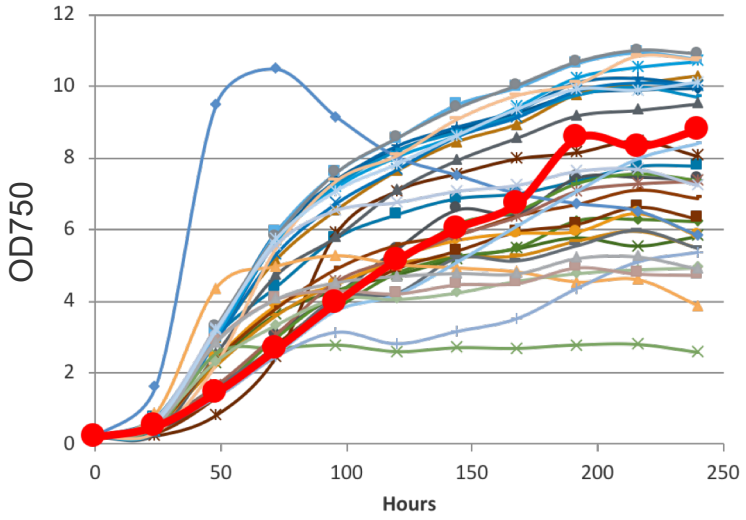
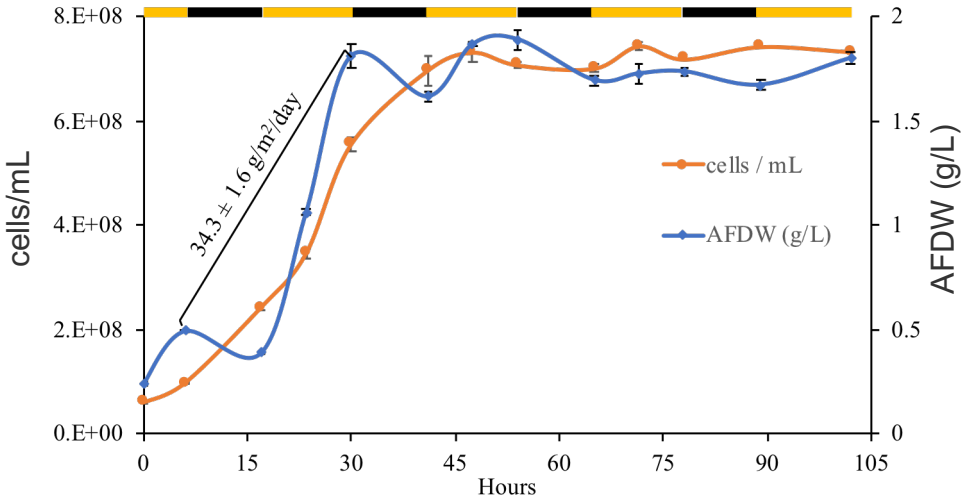
FY16-18 Merit Review Period Accomplishments

I.D. of High-Productivity Halotolerant Strains





- Developed a mid-throughput reactor for **rapid screening of >300 saltwater strains.**
- Two top-candidate Summer and Winter strains down-selected with productivity greater than SOT under simulated outdoor conditions.
- DISCOVR evaluation and outdoor deployment validated results of indoor simulation.
- *Picochlorum sp.* 39-A8 strain selected as model for orthogonal tool development.
  - Rapid growth rate: among fastest doubling time reported to date for a eukaryotic alga
  - High biomass productivity (>40g/m^2/day in PNNL pond simulators) and storage carbon flux
  - Halotolerance (>3X saltwater salinity) and thermo-tolerance (>40^{o}C cultivation capacity)
  - Extensive functional genomic knowledgebase generated
  - Facile genetic tools established

