Algae Biotechnology Partnership

WBS 1.3.1.131-3

BETO Peer Review
April 3, 2023 | Denver, CO
Luke Dahlin, Monica Sanchez, Matt Posewitz,
Chuck Smallwood, Shawn Starkenburg, **Mike Guarnieri**

NREL NATIONAL RENEWABLE ENERGY LABORATORY

Los Alamos NATIONAL LABORATORY

Sandia National Laboratories

COLORADO SCHOOL OF MINES

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
- Genetic engineering has enabled enhanced growth rate and carbon storage accumulation

Problem: Universal genetic engineering approaches are generally lacking for microalgae, substantially hindering the strain development timeline for newly-emerging strains.

Prior Period of Performance: Our team successfully established broad-host range, orthogonal genetic tools in a series of top-candidate deployment strains.

Goal:

1. Develop a high-throughput, automated pipeline for broad-host range algal genetic engineering and phenotyping.
2. Generate publicly accessible, genome-scale libraries in a mass cultivation-relevant algal strain, *Picochlorum renovo.*

Approach - Project Management and Organization

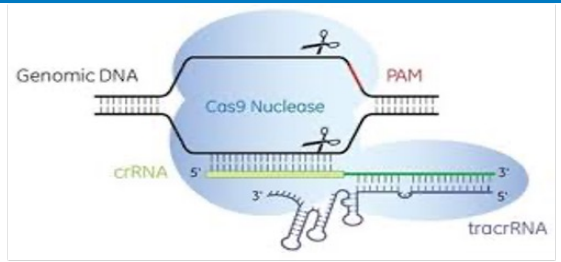
Task 1 – Tool Development
Mike Guarnieri (NREL)

Task 2 – Validation
Chuck Smallwood (Sandia)

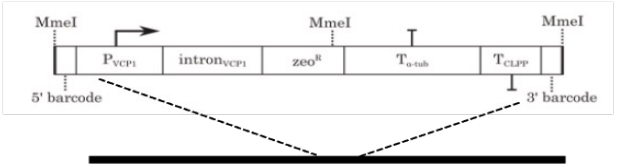
Task 3: Phenotyping
Shawn Starkenburg (LANL)

- Biweekly full team meetings (NREL-LANL-Sandia-CSM)
- Weekly meetings with PI-staff
- Quarterly meetings with BETO PM/TM
- Task managers are responsible for: reporting, tracking, and budgeting

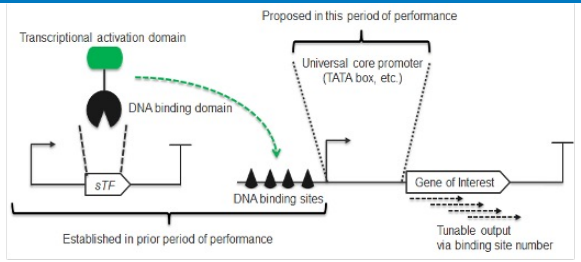
Approach – Task 1: Genetic Tool Development



CRISPR/CAS9



Random Insertional Mutagenesis



Universal Promoter

Task 1 will <u>deliver high-throughput-capable genetic engineering tools suitable for high-efficiency transformation and library generation</u>.

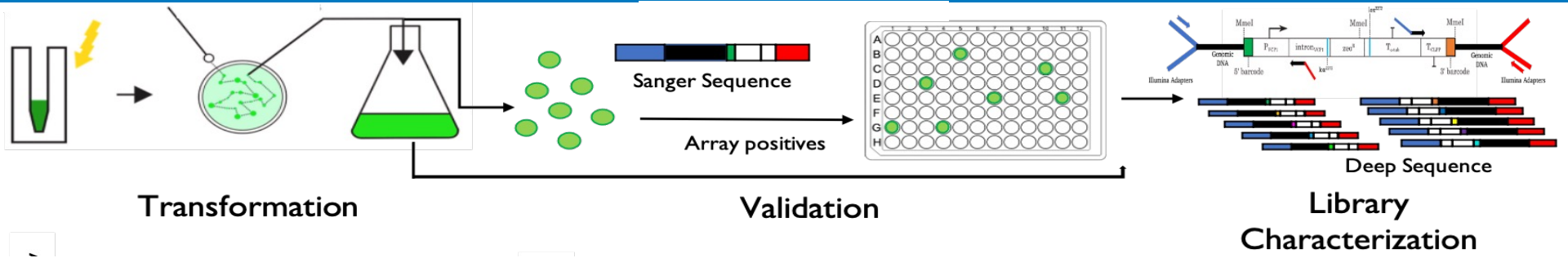
- Random and targeted CRISPR mutagenesis for KO library generation.
- Synthetic control systems for overexpression library generation.

Risks & Challenges:

- Genetic tool transferability between genus/species
- Low transformation efficiency

Major Milestones: (FY22|Q4) Achieved >5X transformation efficiency enhancement in *P. renovo*, exceeding 100cfu/ug DNA. ✓

Approach – Task 2: Implementation and Validation



Task 2 will deliver a publicly accessible, genome-scale mutagenic library of *Picochlorum spp*. for basic and applied science applications.

