NREL
NATIONAL RENEWABLE ENERGY LABORATORY





Algae Biotechnology Partnership
WBS 1.3.2.103

2017 DOE BioEnergy Technologies Office
Project Peer Review
March 7, 2017

Technology Area: Advanced Algal Systems
Principal Investigator: Mike Guarnieri
Organization: National Renewable Energy Laboratory

Goal Statement

- **Project Goals:**
  - Identify novel**, halotolerant algal strains** with exemplary productivity metrics, **superior to current SOT**.
  - Develop enabling genomic and genetic tool boxes for top-candidate strains.
  - Public strain dissemination and data deposition to encourage widespread adoption and development of strains.

- **Outcome:** Development of halotolerant algal strains with economically-viable productivity metrics.
  - Generation of comprehensive knowledgebases and genetic toolkits for top-candidate strains.

- **Relevance to Bioenergy Industry:**
  - ABP activities will explicitly target key cost hurdles identified via TEA:
    - Enhanced algal biomass productivity and value
    - Robustness under extreme winter- and summer-deployment conditions.
  - Addresses sustainability concerns related to fresh vs. saltwater deployment.
  - Encourages the creation of a new domestic bioenergy industry.
  - Enables the sustainable, nationwide production of biofuels that are compatible with transportation infrastructure.

Quad Chart Overview

Timeline

- Project start date: October, 2015
- Project end date: September, 2018
- Percent complete: 50%

Barriers

- **Aft-A:** Biomass Availability & Cost
  - *Identification of strains with enhanced biomass productivity*
- **Aft-B:** Sustainable Algae Production
  - *Halotolerant strains, saltwater cultivation*
- **Aft-C:** Biomass Genetics & Development
  - *Genomics, CRISPR/Cas9 and episomal tool development*

Budget

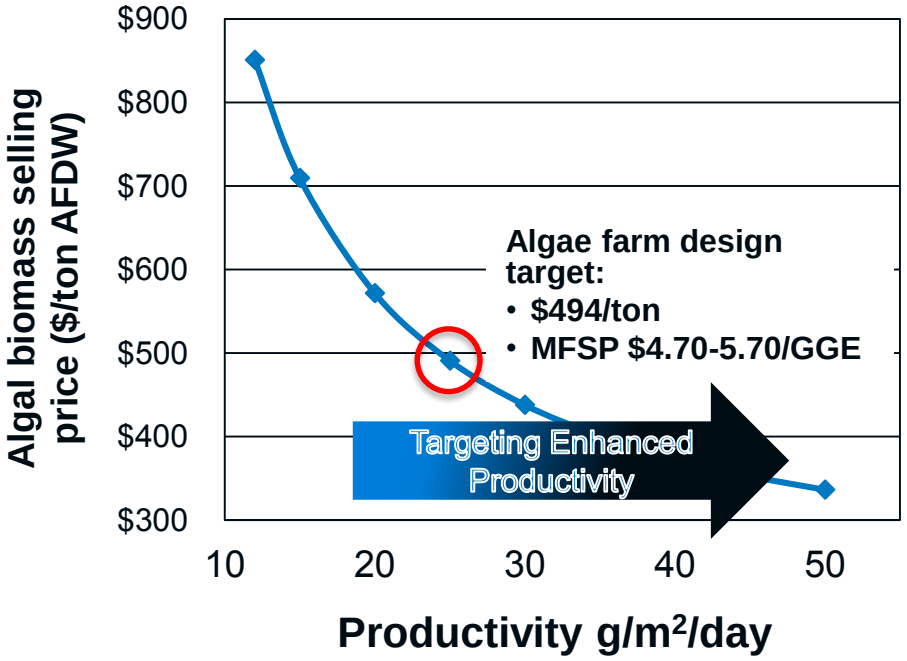
FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17->End Date)
$273K	$300K	$1.2M

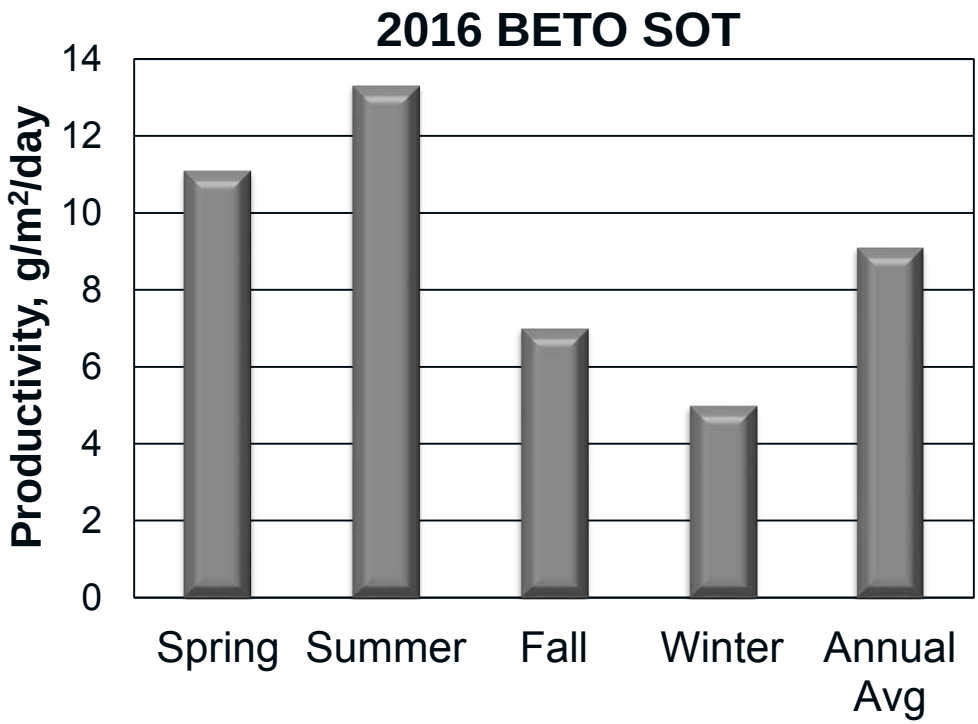
Partners

- **LANL/LBNL:** Functional genomics
- **CSM (25%):** Genetic tool development
- **ATP^3/ASU:** Outdoor deployment

Project Overview

- **Context:** Algal biomass productivity & value enhancements are essential.
  - *Current top candidate strains fall short of target metrics*





Specific Project Goals:

- Screening, down-selection, and characterization of high-productivity halotolerant strains.
- Outdoor deployment and optimization of cultivation parameters.
- Development and optimization of advanced genomic and genetic tools.
- Public dissemination of strains, associated omic and meta-data, & genetic tools.