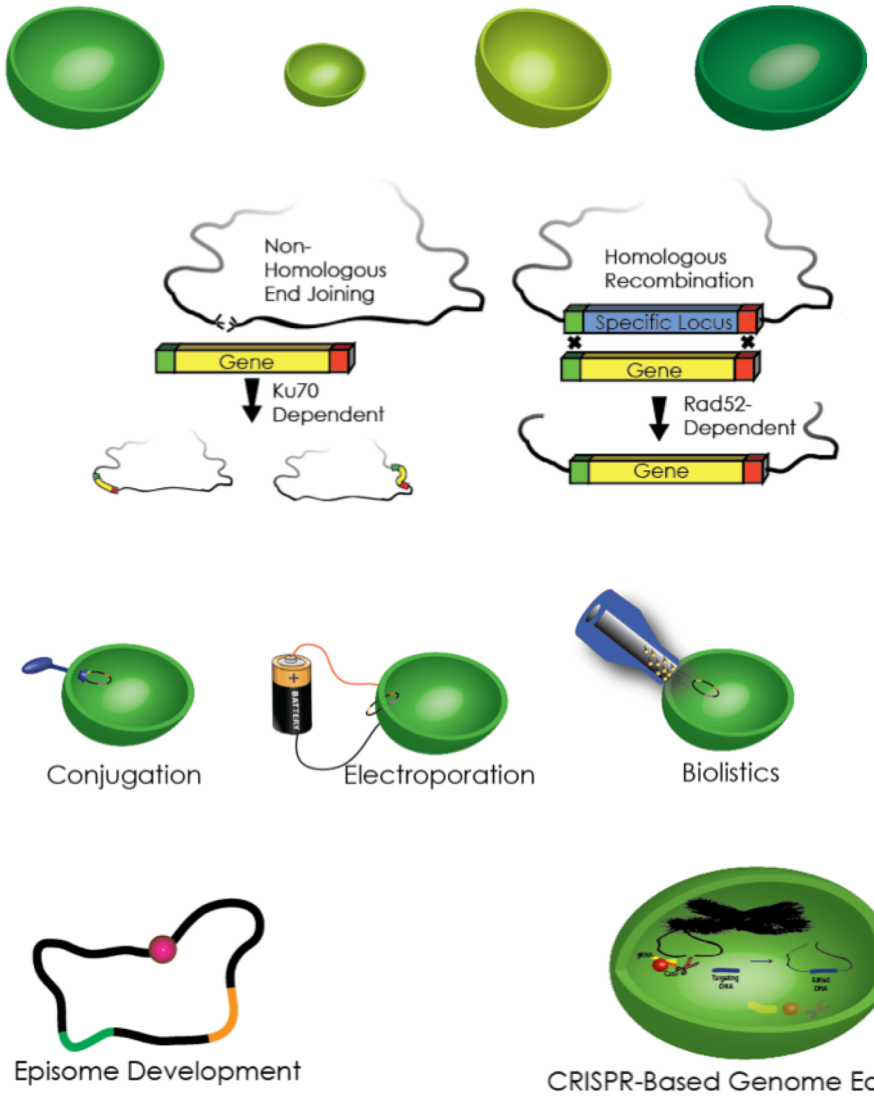
Genetic Tool Development Pipeline

I. Strain Selection

II. Random & Targeted Integration Tool Development

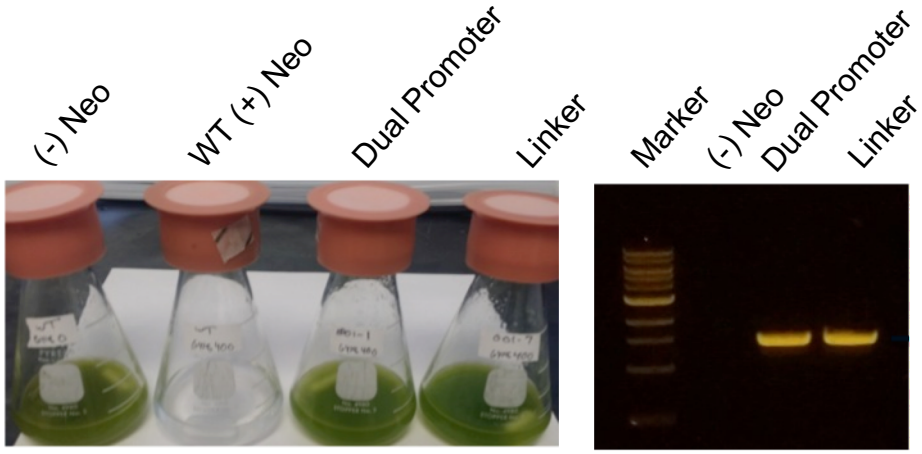
III. Transformation

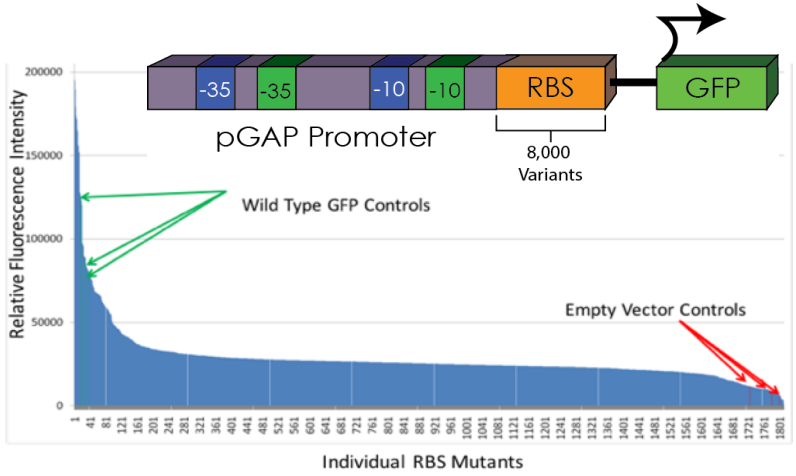
IV. Advanced Genome Engineering

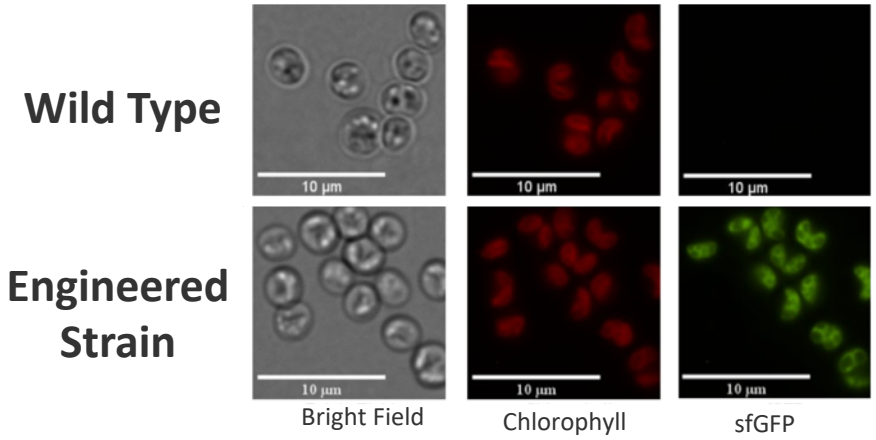


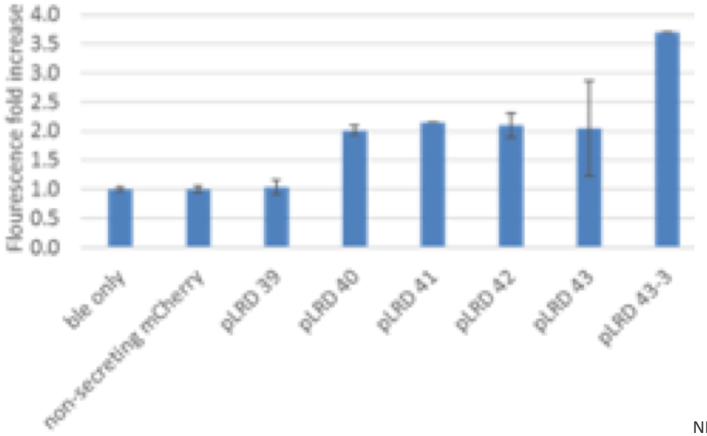
Genetic Tool Development

- Successfully generated nuclear and plastidial transformants in four non-model top-candidates, as well as protein secretion capacity in 39-A8.
- 8,000 RBS library generation for tunable expression: 3 orders of magnitude.
- Reproducible, stable (maintained expression following 8 serials transfers off selective pressure), and rapid (<2 weeks for homoplasmy in 39-A8).