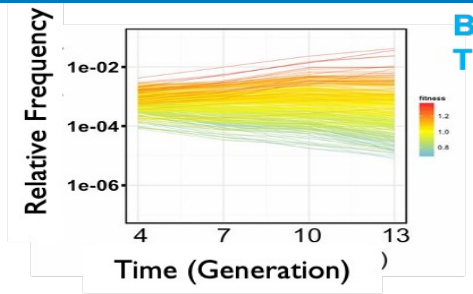
Risks & Challenges:

- Insertional library failure (insufficient genome coverage)
- Truncation of integration cassette

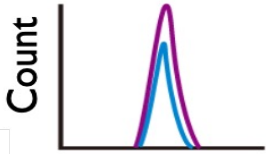
Major Milestones:

- **(Go/No-Go)** Demonstrate genome scale insertion at >1000 unique sites in the *P. renovo* genome (~10% of *P. renovo* genome).
- **(FY23|Q4)** Generate >10,000 mutants targeting 50% of coding regions.

Approach – Task 3: Phenotyping and Characterization



Barcode Tracking

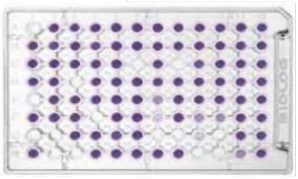


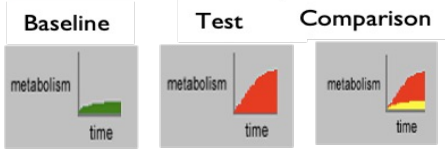
Chlorophyll
Lipids

Flow Cytometry

High Throughput Phenotyping

Candidate Strains





Biolog Phenotyping

Metabolic Characterization

Task 3 will deliver a high-throughput (96-well plate-amenable) pipeline to conduct rapid phenotyping and characterization of mutant libraries.

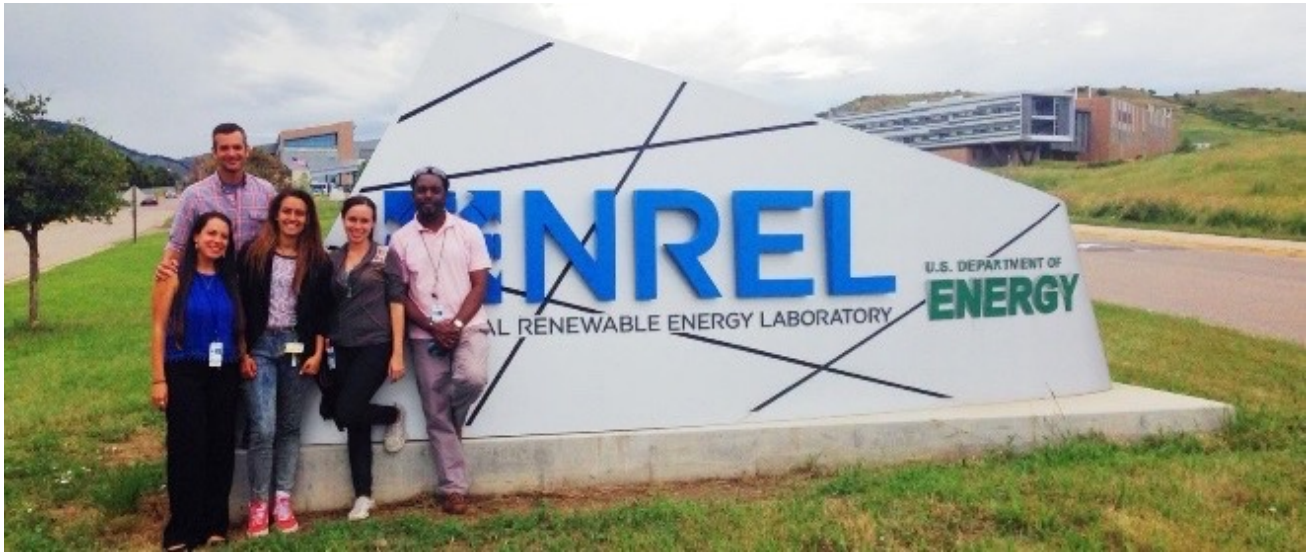
Risks & Challenges:

- High-throughput and/or automated adaptation of established phenotyping methodologies.

Major Milestones: (FY24|Q4) Achieve automated, high-throughput generation and phenotyping of 96 mutants in microtiter format in < 2 weeks.

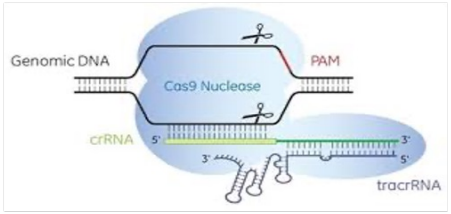
Approach: Diversity, Equity, and Inclusion

- We have established a Memorandum of Understanding with University of Puerto Rico, an HSI graduating the largest number of Hispanic engineers under the US flag
  - Faculty and student exchange internships, providing opportunities to learn microbial genetics, photophysiology, and applied phycology.
  - Algal genomics workshop at UPR to provide hands-on tutorial in algal genome sequencing, data processing, and data analysis.