Management Approach

- Strain Screening, Characterization, and Down-Selection
- Genetic Tool Development

- Functional Genomic Analyses

- Outdoor Deployment

- **Diverse staffing plan:**
  - Molecular and Microbiologists
  - Computational Biologists
  - Analytical chemists
  - Cultivation expertise

- **Extensive Team Interaction:**
  - Regular PI-technical staff interaction
  - Monthly NREL team, platform, and external ABP meetings
  - Quarterly BETO meetings

- **Industry Engagement & Exchange**

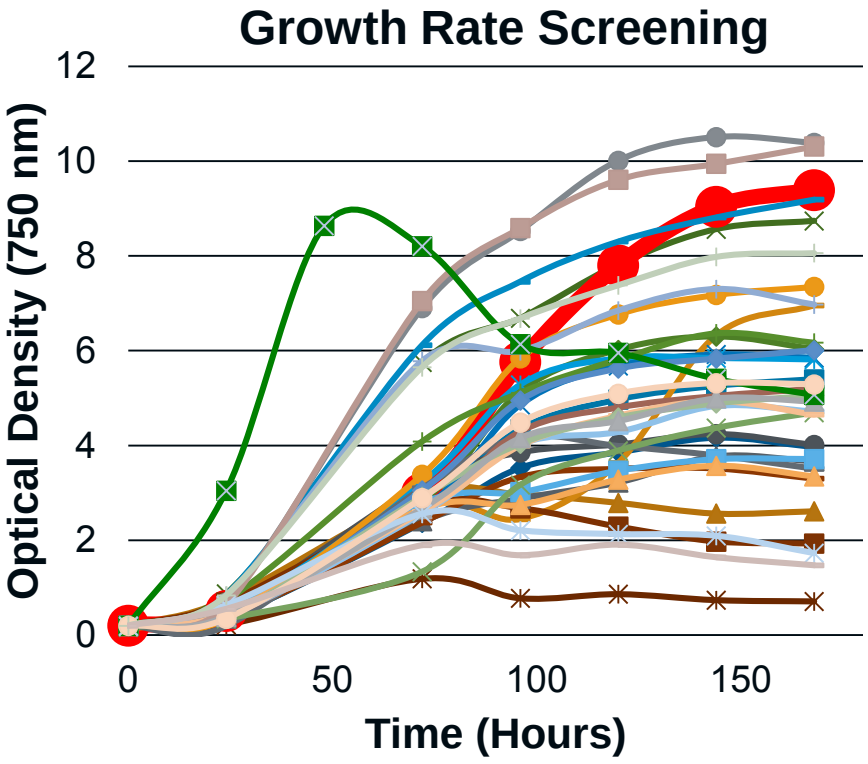
Technical Approach

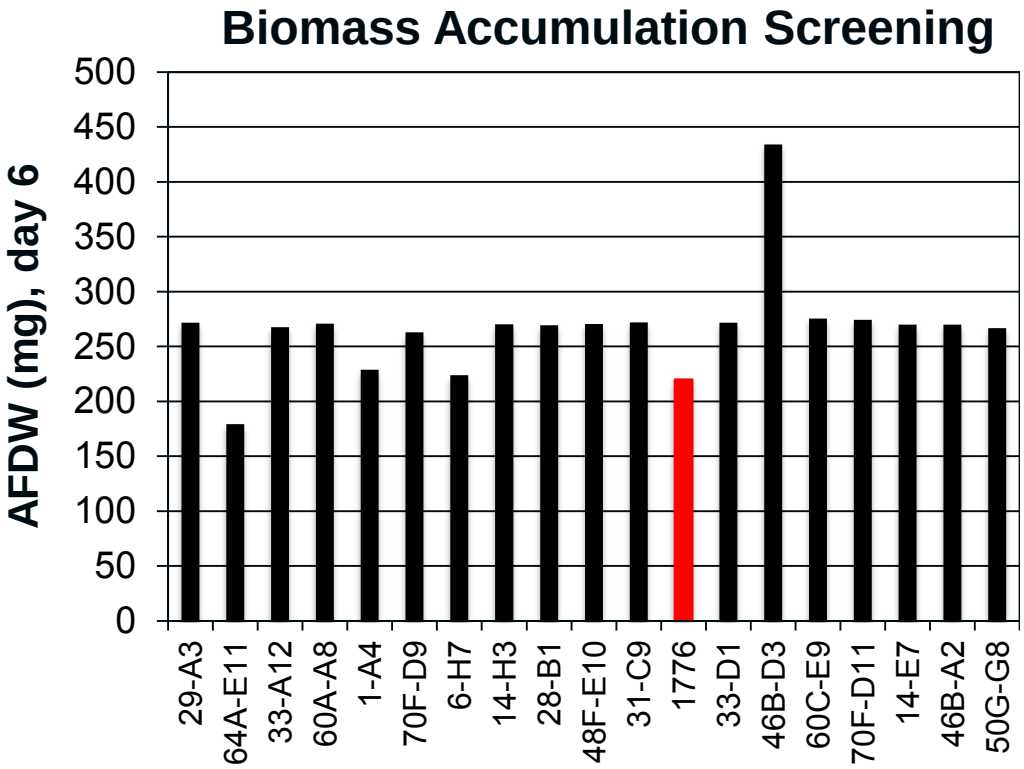
- **Approach:** Leverage core capabilities of partner institutes in i) molecular strain development, ii) *omic* analyses, and iii) algal cultivation, in order to identify and develop deployment-viable algal production strains.
  - Integrated and iterative simulated outdoor-outdoor screening and evaluation approach will mitigate risk associated with transition from lab to commercial pond deployment.

- **Major challenges:**
  - **Technical:** (i) identification of high-productivity algal strains, (ii) generation of reproducible, high-efficiency genetic tools
  - **Market:** Conduct laboratory testing and outdoor field demonstrations to reduce risk to early adopters.

- **Critical success factors:**
  - Demonstrate robust, high-productivity saltwater outdoor deployment.
  - Establish comprehensive strain knowledgebases.
  - Achieve routine, facile genetic transformation.
  - Widespread adoption of strains and associated tools.

