DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Algae Biotechnology

March 23, 2015
Algae Feedstocks

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Goal Statement

- Perform applied, precompetitive R&D in Algae Biotechnology and Bioenegineering.
- The activities will be divided into two main areas of R&D:
  - 1) molecular tools, technologies, and resources for strain improvement; improved strains
  - 2) bioenegineering technologies for increasing algae biomass productivity and the energy-efficiency of algae processing steps
- The results will:
  - be broadly applicable to the algae biofuel community;
  - will strengthen the knowledge base for advancements in algae biotechnology and bioengineering to support algae feedstock logistics operations; and
  - will help position the algae biofuels industry for further growth.

Quad Chart Overview

Timeline

- Project start date: June, 2012
- Project end date: September 2015
- Percent complete: 85%

Barriers

- Barriers addressed
  - AFt-C. Biomass Genetics and Development
  - AFt-D. Sustainable Harvesting
  - AFt-B. Sustainable Algae Production
  - Aft-H. Integration & Scale-up

Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-FY17)
DOE Funded	$0	$2,662,110	$1,379,973	$5,653,816
Project Cost Share (Comp.)*	n/a	n/a	n/a	n/a

*If there are multiple cost-share partners, separate rows should be used.

Partners

- Partners (FY15)
  - NREL
  - PNNL

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1 - Project Overview

- This project was initiated in 2012, during the final year of the National Alliance for Advanced Biofuels and Bioproducts (NAABB) algae consortium.
- The project aims to leverage key achievements from NAABB and advance their technology readiness level.
- Specific outcomes:
- *Strain improvement*
  - Demonstrate improved performance of genetically modified algae production strains.
  - Assemble and integrate genomics, transcriptomics, proteomics, and cultivation metagenomics data from NAABB and other sources on algae production strains
- *Productivity improvement*
  - Demonstrate scale-up feasibility of energy-efficient algae harvesting, extraction, and separations using ultrasound technology
  - Development of an integrated CO_2 delivery system

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2 – Approach (Technical)

- Address major challenges to conducting R&D in this field, specifically:
- 1) Algae biofuels industry is small and relatively immature, which results in risk aversion.
  - Limits opportunities for collaborations with industry.
  - *We take a precompetitive approach to R&D*
  - Transition R&D efforts to competitive projects. Two projects were transitioned to Algae Biomass Yield (ABY) projects:
    - *Chlorella* strain improvement to REAP/NMSU (Fall 2013)
    - Ultrasonic harvesting to Cellana (Fall 2014)
  - Ongoing effort to transition other R&D efforts for competitive opportunities as they arise.
- 2) Lack of opportunities for TRL-1-2 funding-from BETO or anywhere else.
  - E.g., Foundation in algal biology still needs significant strengthening.
  - The industry depends on highly productive strains (AFt-C. Biomass Genetics and Development) but there is a lack of resources (informational and $$) to find and develop them. Perceived as TRL-1, but is really fundamental applied R&D.
  - *We support a small amount of strategic, exploratory work in this AOP (5-10%).*

2 – Approach (Management)

- FY15: Re-directed 2/3 of this project to the new Algae Biotechnology Hub (LANL, NREL, and PNNL)
  - Bigger project, multiple labs
  - Expected results: Data/information sharing will accelerate progress for BETO and the algae biofuel industry; expand other opportunities, including industrial partnerships; help strengthen knowledge base in algal biology.
- Algae Biotechnology Hub Structure:
  - Each lab has a unique role and unique resources
  - Developed shared milestones and deliverables
  - Developed collaboration mechanisms: meetings, quarterly telcons, inter-lab visits; data and analysis transfer
- Challenge:
  - Staggered start to the Hub
    - LANL was in year 3 of existing project
    - NREL and PNNL projects went to Merit Review

3 – Technical Accomplishments-Overview

Strain Improvement

- Molecular toolbox developed for *Chlorella sorokiniana*
- Applied to develop increase heat tolerance, salt tolerance; light harvesting ability and cellulose digestion activity
- Enhanced bicarbonate uptake in *Nannochloropsis salina*, results in increased biomass and lipid productivity
- Flow sorting improvement in lipid accumulation