2. Progress and Outcomes

Task1: Tool Development



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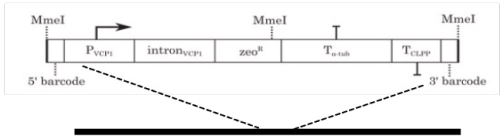

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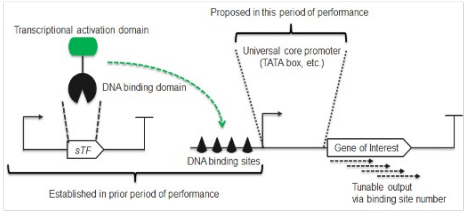
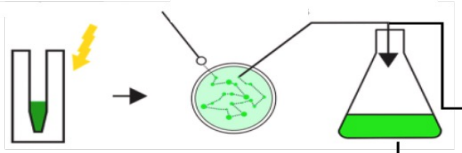


Universal Promoter

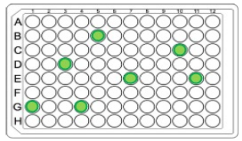
Task2: Implementation and Validation



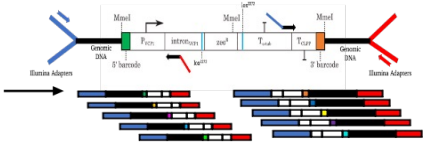
Transformation

Apply to *Picochlorum*

Array positives



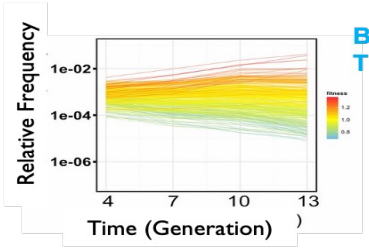
Validation



Deep Sequence

Library Characterization

Task3: Phenotyping and Characterization



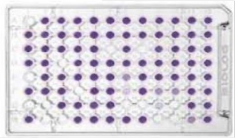
Barcode Tracking

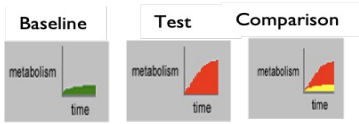
Chlorophyll
Lipids

Flow Cytometry

High Throughput Phenotyping

Candidate Strains

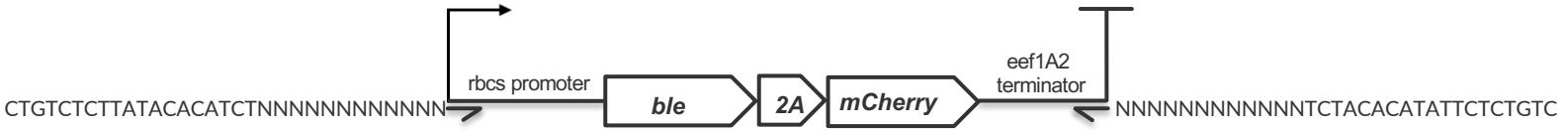




Biolog Phenotyping

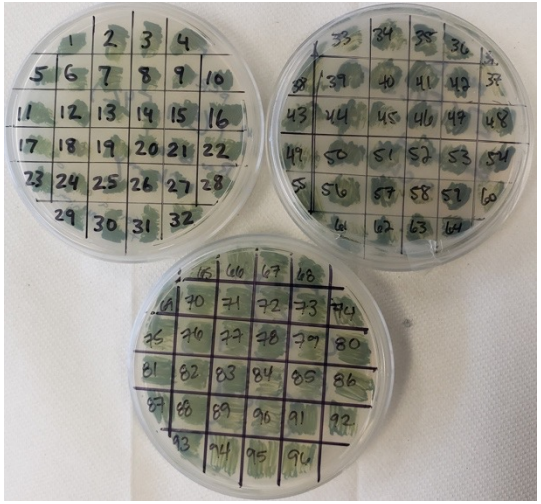
Metabolic Characterization

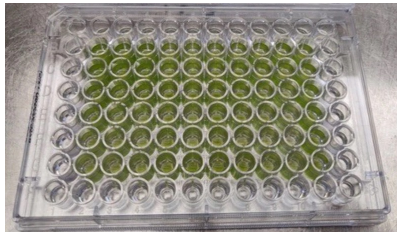
Transformation Optimization Enables High-Throughput Mutant Generation