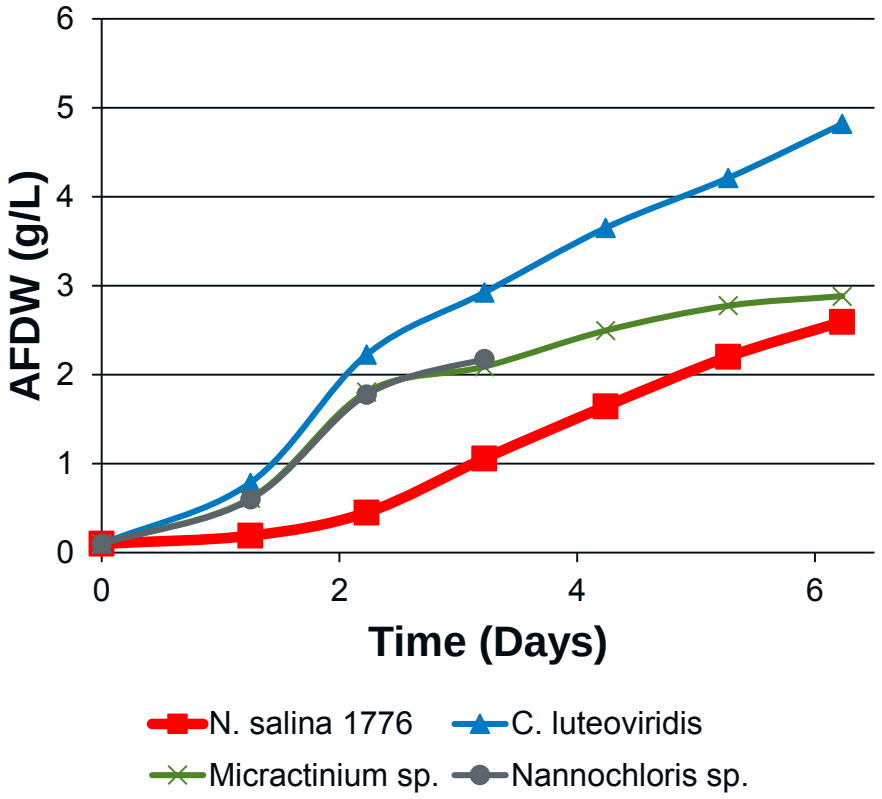
Identification of Halotolerant Strains

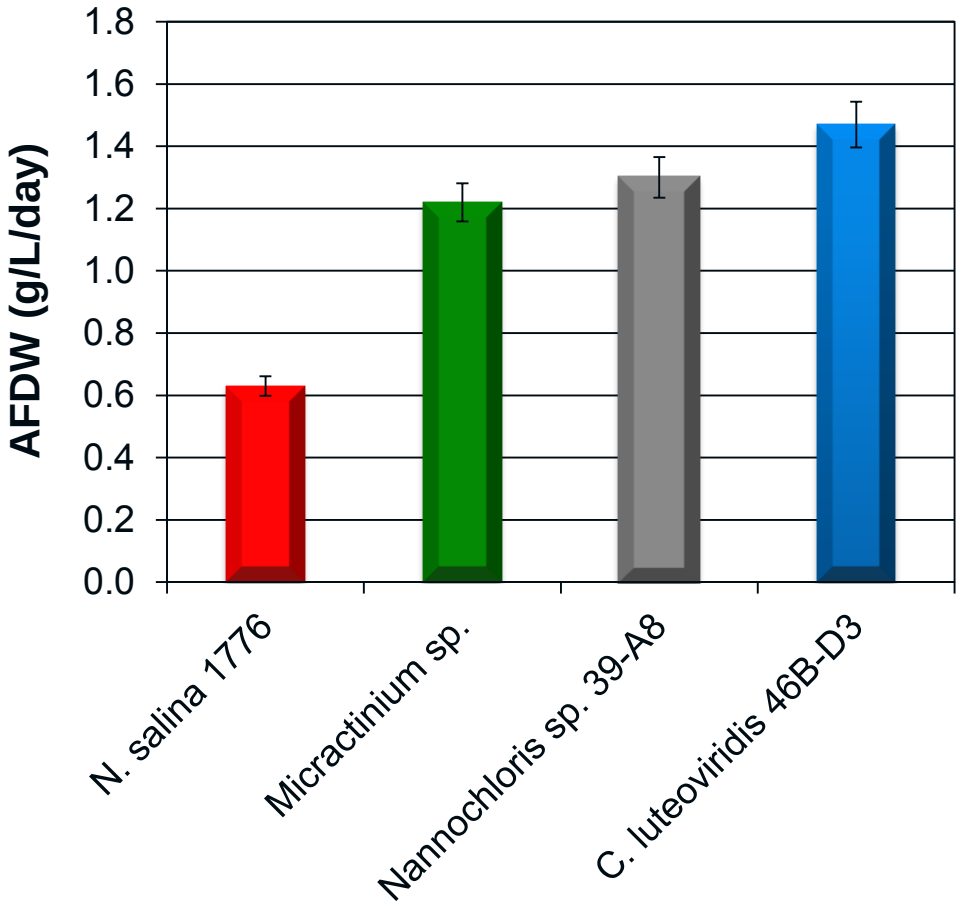




- Developed a mid-throughput reactor for rapid screening of **>300 strains.**
  - Three independent trials conducted to down-select top-candidate strains.
- Simulated average light and temp. over the course of 3-month ATP[3] growth season.
  - A series of strains displayed superior doubling times to NAABB benchmark strain, *N. salina* (highlighted in red) under both summer and winter conditions.
  - *Nannochloris* strain among fastest doubling time reported to date for a eukaryotic alga.

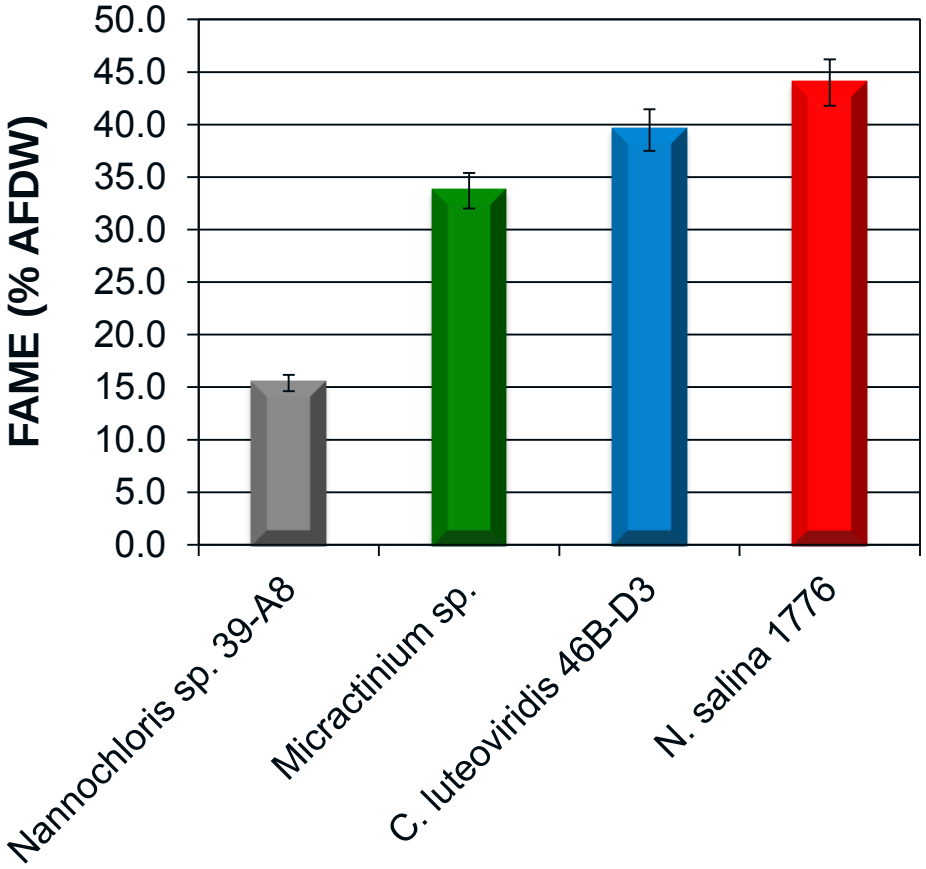
Biomass Productivity (Summer Simulation)