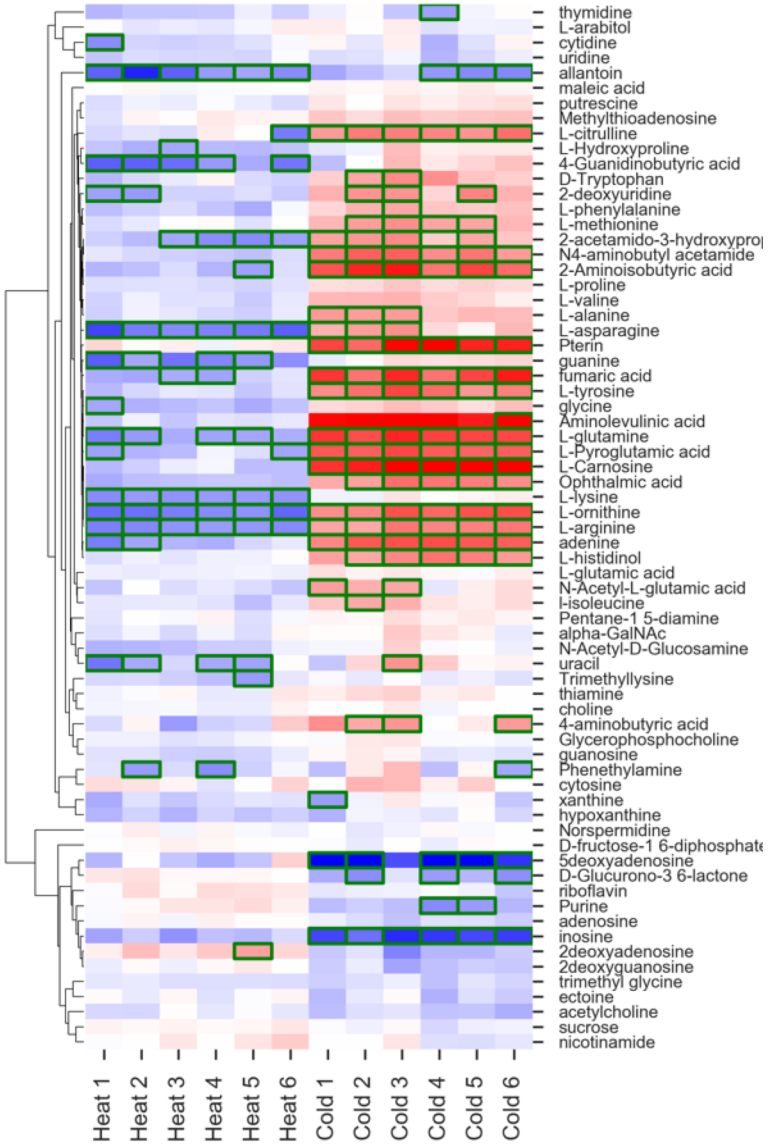








Omics Data Generation and Genetic Target I.D.



- Omic analyses and pipelines will enable pathway mapping, and identification of regulatory elements and strain-engineering targets.
  - Core promoter and UTR elements identified for cassette design
- Complete genome sequence and assembly of top two candidate winter and summer strains completed.
  - *Micractinium, Picochlorum, Scenedesmus,* and *Chlorella*
- Transcriptomic and metabolomic datasets obtained for top summer strains to identify putative strain-engineering targets.
  - A series of transcription factors differentially-expressed under heat- and cold-stress have been identified as potential regulators of lipogenesis.
  - Efforts are currently underway to genetically manipulate targets to define role in stress responsiveness and carbon partitioning.

Relevance

- ABP activities enable targeting of MYPP key cost hurdles:
  - Enhanced algal biomass productivity and value (composition)
  - Robustness under extreme winter- and summer-deployment conditions.
- Addresses sustainability concerns related to fresh vs. saltwater deployment.
- Community-wide need for broad-host range tools = broad impact.
- Rapid dissemination and exchange of strains and meta-data will enable rapid adoption by algal industry.
  - Successful toolbox will prevent the need to “reinvent the wheel” for newly identified deployment candidates
- EPA engagement will establish and target core GMO deployment hurdles.

Relevance

Stakeholder Outreach and Engagement

Three material transfer agreements are in place with commercial algal entities, encompassing

- strain exchange and screening,
- media and deployment evaluation
- strain evaluation for nutraceutical production potential

Exchange of Strains with DISCOVR pipeline and SD-CAB:

- Evaluation of top-candidate ABP strains relative to top-candidate platform strains.

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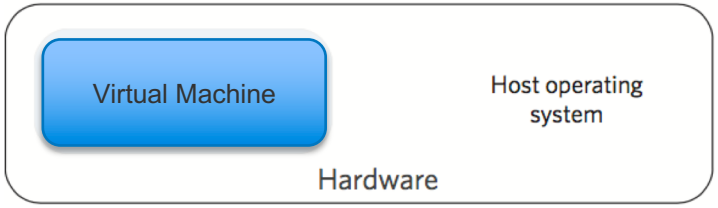
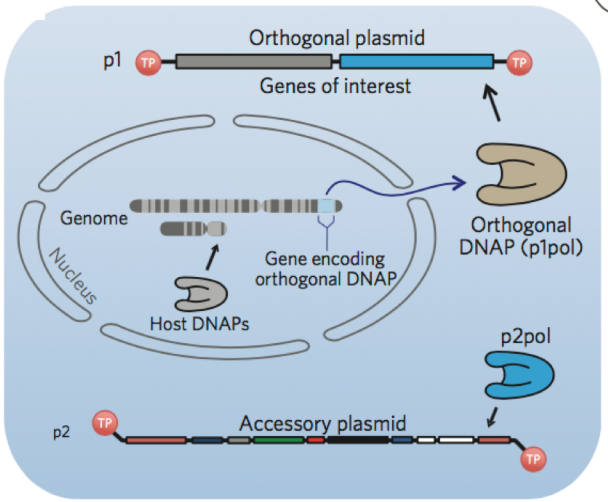
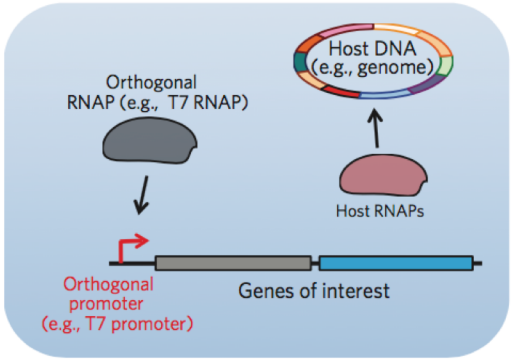
FY19-21 Merit Review Period: Work Scope