- Established a website for algae omics analysis, storage, and bioinformatics

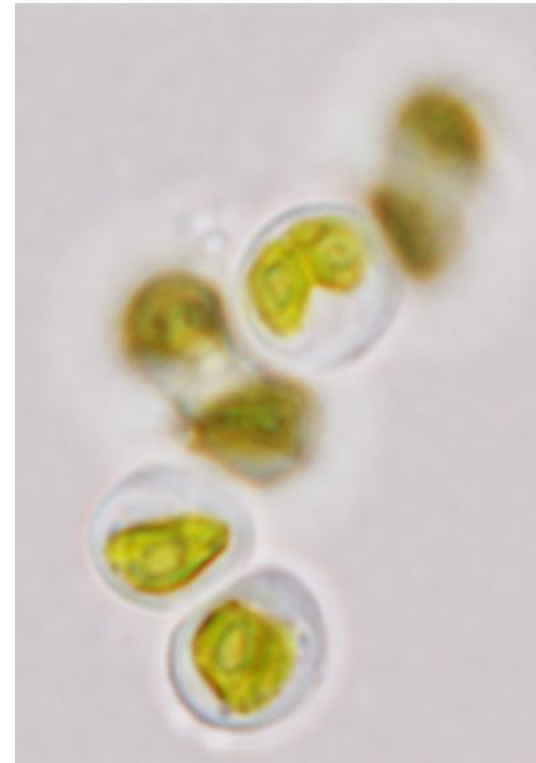
Production Efficiency

- Developed new approach for efficient CO_2 delivery
- Demonstrated a new model for scaling-up low-energy ultrasonic algae harvesters

Strain Improvement to *Chlorella sorokiniana*

Barrier addressed: AFt-C. Biomass Genetics and Development

- Robust freshwater strain (*Chlorella* sp. DOE1412) discovered and evaluated in NAABB
- Closely related *Chlorella sorokiniana* strains were selected for further development and improvement to production performance
- Completed development of a molecular toolbox for *Chlorella sorokiniana*
- Complementary development of other *Chlorella sorokiniana* strains being done as part of REAP ABY project.
- Improvements targeted:
  - Heat tolerance
  - Salt tolerance
  - Light harvesting



Chlorella sp. *DOE1412 (photo from Jurgen Polle, Brooklyn College*

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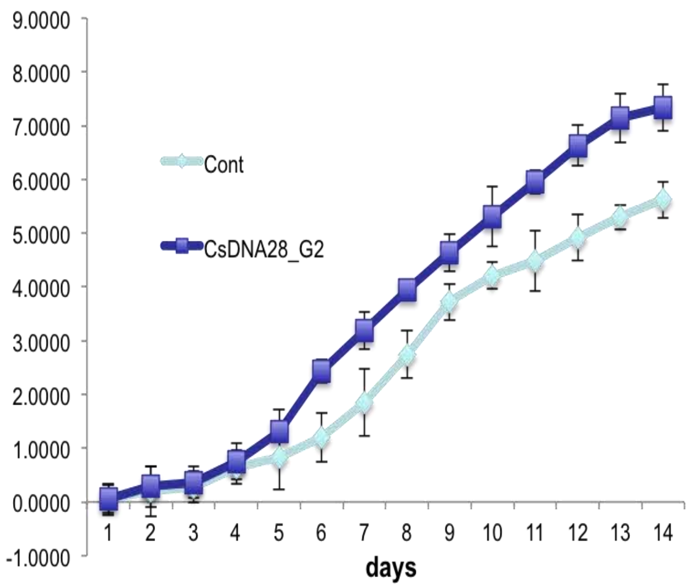
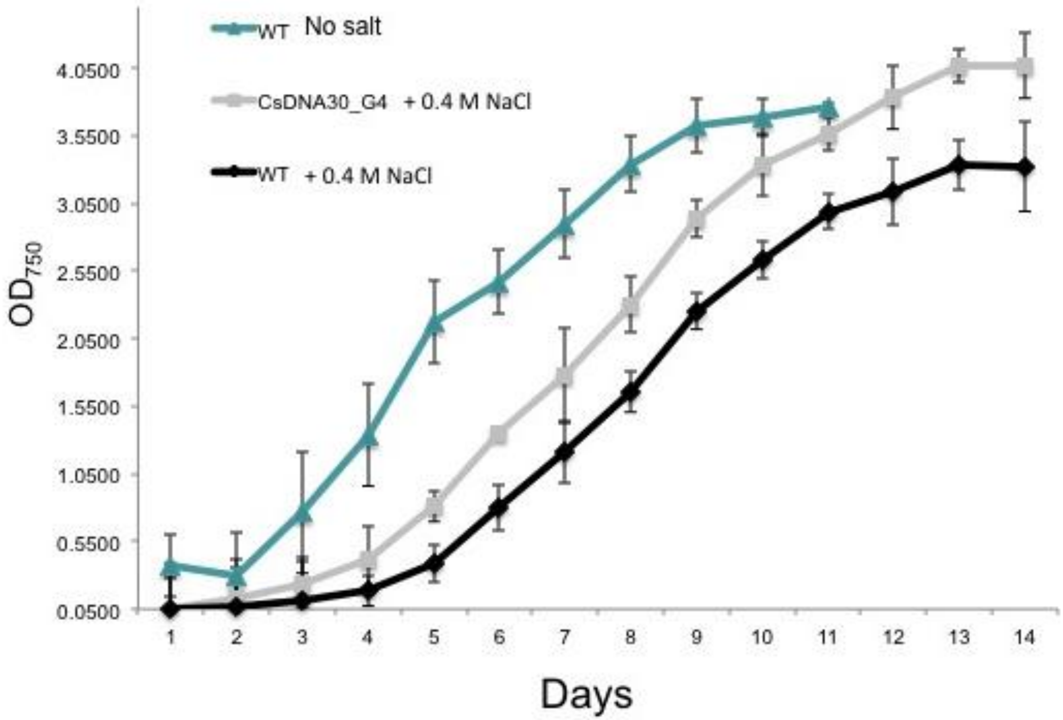
Enhanced heat tolerance in *Chlorella sorokiniana–1230* insertional mutants; improved salt tolerance relative to WT