FY22 | Q4 Milestone ✓

Electroporation → Selection → Indexing/ Patching



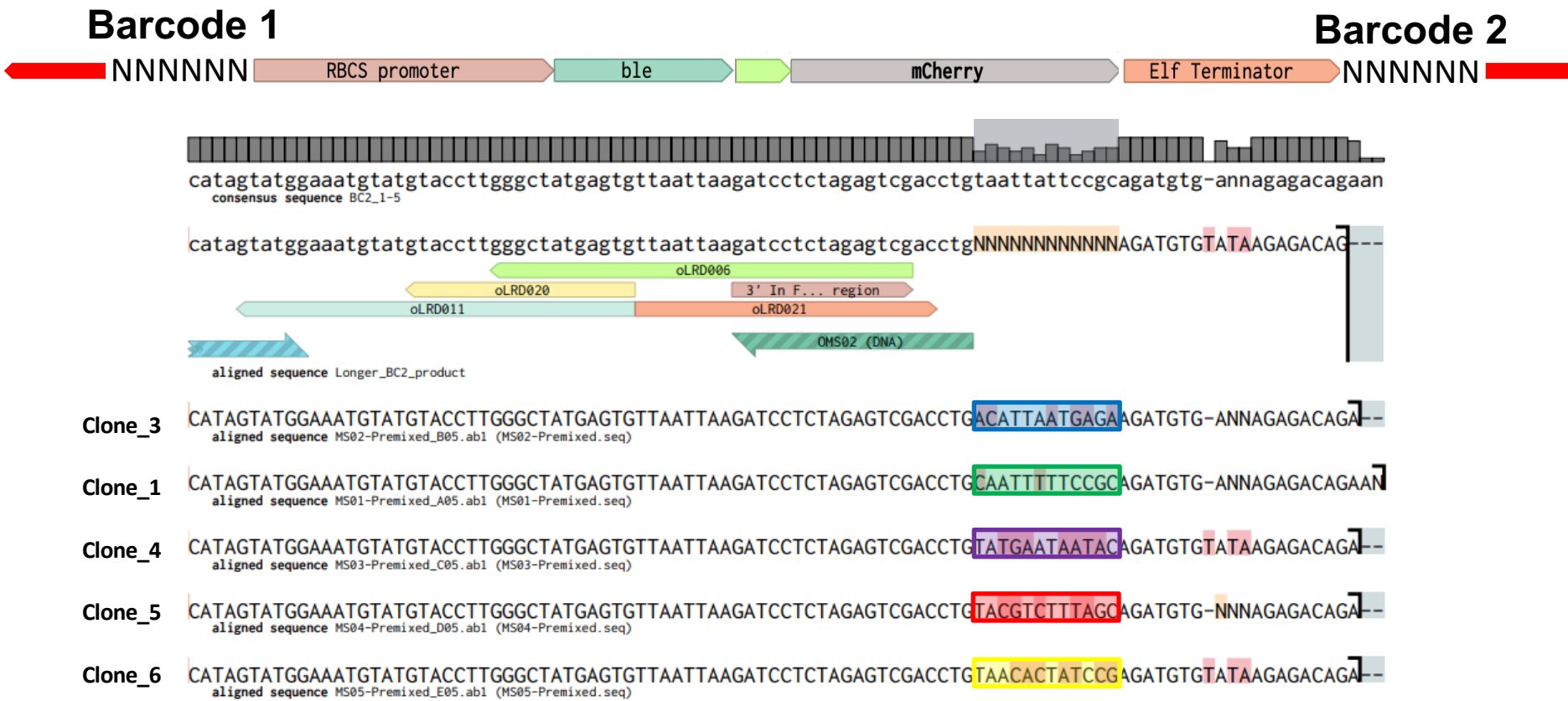


Hand off to Sandia for barcode validation

- Transformation optimization:
  - Cell Number
  - Voltage / Time Constant
  - DNA Dialysis
  - DNA concentration
  - Electroporation Buffer
  - Outgrowth conditions

- *Establishing optimized cryopreservation procedures for stocking 96 well plates of insertional mutants*

Library Validation Enabled via (Next-Gen) Barcode Sequencing



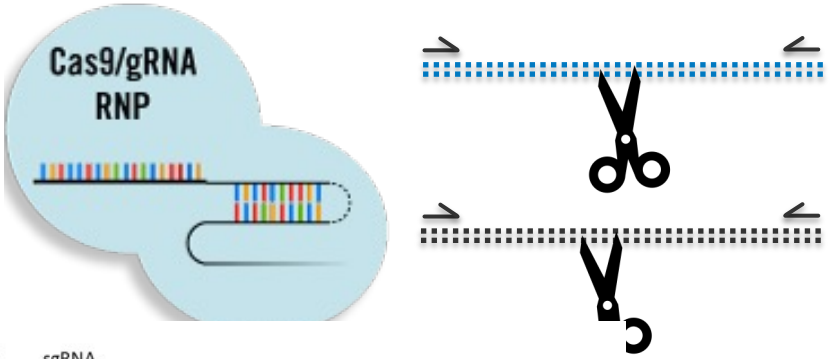
Successful verification of regions of the insertional cassette as well as 96 barcoded loci for 96 different insertional clones.