Future Work: FY19-FY21 Scope

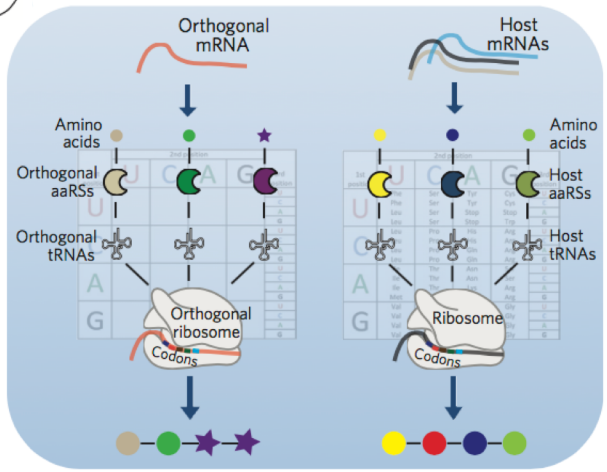




DNA Polymerase



RNAP:promoter



aminoacyl-tRNA synthetases: tRNAs

Liu, et al. 2018. *Nat. Chem. Bio*; 14, 103-6

- Development of a suite of orthogonal tools.
  - Allows for host-independent regulation
- Targeted genome editing (HR- and/or CRISPR-mediated)
- Demonstrate toolbox “universality” (broad-host applicability in 5 or more top-candidate deployment strains currently under evaluation in BETO and/or industry portfolio).

Future Work

- Future work will target advanced, **broad-host range** genetic toolkit development to enable targeted strain-engineering in top-candidate strains.

- FY19 Targets:
  - Achieve heterologous **RNAP plastid localization** and expression
  - Demonstrate targeted incorporation of two **tRNA synthetase-tRNA pairs**
  - Demonstrate **tunable gene expression** across >2 orders of magnitude in 39A8 via deployment of synthetic algal promoter elements.

- FY20 Target:
  - Achieve **functional CRISPR/Cas9 editing** at >5% repair efficiency (or modulating for dCas9) in 39A8 and one additional top-candidate strain selected from the DISCOVR AOP.

- FY21 Targets: **Demonstrate system "universality"** via CRISPR-mediated integration and orthogonally-regulated gene expression of native and heterologous fatty acid biosynthetic pathway genes **in 5 representative deployment organisms.**

Summary

- **Overview**
  - Development of advanced genetic and genomic tools will be ***integral to achieving BETO 2022 biomass productivity and cost targets.***
- **Approach**
  - Algal screening, synthetic biology, functional genomics, and iterative outdoor deployment will be employed to develop broad-host range algal genetic tools.
- **Accomplishments**
  - Strain screening and characterization has led to down-selection of high-potential candidate halotolerant deployment strains.
  - Established nuclear and plastidial engineering, tunable expression, and protein secretion.
  - Genomic blueprints and core regulatory elements established for top-candidates.
- **Relevance**
  - ABP activities enable targeting of MYPP key cost drivers
  - Community-wide enabling tools
- **Future Work**
  - Advanced genetic tool development and orthogonal pathway regulation.
  - Public dissemination of strains, toolkits, and associated meta-data.

Acknowledgements

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

Fanni Davrazou
Calvin Henard
Jeff Linger
Phil Pienkos
Sharon Smolinski
Nick Sweeney
Stefanie Van Wychen
Luke Dahlin (CSM)
Matt Posewitz (CSM)
Shawn Starkenberg (LANL)
Tisza Bell (LANL)
Blake Hovde (LANL)
Igor Grigoriev (LBNL/JGI)
Sarah Calhoun (LBNL)
John McGowan (ASU)
Henri Gerken (ASU)
Michael Heusemann (PNNL)
Scott Edmundson (PNNL)

Thank You

www.nrel.gov
www.nrel.gov/bioenergy/algal-biofuels.html

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Response to Reviewers' Comments 2017

•Overall, this project has substantial merit and potential for the discovery of higher-productivity microalgae species. Integration of simulated outdoor screening blends well with the ability to transition for lab to pilot-scale data collection.
•Identification of fast-growing, halotolerant strains will have clear relevance to industry and BETO goals if successful.
•Regarding genetic tool development, this project is ambitious at the risk of being somewhat unfocused.
•Advanced genetic tools (especially newly developed CRISPR technology) for algae will be important for advancing toward productivity targets and improving strain robustness.

We thank the Review panel for their encouraging and constructive critique. We are optimistic that our progress to date to identify high-productivity, halotolerant algal strains, and develop associated genetic and genomic toolkits, represents a critical advancement for the BETO algae portfolio and the larger algal research community as a whole. We look forward to continued efforts to enhance productivity in top-candidate strains via the further development of robust genetic and functional genomic tools. Additionally, we have strived to more acutely focus the project, transferring all outdoor/strain evaluation to DISCOVR pipeline, and focusing exclusively upon tool development in a single top-candidate non-model algal in years 1 and 2 of our current Merit Review cycles. Following successful toolbox generation, multi-host efficacy will be evaluated.