Growth at 38°C
Insertional mutants have 30% greater productivity than WT



By day 11, transgenic lines had cell numbers equal to no salt WT following a 3 day lag

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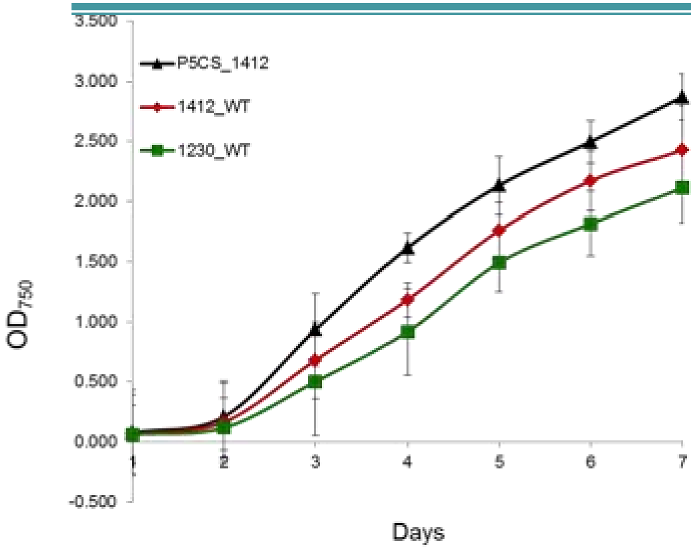
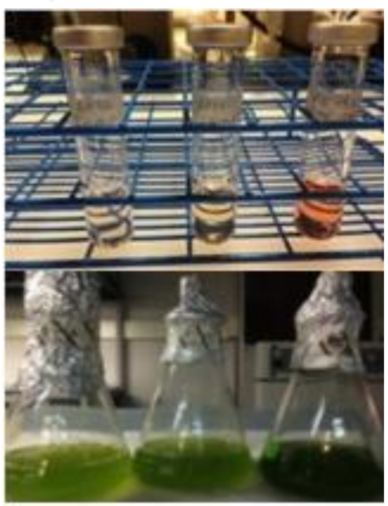
Enhanced heat tolerance in *Chlorella sorokiniana–1412* mutants over-accumulating (5X) proline