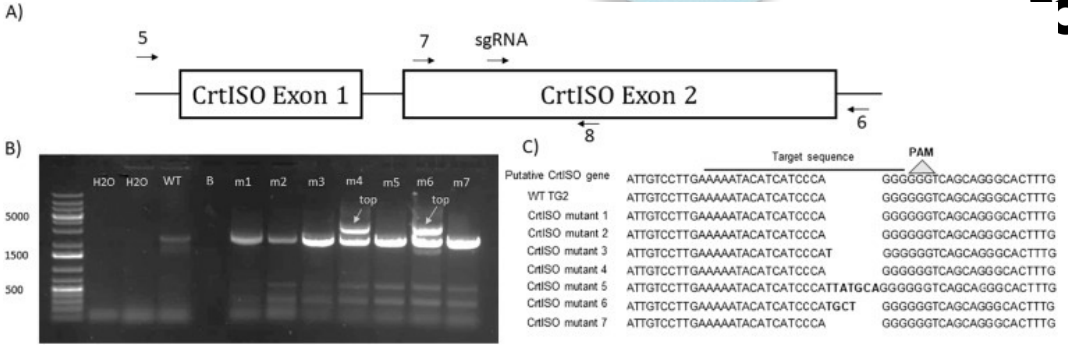
Establishment of CRISPR System Enables Targeted, Diploid Genome Editing

COLORADO SCHOOL OF MINES
Algal Research
Volume 49, August 2020, 101944

Genome editing using Cas9-RNA ribonucleoprotein complexes in the high-productivity marine alga *Picochlorum celeri*

Anagha Krishnan [a], Melissa Cano [a], Tyson A. Burch [a], Joseph C. Weissman [b], Matthew C. Posewitz [a]





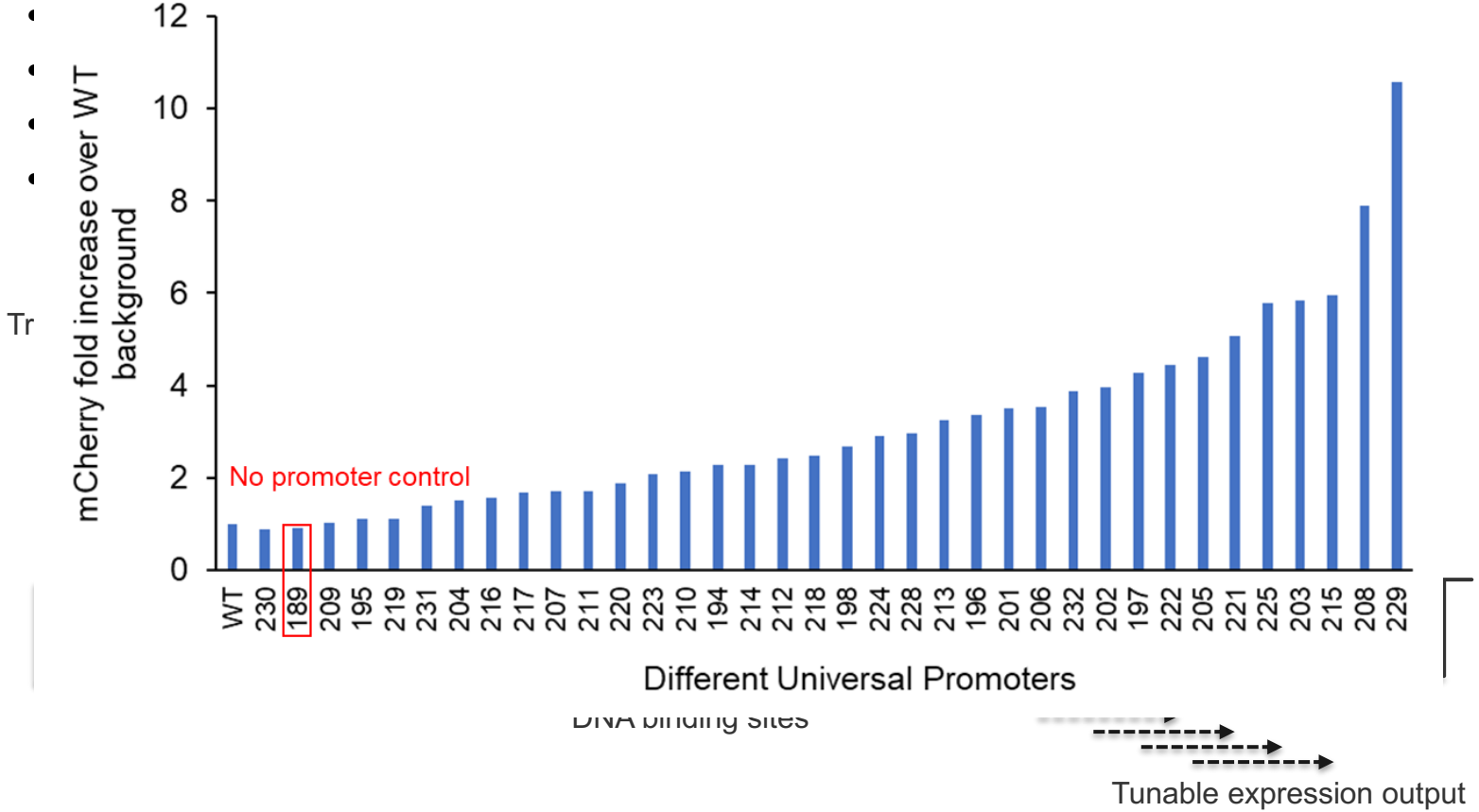
Rapid transfer of CRISPR/Cas methodology from *P. celeri* → *P. renovo*

Next Steps:

- Optimize RNP delivery to increase knockout frequency
- Initiate development of genome-wide gRNA library.