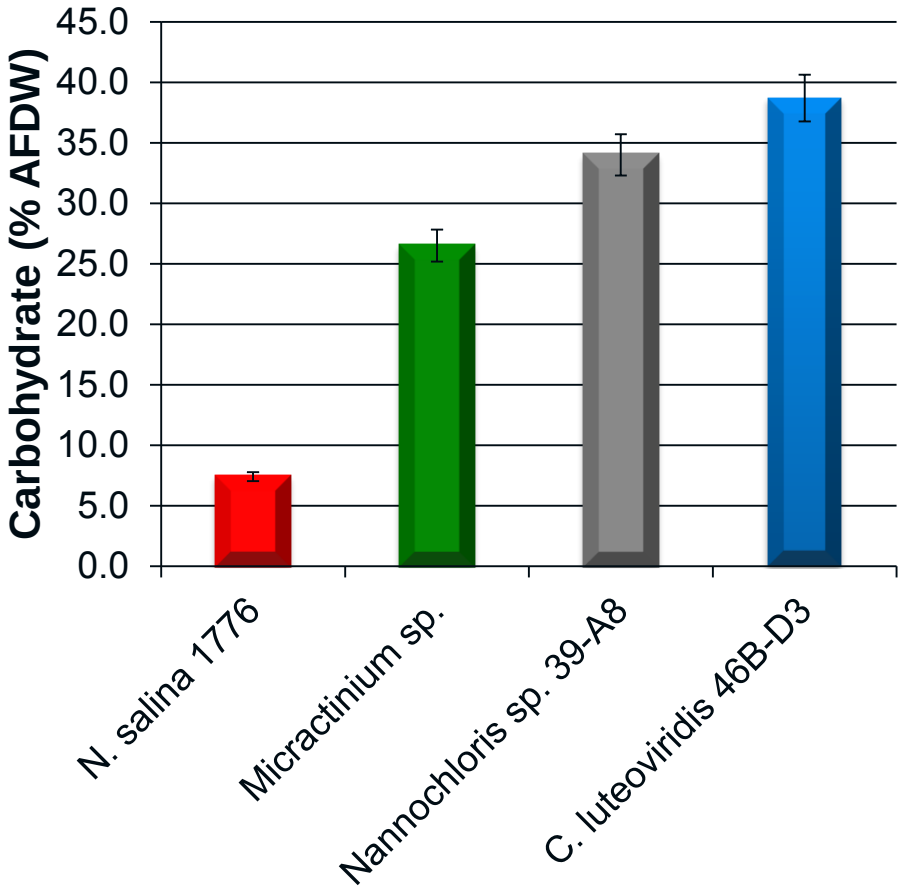




- Top-candidate strains displayed superior max biomass accumulation rates compared to *N. salina.*

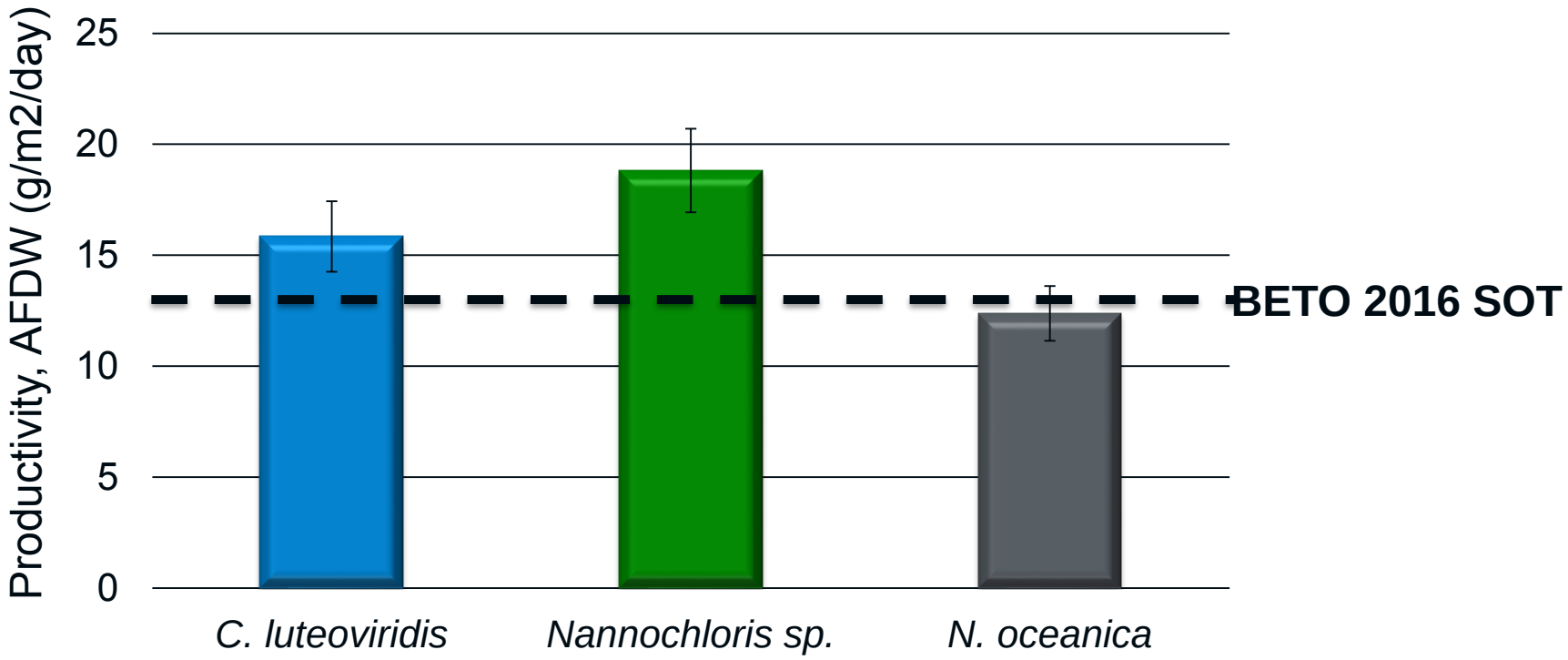
Compositional Analyses (Summer Simulation)