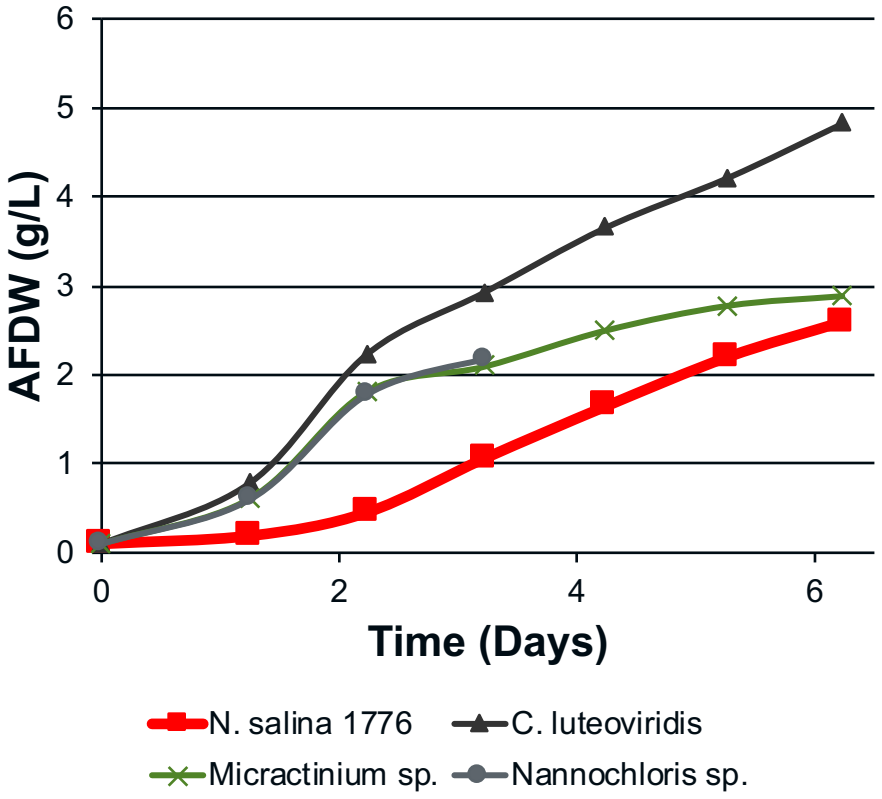
Publications

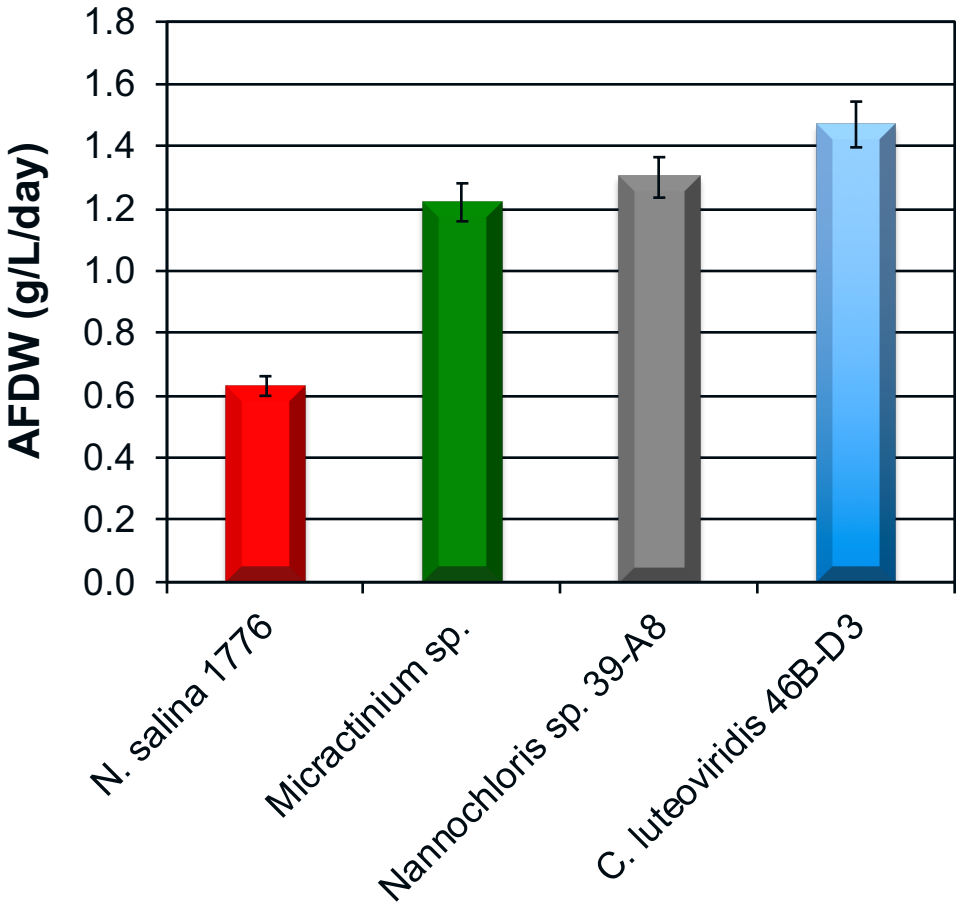
- N Arora, PT Pienkos, V Pruthi, KM Poluri, MT Guarnieri Leveraging algal omics to reveal potential targets for augmenting TAG accumulation. (2018) *Biotechnology advances,* 36(4).
- LR Dahlin, S Van Wychen, HG Gerken, J McGowen, PT Pienkos, et al. Down-selection and outdoor evaluation of novel, halotolerant algal strains for winter cultivation. (2018) *Frontiers in Plant Science 9*
- MT Guarnieri, J Levering, CA Henard, JL Boore, MJ Betenbaugh, et al. Genome Sequence of the Oleaginous Green Alga, Chlorella vulgaris UTEX 395. (2018). *Front Bioeng Biotech* 6(37).
- MT Guarnieri, AT Gerritsen, CA Henard, EP Knoshaug. Phosphoproteome of the oleaginous Green alga, Chlorella vulgaris UteX 395, under Nitrogen-Replete and-Deplete Conditions. (2018) *Front Bioeng Biotech* 6(19).
- C Zuniga, J Levering, MR Antoniewicz, MT Guarnieri, MJ Betenbaugh, et al. Predicting dynamic metabolic demands in the photosynthetic eukaryote Chlorella vulgaris. (2018) *Plant phys.* 176 (1)
- CA Henard, MT Guarnieri, EP Knoshaug. The Chlorella vulgaris S-nitrosoproteome under nitrogen-replete and-deplete conditions (2017) *Front Bioeng Biotech* 4(100)
- J Liu, X Mao, W Zhou, MT Guarnieri. Simultaneous production of triacylglycerol and high-value carotenoids by the astaxanthin-producing oleaginous green microalga Chlorella zofingiensis. (2017) *Bioresource technology* 214, 319-327.
- C Zuñiga, CT Li, T Huelsman, J Levering, DC Zielinski, BO McConnell, et al. Genome-scale metabolic model for the green alga Chlorella vulgaris UTEX 395 accurately predicts phenotypes under autotrophic, heterotrophic, and mixotrophic growth conditions. *Plant physiology*, pp. 00593.2016
- Dahlin, L. and Guarnieri, MT. Recent Advances in Algal Genetic Tool Development (2016) *Curr Biotech*. 5(3); 192-197.