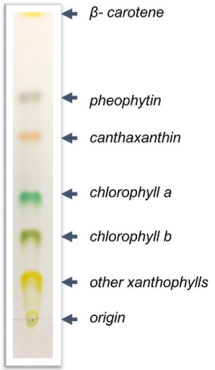
Genetic Tools Expand Library Generation Capabilities

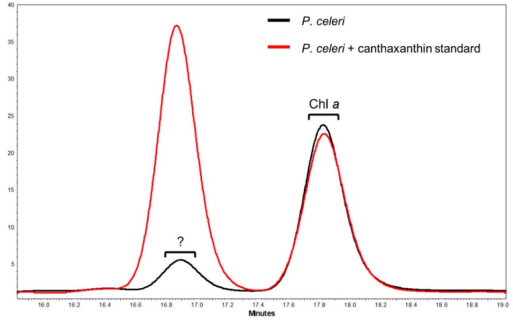
Synthetic Transcription Factor-Universal Core Promoter system enables:



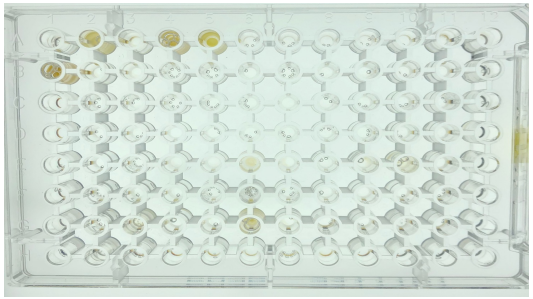
Next Steps: High-Throughput Phenotyping Pipeline Initiation

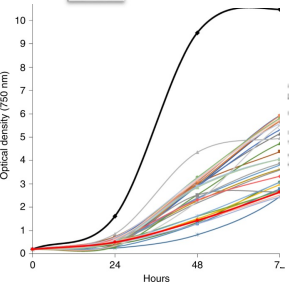
- 96-well plate carbon and nitrogen utilization assays complete.
- Starch, lipid, chlorophyll, and pigments assays are being optimized for 96-well plate detection.