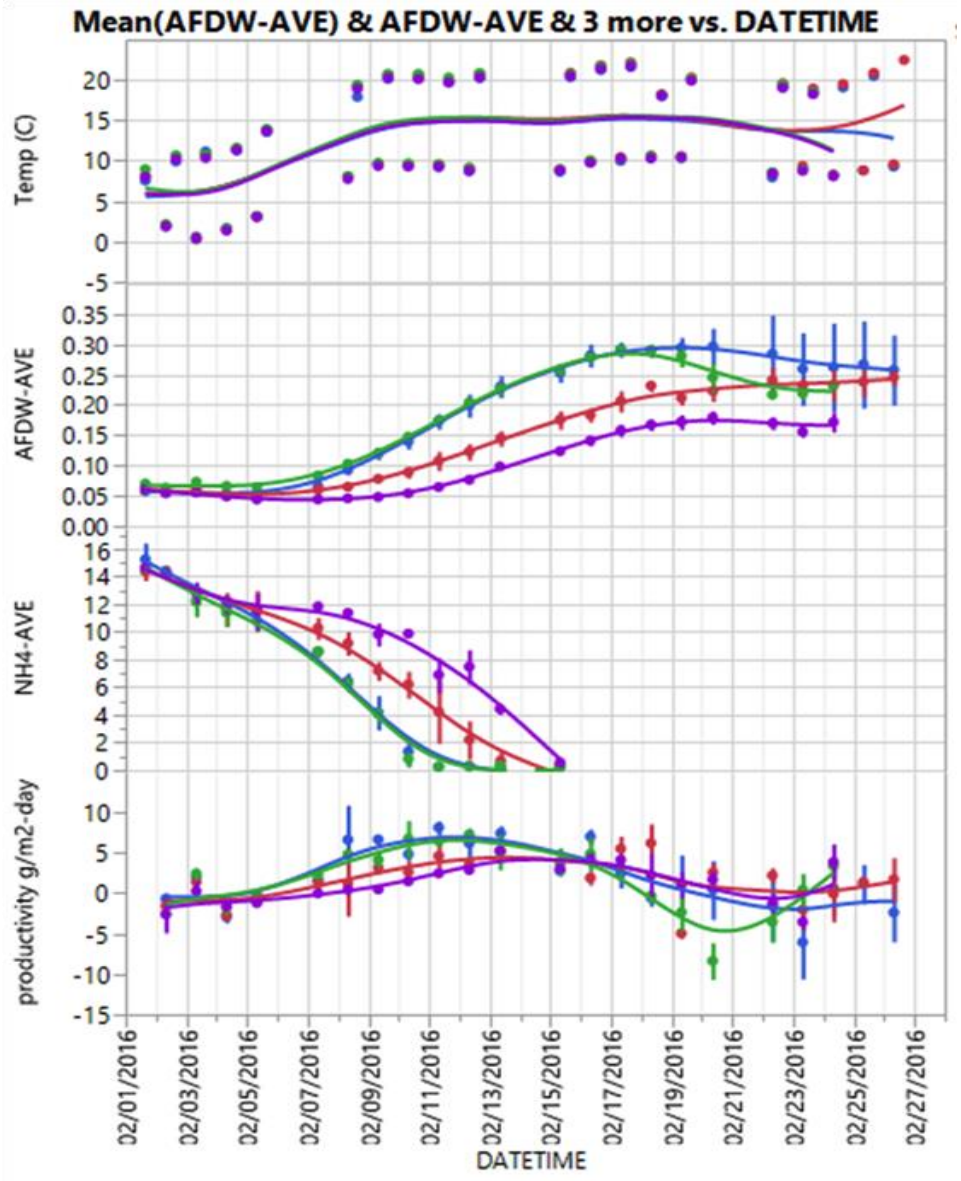
- *C. luteoviridis* and *Micractinium sp.* display robust lipid accumulation compared to benchmark.
  - Both strains represent robust lipid and carb producer
- Strains suitable for CAP and/or HTL processes.

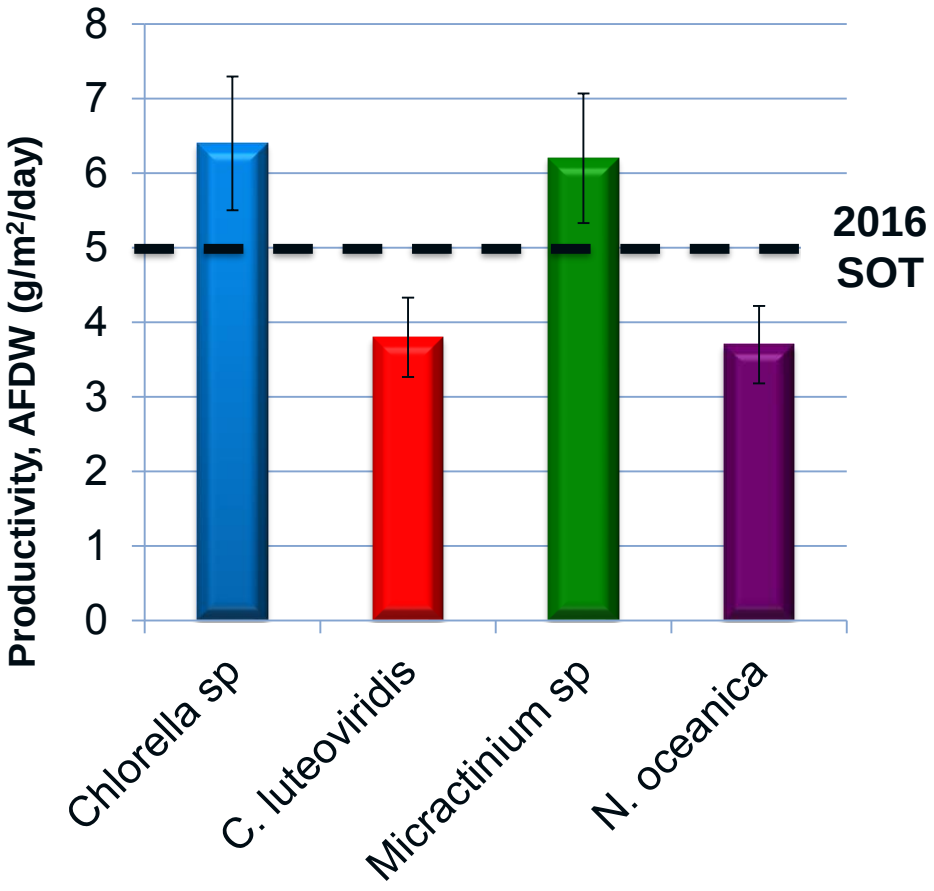
Summer Deployment at ATP3 Testbed



- Outdoor deployment validated indoor screening.
  - Top candidate strains outperformed *Nannochloropsis oceanica*.
  - *Nannochloris* sp. 39-A8 contaminated a series of ponds at ATP3, indicating “weedy” nature and strain robustness.
- **Algal biomass productivity > SOT.**

