BERKELEY LAB
Bringing Science Solutions to the World

U.S. DEPARTMENT OF ENERGY

# U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2017 Project Peer Review

## Genetic blueprint of microalgae carbon productivity

March 7, 2017
Advanced Algal Systems

Igor Grigoriev
LBNL

# Goal Statement

*Goal:* Develop a functional genomics pipeline with multi-state perturbations experiments (dynamic time course, multi-condition perturbation) to baseline strain performance.

*Outcomes:*

- standardize functional genomics experimentation to *reduce strain improvement development time by 50%* to accelerate the ***identification of both conserved and strain-specific pathways for enhancements***

- select gene targets in *Chlorella luteoviridis* to improve biomass production rates by 25%

# Quad Chart Overview

## Timeline

Project start date: 10/1/2016

Project end date: 9/30/2019

Percent complete: 5%

## Barriers

Aft-B. Sustainable Algae Production

Aft-C. Biomass Genetics and Development

## Budget

| | Total Costs FY 17 –FY 19 | FY 17 Costs | FY 18 Costs | Total Planned Funding (FY 17-Project End Date) |
|---|---|---|---|---|
| DOE Funded | 1,350K | 350K | 450K | 1,350K |

## Partners

- FY17: LBL (33%)+ LANL (66%)
- LANL(1.3.1.600; 1.3.2.100), NREL(1.3.2.102), DISCOVR(1.3.2.502)

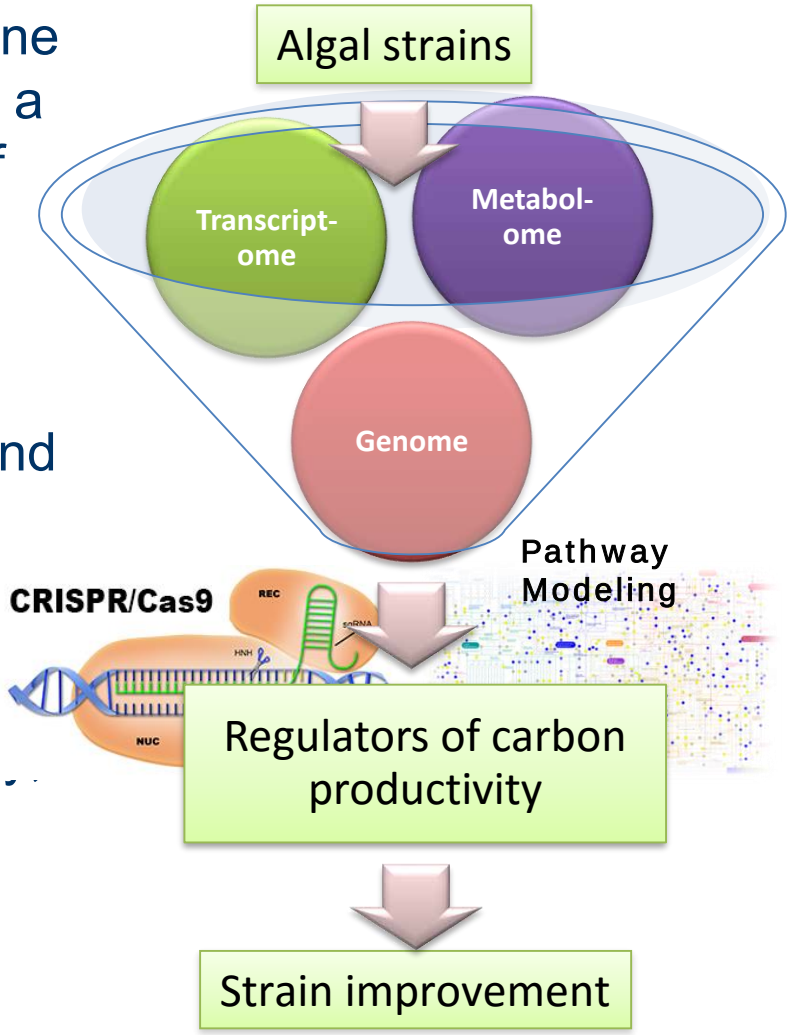
# 1 - Project Overview

**Goal:** Develop a functional genomics pipeline with multi-state perturbations experiments , a platform for production and interpretation of multi-omics measurements to accelerate strain improvement and commercialization.