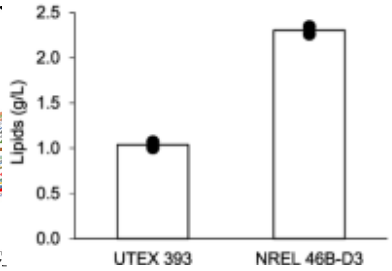


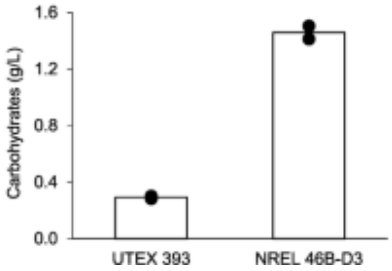


Automate







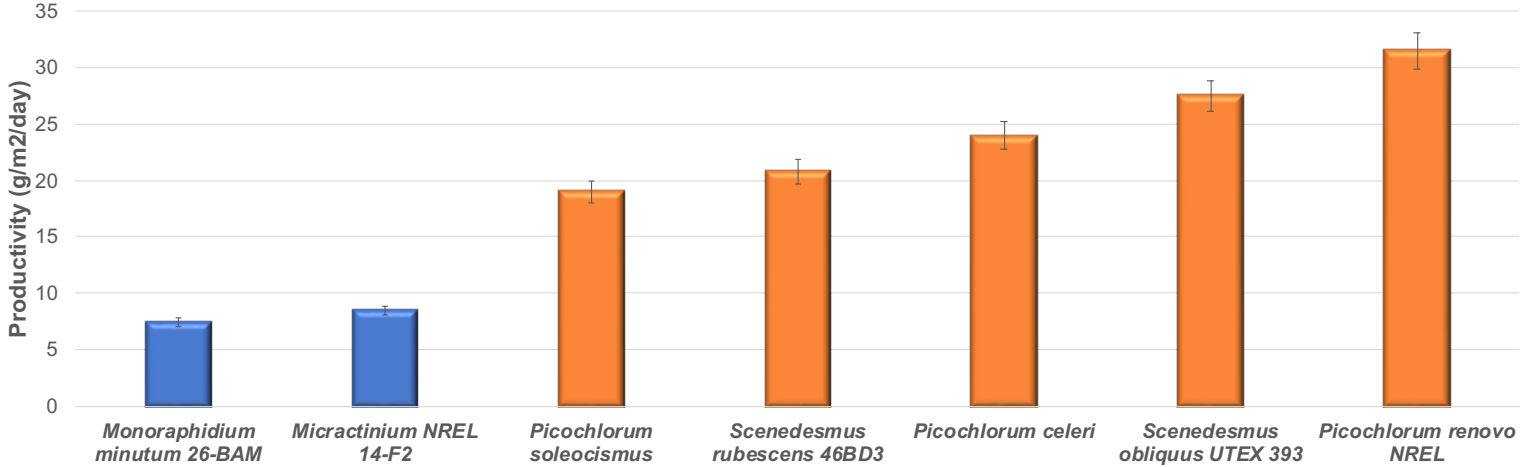


Pipeline will enable rapid library characterization and genotype-phenotype correlation.

Calhoun, et al. (2021) *Comm Bio* 4, 333.

Impact: De-risking and Accelerating the Path to BETO Targets

- ABP activities enable **targeting of MYPP key cost hurdles**:
  - Feedstock 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.**



- *Picochlorum* libraries and high-throughput pipelines represents **first-in-class tools for basic and applied research in deployment-relevant algae**.

Impact: Tool and Data Dissemination to the Algal Community

- **Strain & Tool Dissemination**
  - Publicly accessible strains and DNA sequences
  - Over *20 Material Transfer Agreements* executed with academic and industrial partners
- **Data Dissemination**
  - JGI, NCBI, Phycocosm
- **Patents & Pubs**
  - 2 patents, 7 publications since last Peer Review (2021)
- **Platform and Commercial Partnership**

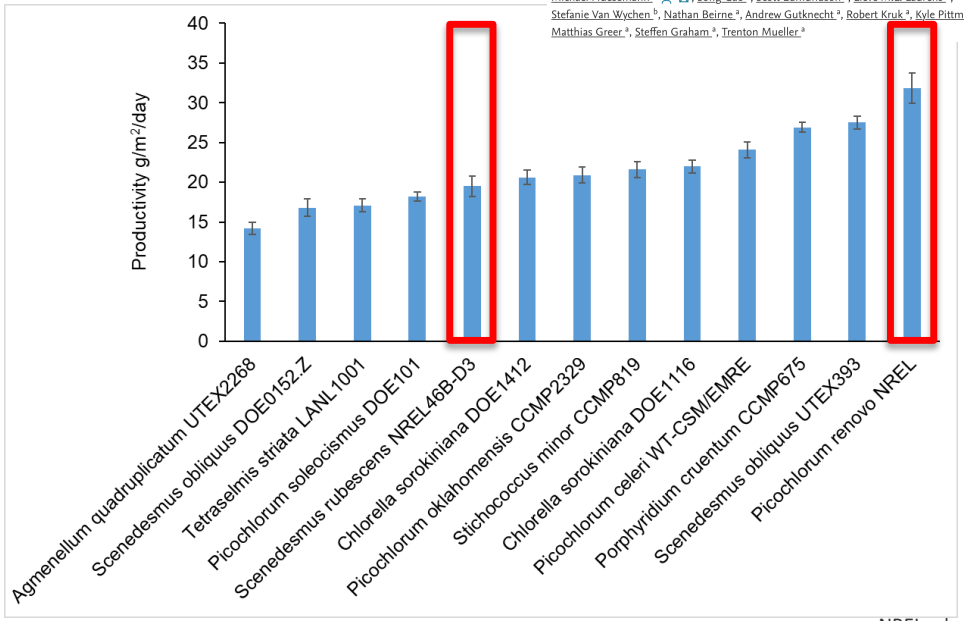
NCBI

JGI PhycoCosm
THE ALGAL GENOMICS RESOURCE

ELSEVIER
Algal Research
Available online 17 December 2022, 102948
In Press, Journal Pre-proof What's this?

DISCOVR strain pipeline screening – Part II: Winter and summer season areal productivities and biomass compositional shifts in climate-simulation photobioreactor cultures

Michael Huesemann [a], Song Gao [a], Scott Edmundson [a], Lieve M.L. Laurens [b], Stefanie Van Wychen [b], Nathan Beirne [a], Andrew Gutknecht [a], Robert Kruk [a], Kyle Pittman [a], Matthias Greer [a], Steffen Graham [a], Trenton Mueller [a]



Impact: Tool Development Enables Applied Pursuits

Algae Biotechnology Partnership tools have enabled and support Platform expansion across multiple DOE offices:

- **FECM –** integrated electro-bioconversion for next-gen algal cultivation
- **ARPA –** development of algal-derived cement
- **NREL LDRD –** photoreactive capture for SAF production
- **BETO FOA –** atmospheric CO_2 capture and conversion
- **BETO FOA –** carbon utilization efficiency enhancements

Summary

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

Goal: Deliver a high-throughput, automated pipeline for broad-host range algal genetic engineering and phenotyping

Approach: Develop enabling genetic tools and high-throughput geno- and phenotyping pipeline that is applicable across diverse deployment-relevant algae.