Winter Deployment at ATP3 Testbed

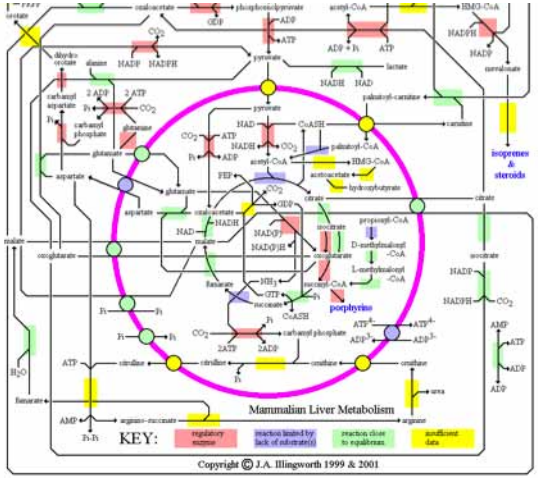


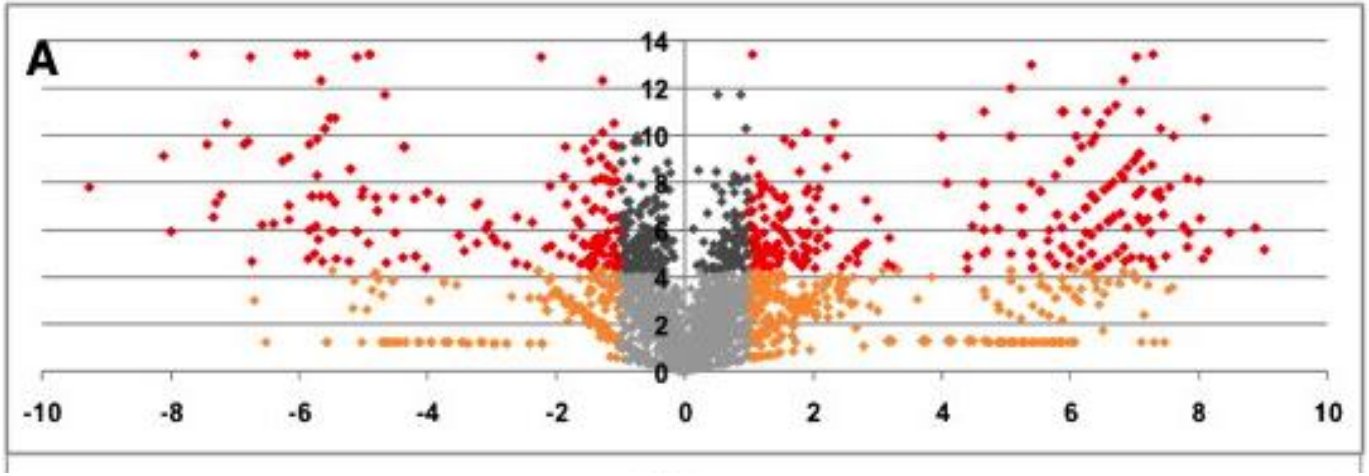


- Outdoor deployment validated indoor screening.
- **Algal biomass productivity > SOT**

Genomic & Transcriptomic Analyses Completed

- Complete genome sequence and assembly of top two candidate winter and one summer strain completed.
  - *Micractinium sp*., *Nannochloris sp*., and *Chlorella sp*.
  - +/- Nitrogen omic datasets obtained for top summer strain.
  - Combined, these omic analyses will enable pathway mapping, and identification of regulatory elements and strain-engineering targets.





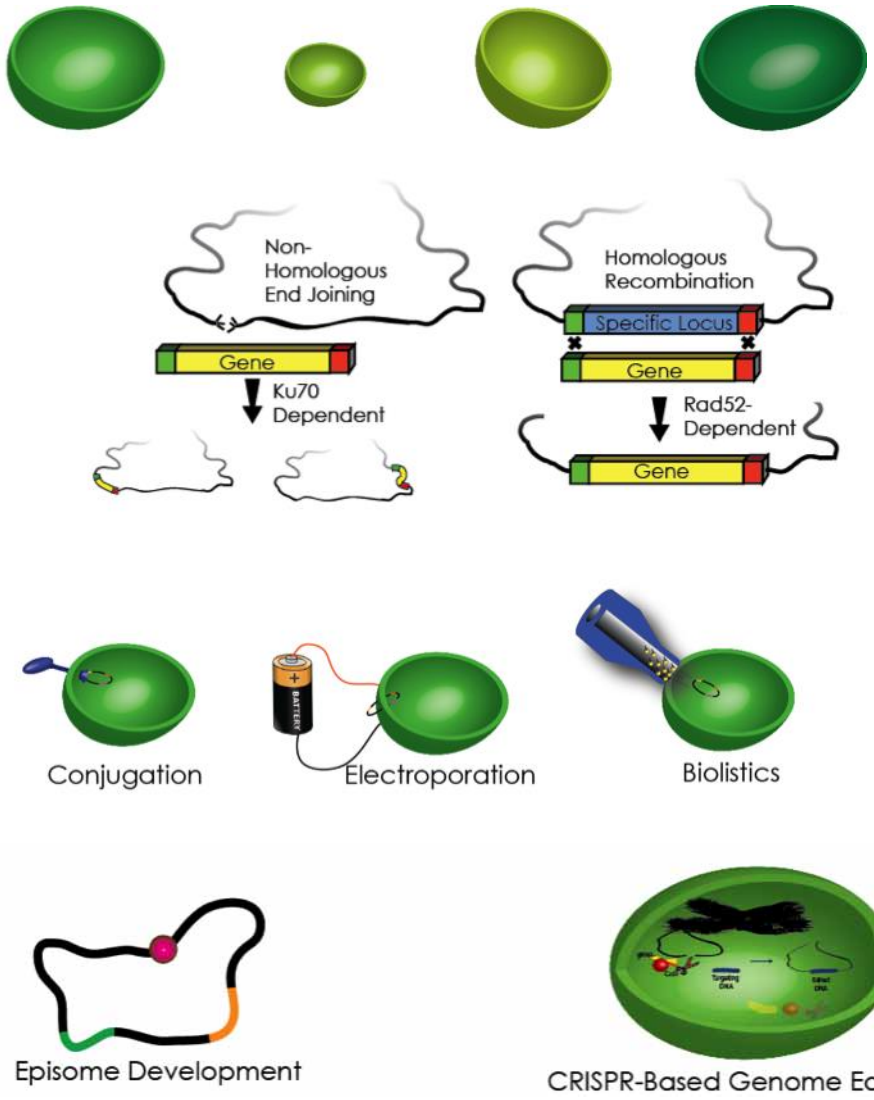
Genetic Toolbox Development

I. Strain Selection

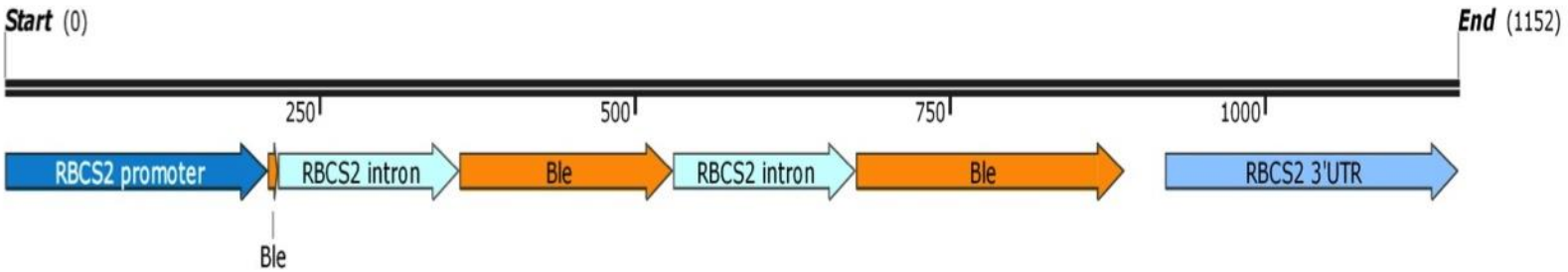
II. Random & Targeted Integration Tool Development

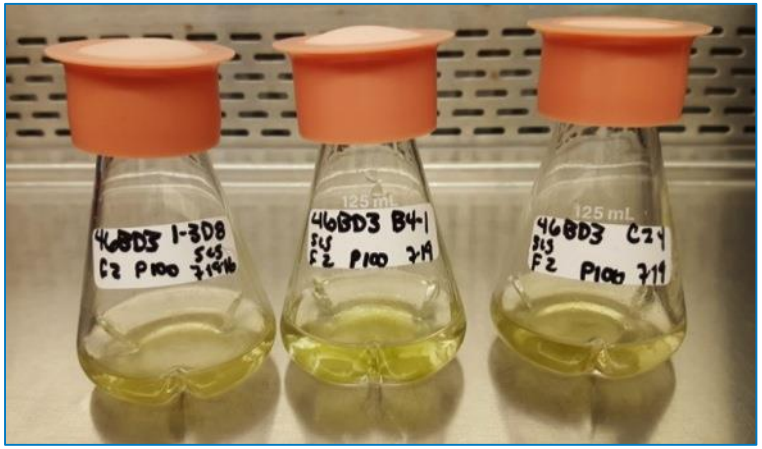
III. Transformation

IV. Advanced Genome Engineering



Successful Genetic Transformation





- **Initial transformation trials yielded viable transformants for 3-of-4 top candidate strains.**
  - Random integration cassettes coupled w/ electroporation
  - Stable following two serial transfers.
  - Targeted (HR) integration trials underway.