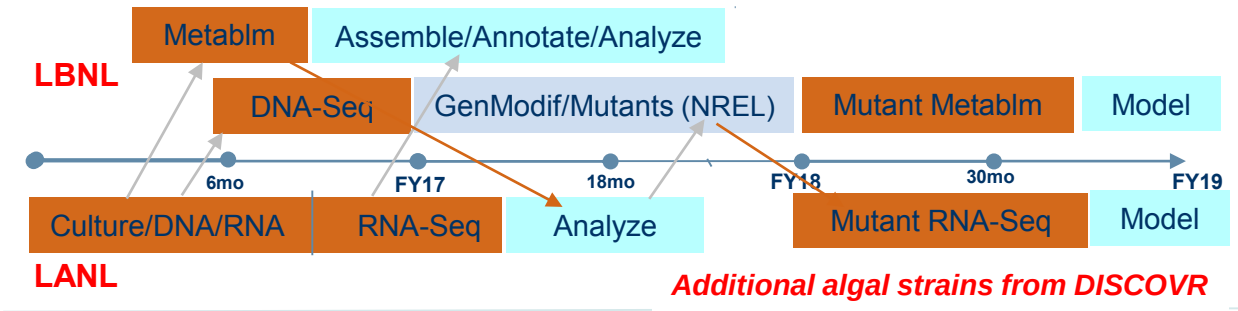
**First target:** *Chlorella luteoviridis* , which outperforms both *Nannochloropsis salina* and *N. oceanica* in lab-based and outdoor summer cultivation (NREL, ATP$^3$)

**Objectives:**

- Produce high quality sequence, assembly and annotation of *C.luteoviridis* genome
- Generate omics data for functional genome analysis and modeling
- Identify gene targets for mutagenesis for 25% improved biomass production rates



# 2 – Approach (Management)




***NREL**: Chlorella luteoviridis strain*(WBS *1.3.2.102*; Guarnieri, PI)

***LANL** (Starkenburg)**:** Cultivation studies, DNA/RNA extraction, transcriptomics sequencing and analysis*

***LBNL:***

*Grigoriev: sequencing/assembly/annotation/analysis*
*Northen: metabolomics of wild type and mutants*

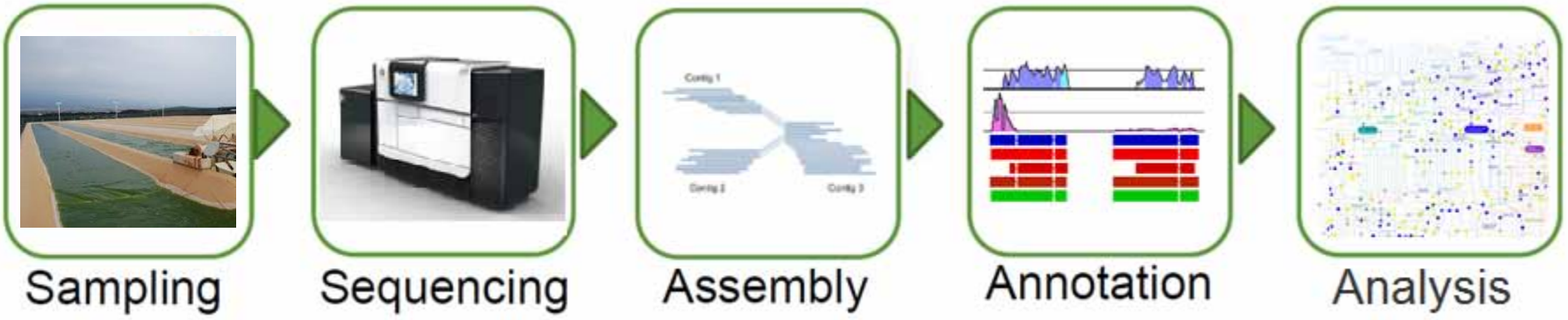
*Sequencing will be subcontracted to JGI*