Invited Presentations

- Guarnieri MT, et al. Gordon Research Conference: Molecular Basis of Microbial C1 Metabolism. Sunday River, ME 2018.
- Guarnieri MT, et al. The 9th International Conference on Algal Biomass, Biofuels and Bioproducts. San Diego, CA. 2017.
- Dahlin, L, et al. Western Photosynthesis Conference, Oracle, AZ 2017.
- Guarnieri, MT, et al. 8th Annual Conference on Algae Biomass, Biofuels, and Bioproducts, San Diego, CA, USA. 2016
- Guarnieri MT, et al. Algae Biomass Summit, Phoenix, AZ, USA. 2016
- Dahlin, L, et al. Algae Biomass Summit, Phoenix, AZ, USA. 2016.

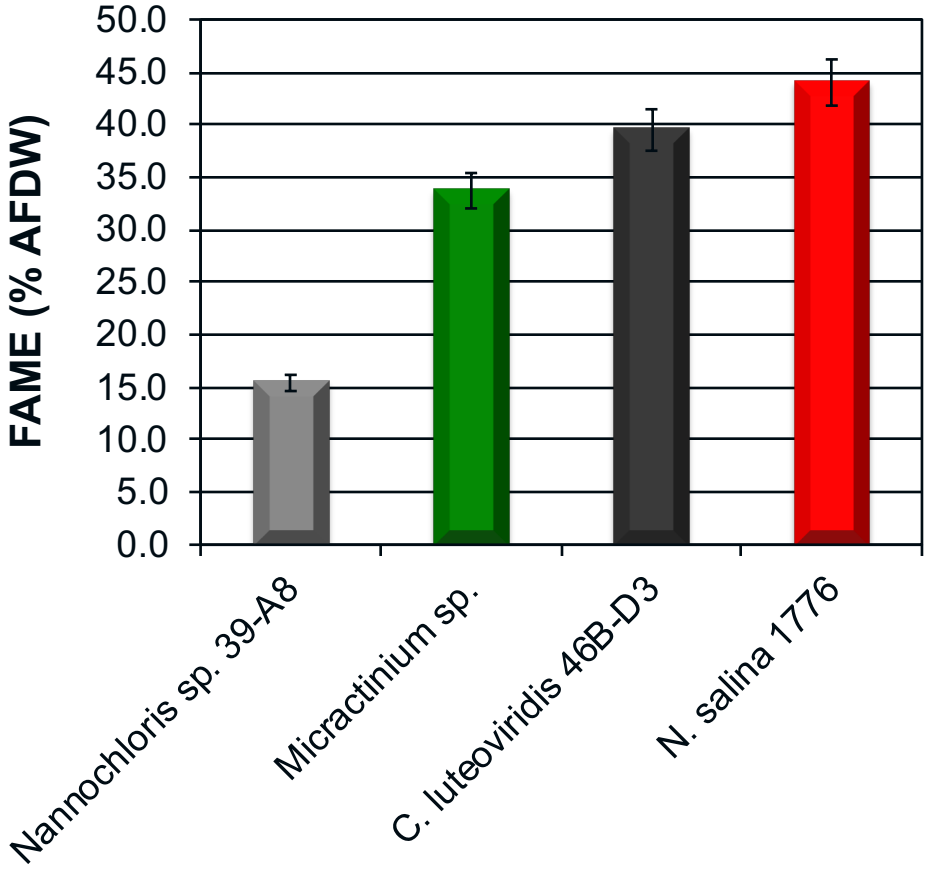
Biomass Productivity (Summer Simulation)