Relevance

- Development of high-productivity, high-value, halotolerant strains
  - Reduces reliance on freshwater deployment.
- Rapid dissemination and exchange of strains and meta-data will enable rapid adoption by algal industry.
  - Three MTAs in place with commercial algal entities.
- Addresses MYPP barriers
  - Targets techno-economic AND sustainability barriers
- Genetic and genomic tool development allows for targeted enhancement of strains for biomass, fuel and high-value co-product pursuits.

Future Work

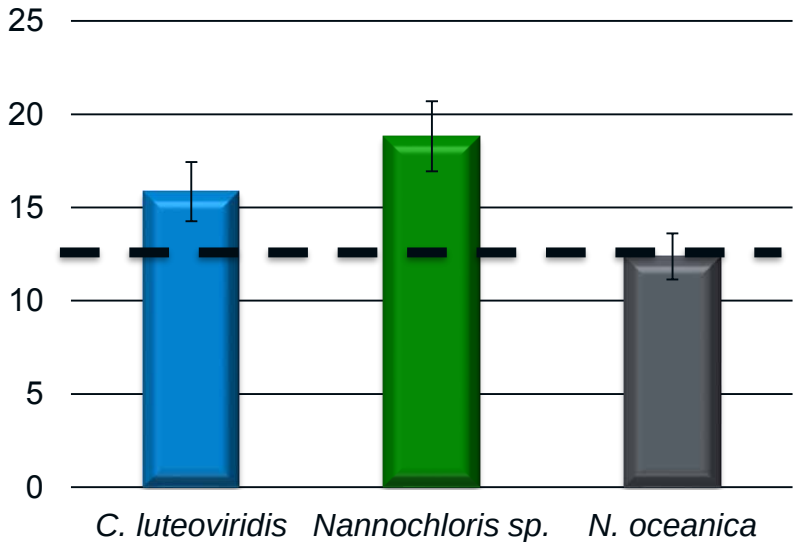
- Future work will primarily target advanced, broad-host range genetic toolkit development to enable targeted strain-engineering.
  - CRISPR/Cas9 and episomal artificial chromosome design.

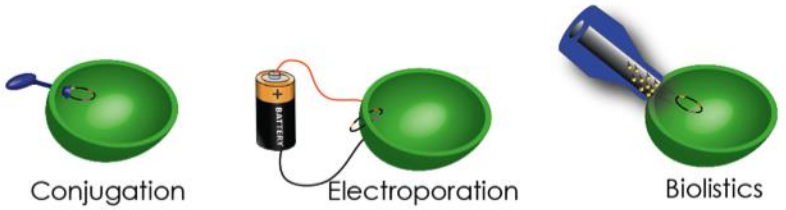
- **FY17 Annual Target:** Down-selection and optimization of advanced genetic tools, demonstrating stable transgene expression following serial transfer.
  - Complete genomic sequencing and annotation of top candidate summer and winter strains.
    - Second summer strain to be sequenced in FY17 in coordination with LANL/LBNL.
  - Outdoor deployment of top candidate strains at the ATP^3 Arizona test bed employing optimized parameters.

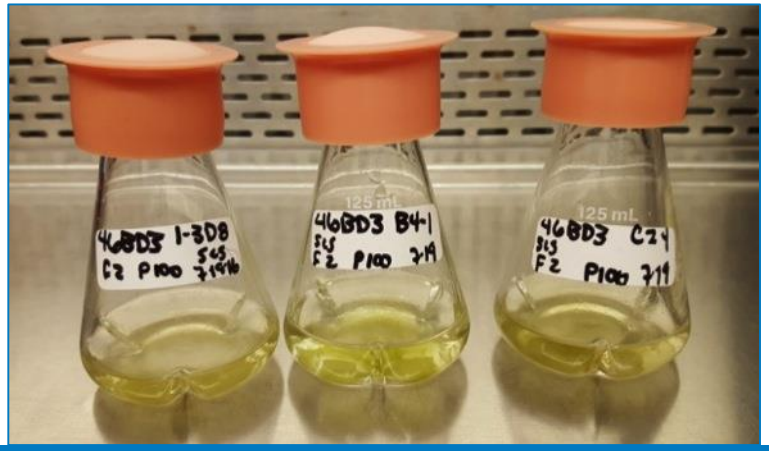
- **FY18 Targets:** Maximize biofuel production potential and increased outdoor cultivation robustness (as related to extended cultivation and pond-crash mitigation) via targeted strain-engineering strategies.
  - Iterative outdoor deployment to assess strain enhancements.

Summary

- Extensive algal strain screening and characterization has led to down-selection of high-potential candidate deployment strains.
  - ***Strains display robust outdoor biomass productivity > SOT.***
- Complete genomic blueprints established for top-candidate strains in collaboration with LANL/LBNL.
- Baseline genetic tools have been established enabling random integration.
- Public dissemination of strains & metadata via greenhouse.lanl.gov will enable rapid adoption.
- Future efforts will encompass advanced genetic tool development and targeted strain improvements.







Acknowledgements

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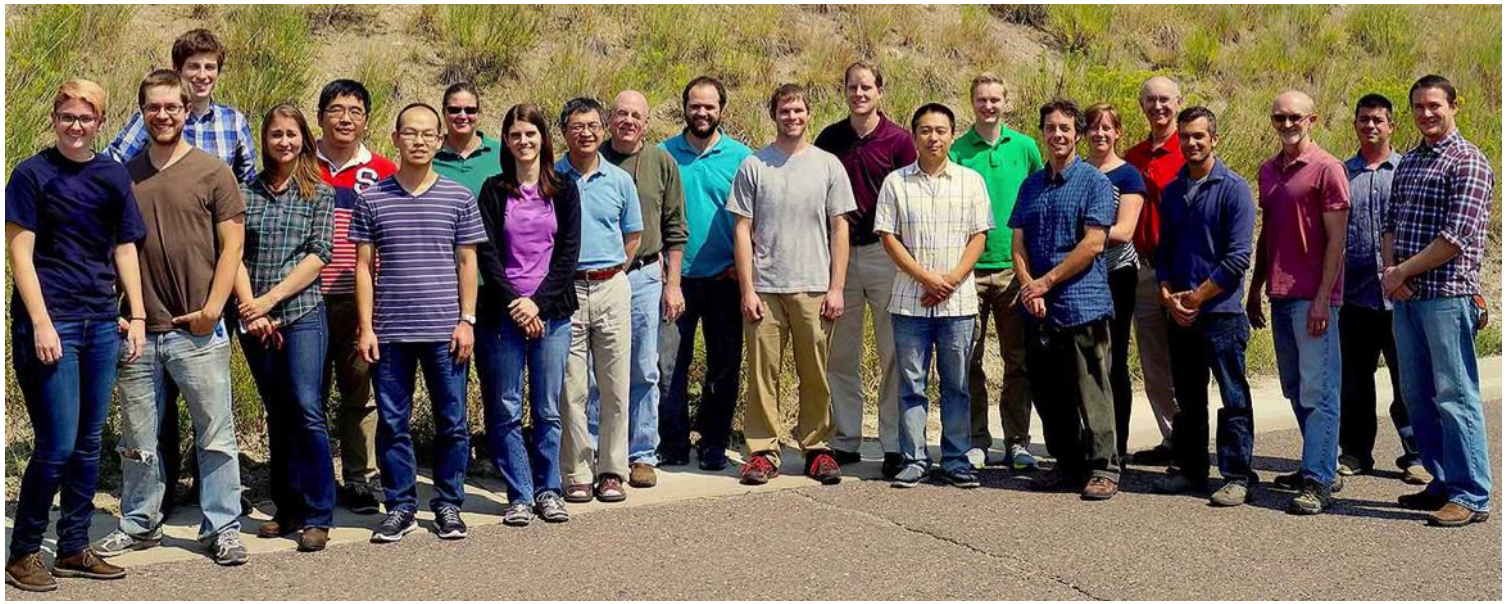