**Communications:** *Monthly conference calls*

# 2 – Approach (Technical)

**Task: *Chlorella luteoviridis* genome sequencing, assembly, and annotation (Grigoriev, LBNL/JGI)**

- Sequencing: PacBio
- Assembly: Falcon
- Annotation: JGI pipeline




***Dependencies:***

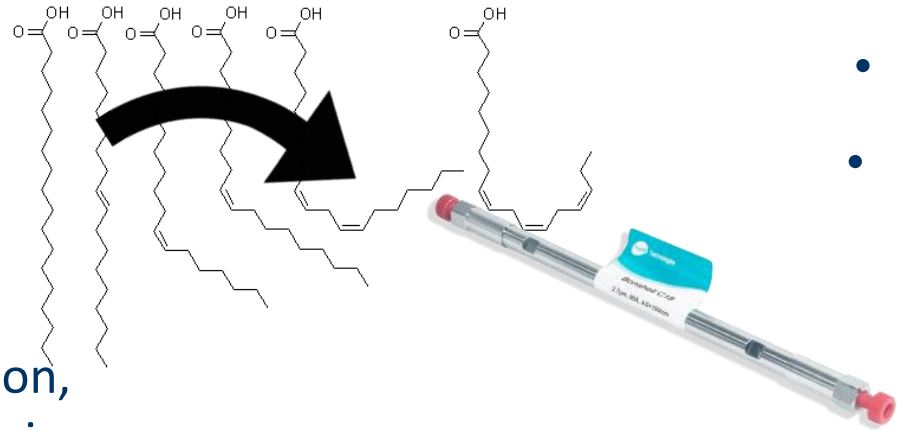
- *C.luteoviridis* strain from NREL
- High molecular weight DNA and RNA-Seq data from LANL
- Additional DISCOVR strains