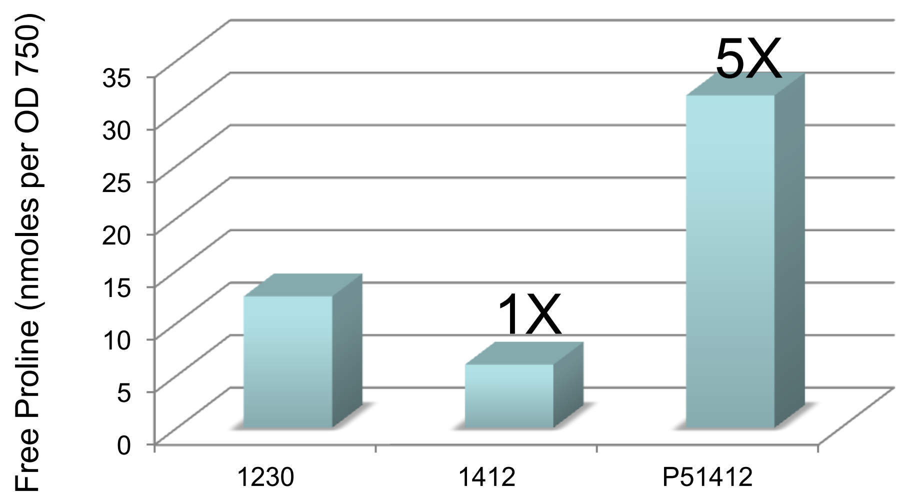




1412 1230 P51412

Growth at 40^{o}C

- P5CS transgenics have 20% greater productivity than the wild-type parent 1412.
- Strain 1412 is more heat tolerant than strain 1230



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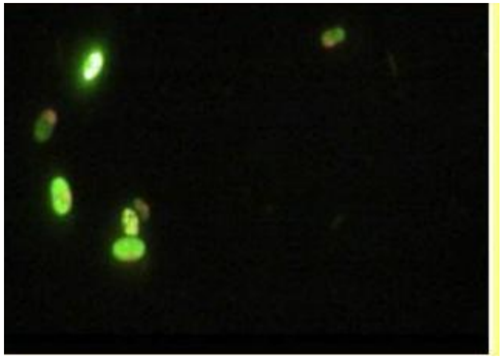
Strain improvement to *Nannochloropsis salina (1776)*

Barrier addressed: AFt-C. Biomass Genetics and Development

- Marine algae, Stramenopile; robust high-lipid-producing strain; productivity is amply demonstrated in commercial outdoor systems
- 20g/m^2/day DW in lab; 9-12 g/m^2 outdoors
- But, biological productivity is still greatest barrier to lower the cost of algae biofuel, and lower cost of algae feedstocks



N. salina *stained with BODIPY lipid stain*

- *Nannochloropsis salina* has no CCM gene homology and no pyrenoid production for carbon storage
  - Inefficient CO_2 assimilation system in *N. salina* may be a good target for directly improving biomass and lipid production in GMO approach
- Flow sorting, non-GMO approach to improvement (also provides insight into new gene targets for GMO improvement)
  - *CRADA/Tech transfer agreement under development for flow sorting*

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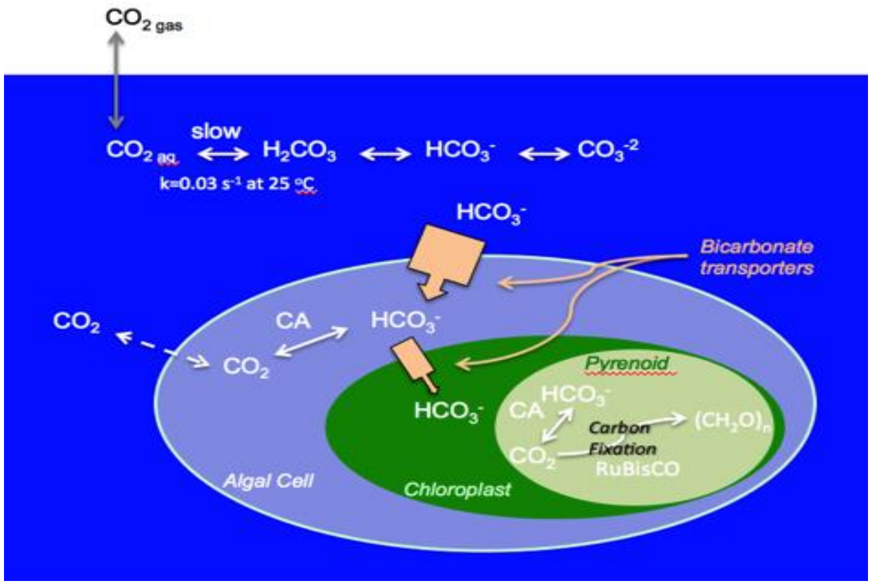
Improving CO_2 Dynamics