Sharon Smolinski
Jeff Linger
Calvin Henard
Nick Sweeney
Phil Pienkos
Stefanie Van Wychen
Ryan Davis
Matt Posewitz (CSM)
Luke Dahlin (CSM)
Shawn Starkenberg (LANL)
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Algae Strain Characterization and Improvement

Characterization of Productivity and Robustness

DISCOVR

Genome Sequencing, Functional 'Omics, Metabolic Mapping

Greenhouse: Comprehensive Knowledge Base of Algal Feedstocks

Genetic Blueprint of Microalgae Carbon Productivity

Strain Improvement: Genetic Modification & Non-GM Strategies

Algae Biotechnology Partnership

Functional Characterization of Cellular Metabolism

Multi-scale Characterization of Improved Algae Strains

Breeding Algae for Long Term Stability& Enhanced Biofuel Prod'n

Strategy

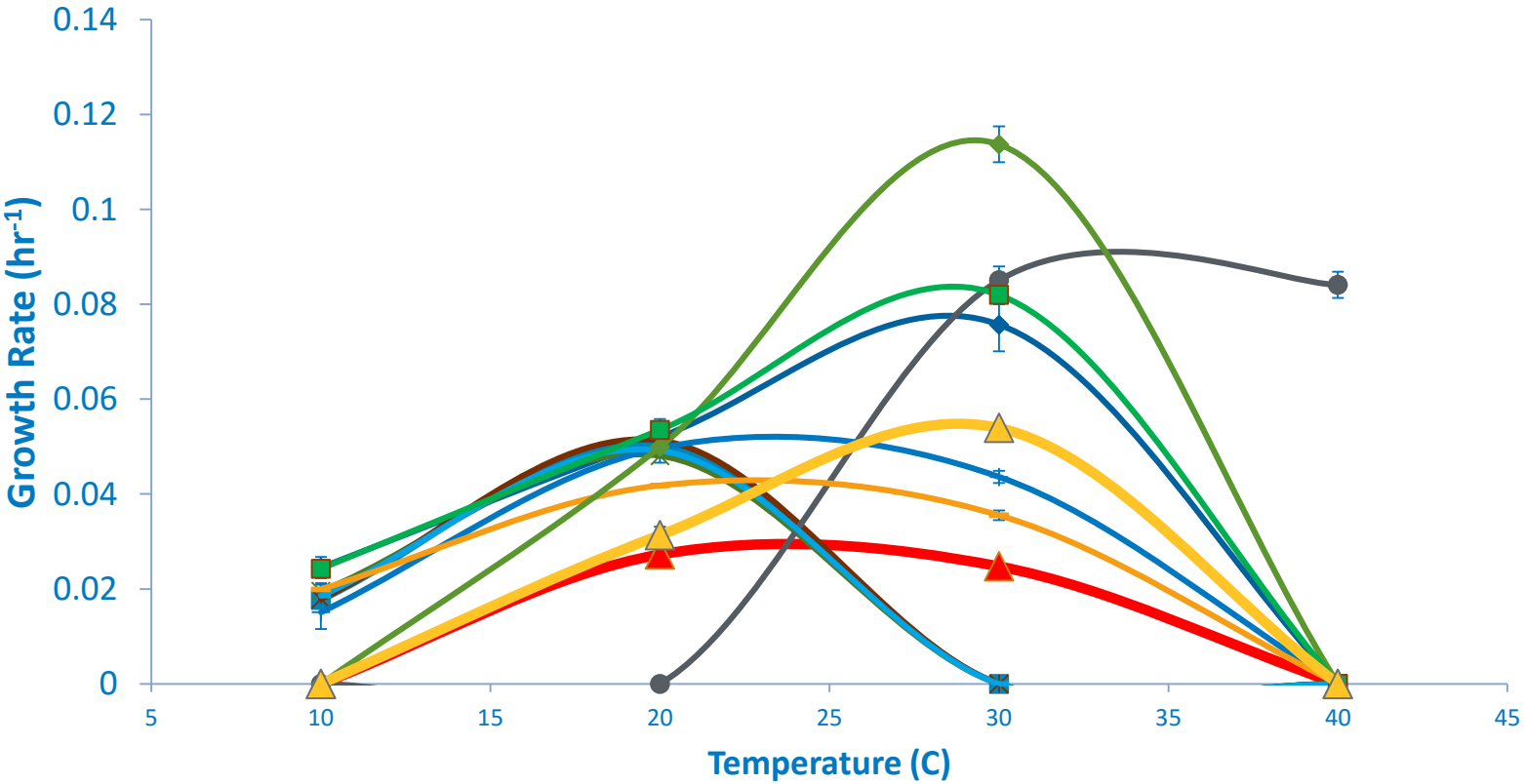
Use a multi-lab consortium approach to establish a state-of-the-art platform for the deep *characterization of new strains under outdoor-relevant conditions*

Leverage the expertise at LANL and LBNL/JGI to deliver the *data and tools* required to understand algae metabolism and develop GM tools

Use a range of expertise & approaches across Labs to deliver *improved algae strains, and a suite of tools* that are effective in our top strains of interest and are also broadly applicable to new strains of interest (from DISCOVR or external stakeholders)

GOAL: *Deliver deeply characterized and improved strains, with accompanying data and tools, to stakeholders including industry, academics, and other BETO projects (e.g. BioFoundry)*

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.

Response to Reviewers' Comment

- This project was not subjected to prior review.

Publications, Patents, Presentations, Awards, and Commercialization

- Publications:
  - Dahlin, L. and Guarnieri, MT. 2016, *Curr Biotech*. 5(3); 192-197.
  - Henard, C., et al. 2017, *Frontiers in Bioeng*. *In Press*.

- Presentations:
  - Guarnieri, MT, et al. 6th Annual Conference on Algae Biomass, Biofuels, and Bioproducts, San Diego, CA, USA. 2016
  - Dahlin, L, et al. Algae Biomass Summit, Phoenix, AZ, USA. 2016.

- Commercialization Efforts:
  - Three material transfer agreements are in place with commercial algal entities, encompassing i) strain exchange and screening, ii) media and deployment evaluation, and iii) halotolerant strain evaluation for nutraceutical potential