# 2 – Approach (Technical)

## Task: *Chlorella luteoviridis* metabolomics (Northen, LBNL)

Cells

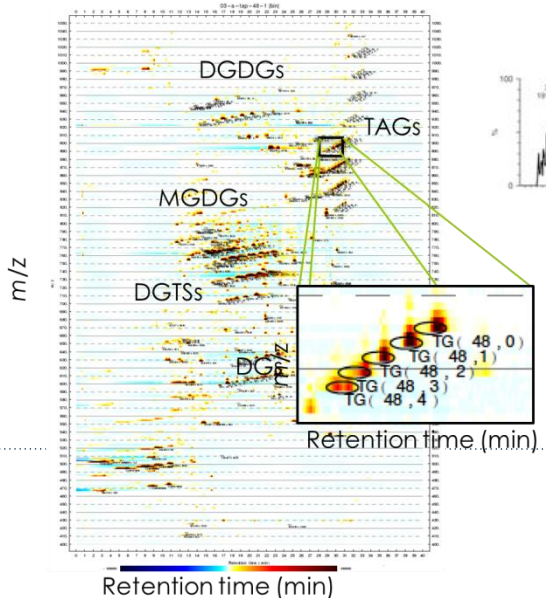
Metabolite Extraction

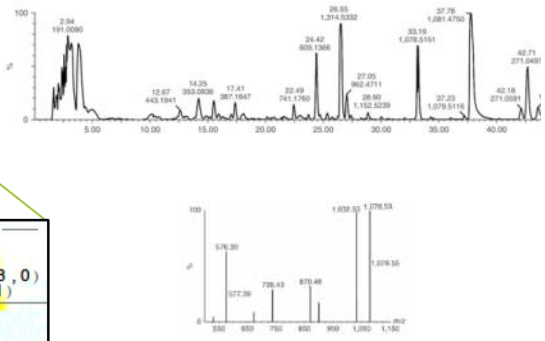


Liquid Chromatography

- C18—Lipids
- HILIC—Polar metabolites

Metabolite identification, Quantification & comparison



Tandem Mass Spectrometry


Mass Spectrometry

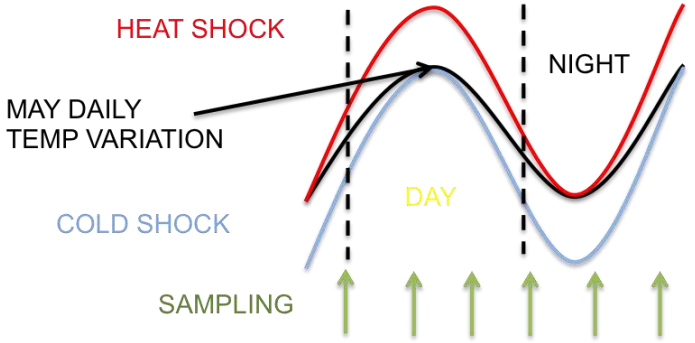
BERKELEY LAB

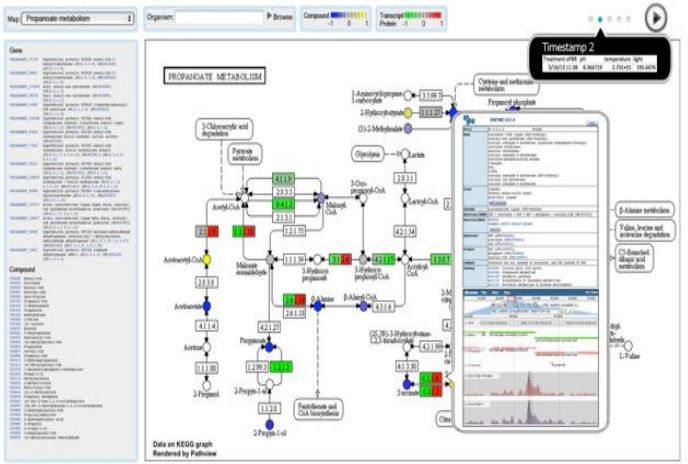
# 2 – Approach (Technical)

## Task: Perturbation Challenge; Transcriptomics and OMICS integration (Starkenburg, LANL)

*C.luteoviridis* strain is grown under 3 conditions in ***environmental PhotoBioReactors (ePBRs)*** :

- To replicate the growth conditions of outdoor cultivation studies at Mesa, AZ
- To compare the growth response to two perturbation simulations: low temperature perturbation and a high temperature perturbation
- To determine the ability to tolerate suboptimal environmental conditions at the 'cross over' point of the crop rotation (spring/fall).




(WBS 1.3.1.600, Starkenburg).

# 3 – Technical Accomplishments/ Progress/Results

***Started in October 2016:***

- *C.luteoviridis* strain acquired from NREL in November 2016
- HMW DNA extraction complete and sent to LBNL/JGI for sequencing
- DNA QC in progress (LBNL/JGI)

# 4 – Relevance

Improving the productivity and robustness of algal strains against perturbations requires extensive advanced genetic, genomic, and molecular biology tools, which are currently lacking for most algal species.

These would allow us to understand:

1) regulation of carbon production and switches from rapid growth to stress-induced carbon storage

2) growth responses at varied temperatures, critical for 'seasonal' strains

In addition to improved *C.luteoviridis* productivity, we build a functional pipeline for omics data production and targeted improvement of other algal strains (e.g., from DISCOVR)

# 5 – Future Work

| Lab | Performance Measure | Completion |
|---|---|---|
| **LANL** ✓ | Strain acquired from NREL; cultivation conditions established, high molecular weight genomic DNA transferred to LBNL for sequencing | 1/31/2016 |
| **LBNL** | DNA quality assessed for samples from LANL to initiate sequencing | 3/31/2017 |
| **LANL** | *C. luteoviridis* grown in ePBRs under 3 outdoor cultivation perturbation simulation experiments, samples prepared for RNA-Seq and metabolomics, transferred to LBNL for metabolomics profiling | 6/30/2017 |
| **LBNL** | Genome sequenced and assembled; Metabolome profiled | 9/30/2017 |
| **LANL** | Transcriptomes sequenced from outdoor cultivation simulation experiments for under 3 perturbation conditions at 6 time points | 12/31/2017 |
| **LBNL** | Transcriptome enabled genome annotation | 3/31/2018 |
| **LANL/ LBNL** | Metabolome and transcriptome datasets integrated for metabolic reconstruction and gene network analysis | 6/30/2018 |
| **LBNL/ LANL** | Two gene targets identified for targeted strain improvement | 9/30/2018 |

# Summary

1. **Goal**: Develop a functional genomics pipeline with multi-state perturbations experiments, a platform for production and interpretation of multi-omics measurements to identify gene targets for strain improvement and commercialization (25% improved biomass production rates for *Chlorella luteoviridis*).
2. **Approach**: Genome sequencing and omics profiling during perturbation experiments to enable metabolic modeling and gene network analysis
3. **Accomplishments:** Most tools developed and tested on other algae; since October 2016, acquired the strain from NREL, grown in ePBRs, extracted and shipped DNA to LBNL/JGI for sequencing
4. **Relevance:** Addresses several barriers (Aft-B, Aft-C) outlined in the 2015 MYPP in Algal Feedstocks Production and Logistics.