Progress: We have established broad-host range, advanced genetic engineering capabilities and initiated generation of *P. renovo* genome-scale mutant libraries and high-throughput phenotyping methodologies.

Impact: Tools and pipelines developed here will accelerate the path to algal biofuels commercialization.

Acknowledgements

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Lukas Dahlin
Jeff Linger
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Venkat Subramanian
Benton Wachter
Ellsbeth Webb
Stefanie Van Wychen

Sandia National Laboratories

Monica Sanchez
Chuck Smallwood

Los Alamos
NATIONAL LABORATORY

Matthew Green
Shawn Starkenburg

COLORADO SCHOOL OF MINES

Matt Posewitz
Anagha Krishnan
Galen Dennis
Tyson Burch

UPRM LA IUPI

Clifford Louime

Additional Slides

Quad Chart Overview

Timeline
- Project start date: 10/1/21
- Project end date: 9/30/24

	FY22 Costed	Total Award (FY22-24)
DOE Funding	$685,115	$2.55M ($1.1M NREL; $650K LANL; $575K Sandia; $225K Mines)

TRL at Project Start: 1
TRL at Project End: 2-3

Project Goals
- Develop a high throughput pipeline for algal genetic engineering and phenotyping in industrially-relevant microalgae.
- Generate publicly accessible, phenotypically-characterized, genome-scale libraries in mass cultivation-relevant microalgae.

End of Project Milestone
Achieve automated, high-throughput generation and phenotyping of 96 mutants in microtiter format in < 2 wks.

Funding Mechanism: FY22 Algae Lab call – Topic Area 1

Project Partners: National Renewable Energy Laboratory, Los Alamos National Laboratory, Sandia National Laboratory, Colorado School of Mines (subcontract from NREL)

Responses to Previous Reviewers' Comments

Regarding likelihood of achieving project end goals (engineering orthogonal regulatory mechanisms in four deployment relevant strains), ***our team successfully achieved our end-project milestone*** *from the prior period of performance, demonstrating toolbox efficacy and transferability to four top-candidate strains currently under evaluation in the BETO deployment portfolio. Additionally, tools have since been transferred to additional hosts under evaluation in the DISCOVR pipeline, ultimately exceeding our end-project goal in the prior period of performance.*

Publications, Patents, Presentations, Awards, and Commercialization

Publications

- Dahlin LR, Guarnieri MT. (2022). Heterologous expression of phosphite dehydrogenase in the chloroplast or nucleus enables phosphite utilization and genetic selection in Picochlorum spp. *Algal Research* 62, 102604.
- Wen J, Rapp K, Dahlin LR, Li CT, Sebesta J, Barry AN, Guarnieri MT, et al. (2021) Mapping the path forward to next generation algal technologies: Workshop on understanding the rules of life and complexity in algal systems. *Algal Research* 60, 102520.
- Dahlin LR, Guarnieri MT. (2021) Development of the high-productivity marine microalga, Picochlorum renovo, as a photosynthetic protein secretion platform. *Algal Research* 54, 102197
- Calhoun S, Bell TAS, Dahlin LR, Kunde, et al. (2021) A multi-omic characterization of temperature stress in a halotolerant Scenedesmus strain for algal biotechnology. *Communications Biology* 4 (1), 1-15.
- Knoshaug EP, Gerritsen AT, Henard CA, Guarnieri MT. (2020) Methods for Algal Protein Isolation and Proteome Analysis. *Met. Pathway Engineering*, 51-59.
- Dahlin L, et al. (2019) Development of a halophilic, thermotolerant model microalga, *Picochlorum renovo. Communications Biology*, 2 (1), 1-9.
- Arora N, Pienkos PT, Pruthi V, Poluri KM, Guarnieri MT. (2018). Leveraging algal omics to reveal potential targets for augmenting TAG accumulation. Biotechnol Adv. 36(4):1274-1292.
- Dahlin L, et al. (2018). Down-selection and outdoor evaluation of novel, halotolerant algal strains for winter cultivation. Front Plant Sci 9, 1513.

Patent Applications

- Heterologous expression of phosphite dehydrogenase in Picochlorum spp. US Patent App. 63/248,213.
- Broad host range genetic tools for engineering microalgae. US Patent App. 16/989,549.
- Photosynthetic protein secretion platform. US Patent App. 17/666,345

Presentations

- Dahlin LR. International Conference on Algal Biomass Biofuels, & Bioproducts. Waikoloa, HI, 2023.
- Guarnieri MT, et al. SIMB SBFC, Portland, OR, 2023.
- Guarnieri MT. The 9th International Conference on Algal Biomass, Biofuels & Bioproducts, Boulder CO, 2019.
- Guarnieri MT. Colorado Renewable Energy Society, Golden, CO, 2019.
- Guarnieri MT, et al. Gordon Research Conference: Molecular Basis of Microbial C1 Metabolism, Newry, ME, 2018.
- Dahlin, L, et al. Western Photosynthesis Conference, Oracle, AZ 2017.