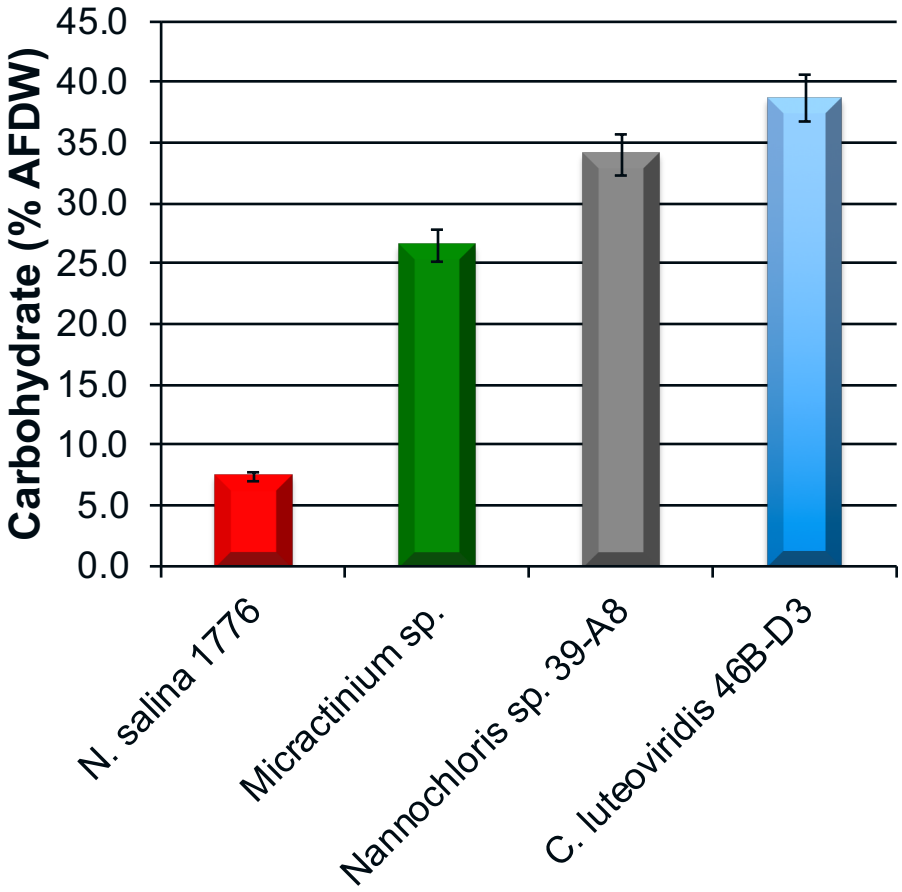




- Top-candidate strains displayed superior max biomass accumulation rates compared to *N. salina.*

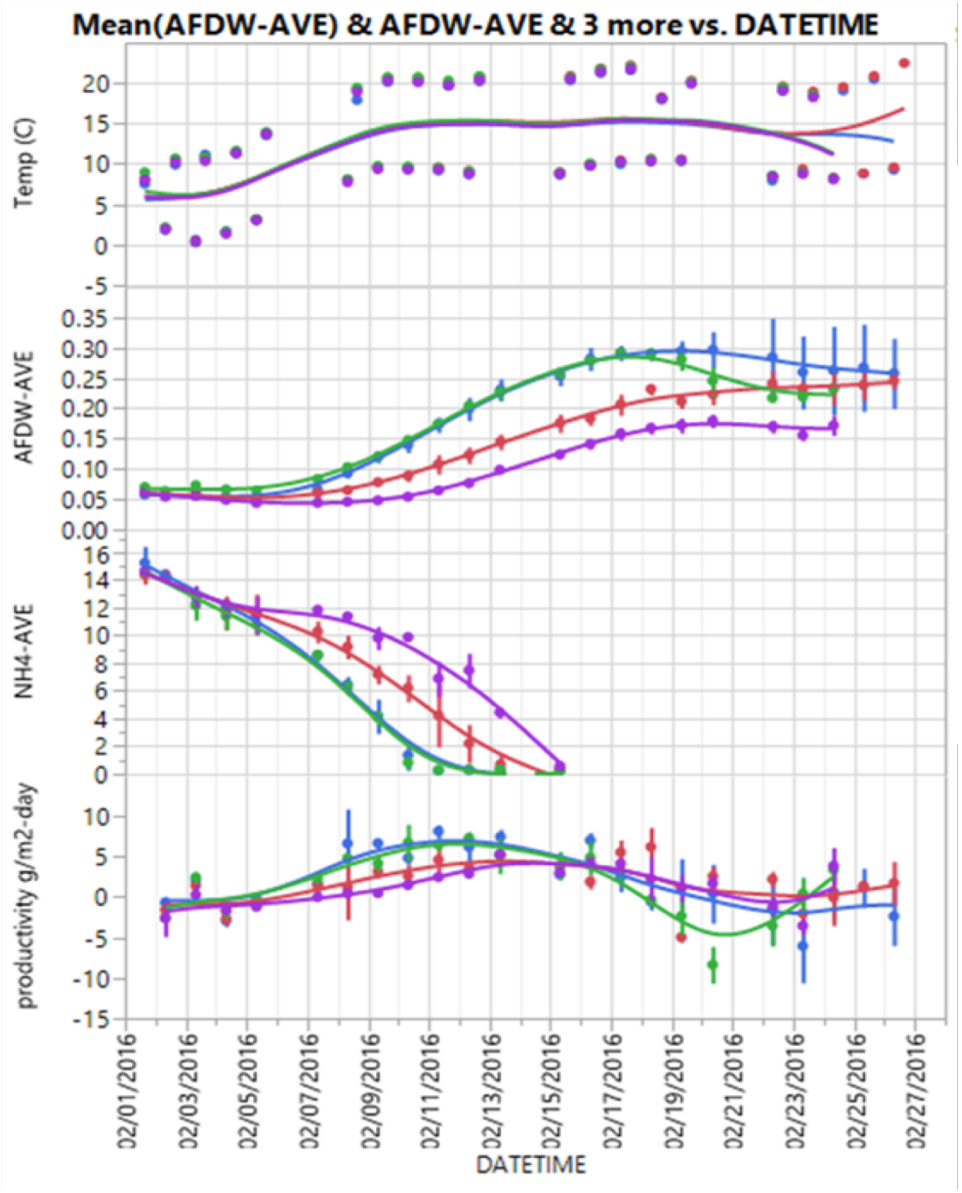
Compositional Analyses (Summer Simulation)

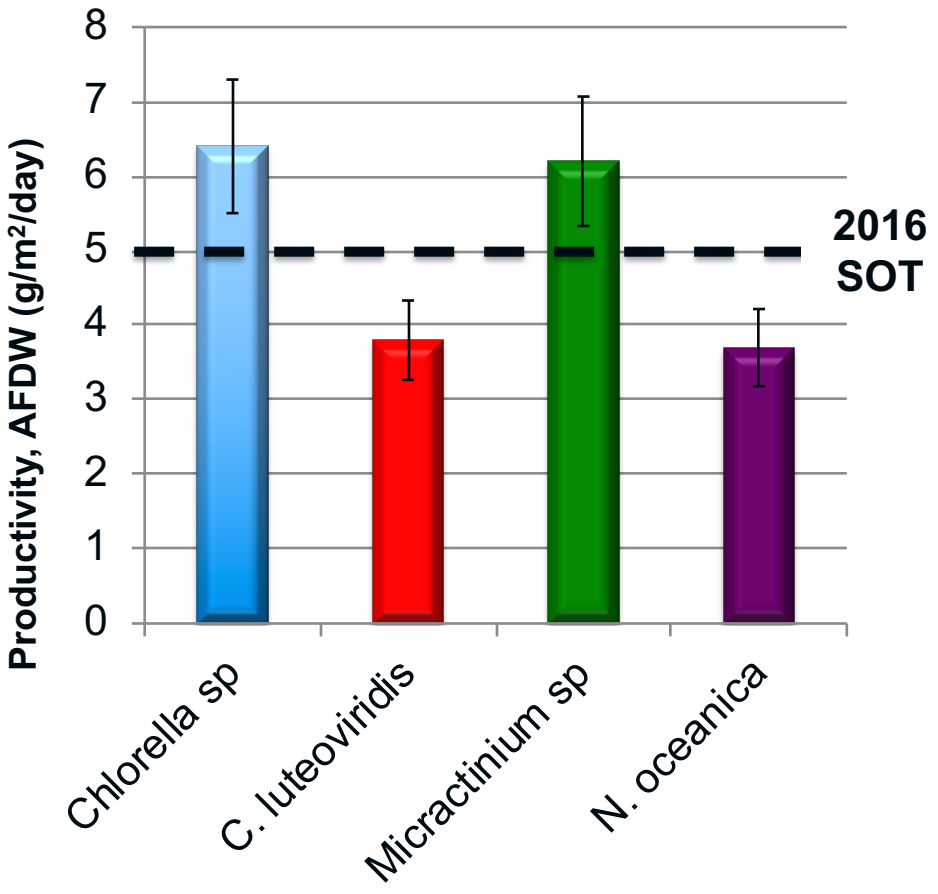




- *C. luteoviridis* and *Micractinium sp.* display robust lipid accumulation compared to benchmark.
  - Both strains represent robust lipid and carb producer
- Strains suitable for CAP and/or HTL processes.

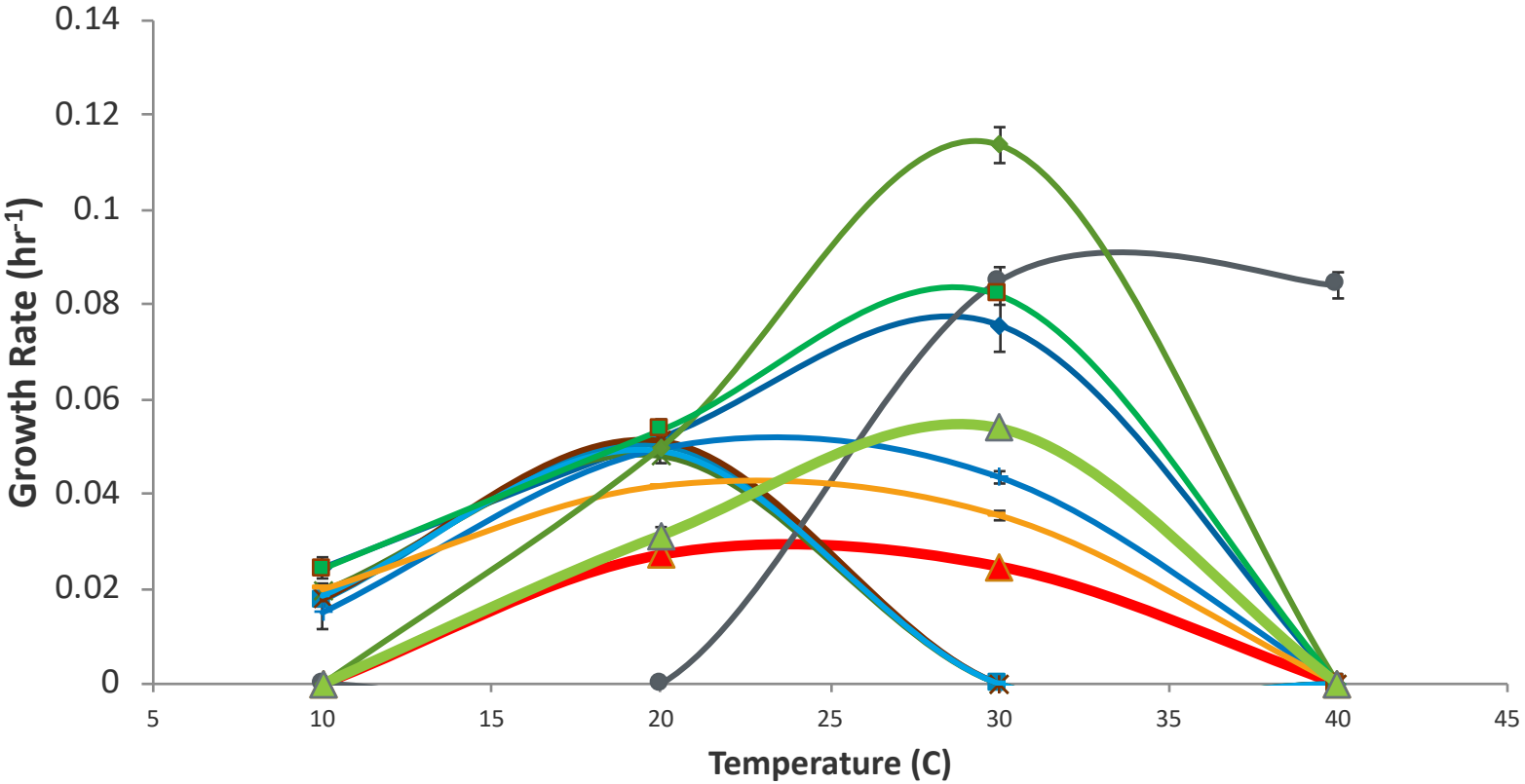
Winter Deployment at ATP3 Testbed





- Outdoor deployment validated indoor screening.
- **Algal biomass productivity > SOT**

Temperature Tolerance Analyses



- Strains displayed distinct temperature optima:
  - ~20°C optima for winter strains, with growth capacity from 10-25°C
  - ~30°C optima for summer strains, with growth capacity from 10-40°C (+)
  - All strains demonstrated superior growth rate to *N. salina* and *N. oceanica* baselines.