Barriers addressed: AFt-B. Sustainable Algae Production and AFt-C. Biomass Genetics and Development

The problem:

- Conversion of CO_2 to carbonic acid in media during cultivation is slow
- Concentration of carbon dioxide species (CO_2 + H_2CO_3) is related to partial pressure of CO_2.
- Therefore, CO_2 delivery by gas sparging is lossy; can lose up to 80% of delivered gas

The 2-pronged solution:

- *Improve delivery efficiency*. Over pressure water with 45 ATM of CO_2 to make the concentration of CO_2 + H_2CO_3 in solution ~0.9 molar. This is 30 times greater than air and water.
- *Improve cell uptake efficiency.* Bicarbonate uptake biologically enhanced by overexpression of membrane bicarbonate transporter *(BICa)*
- RuBisCO substrate loading enhanced by overexpression of chloroplast bicarbonate transporter *(ChlorBIC)*

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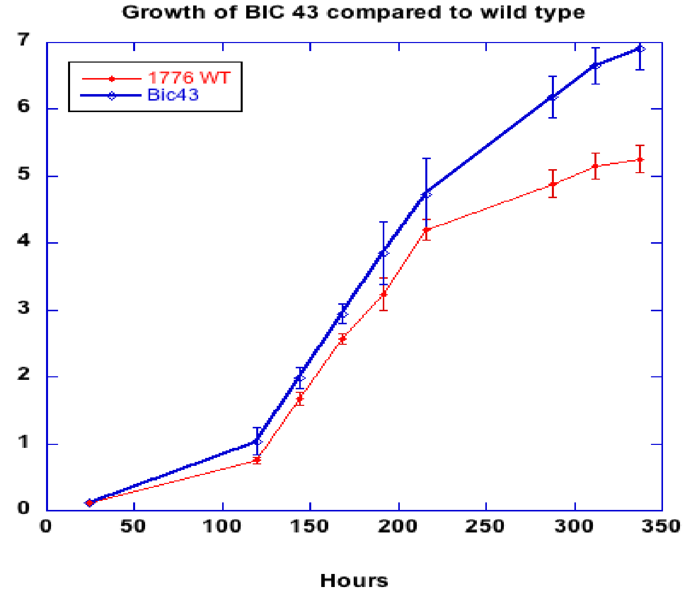
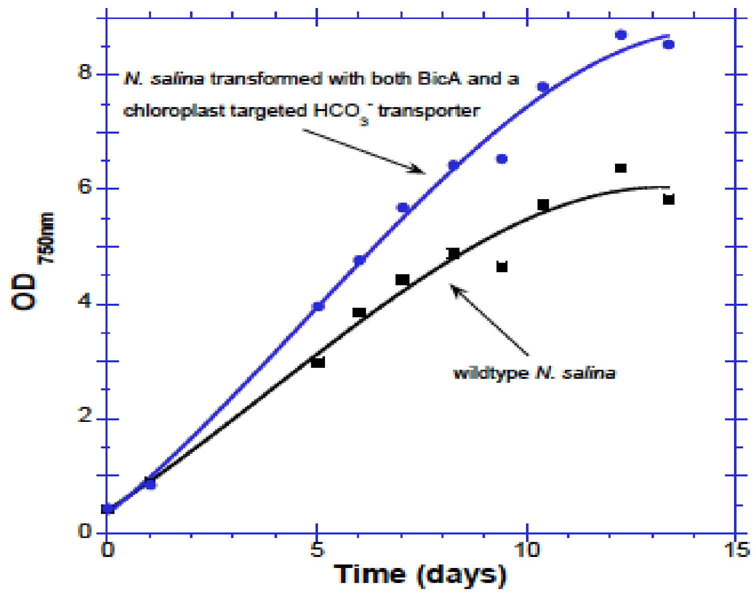
Genetic Engineering of *Nannochloropsis salina*

- Increase biomass and lipid productivity through enhanced carbon assimilation
  - BicA transformants increased biomass production by 30%
  - BicA/ ChlBic double transformants increased biomass production by 46%
- Direct carbon flux to TAGs through increased sink strength
  - ACCase transformants increased lipid concentration by 24% but resulted in slower cell growth
  - *In progress*: Phenotyping ACCase/ BicA/ ChlBic stacked transformants





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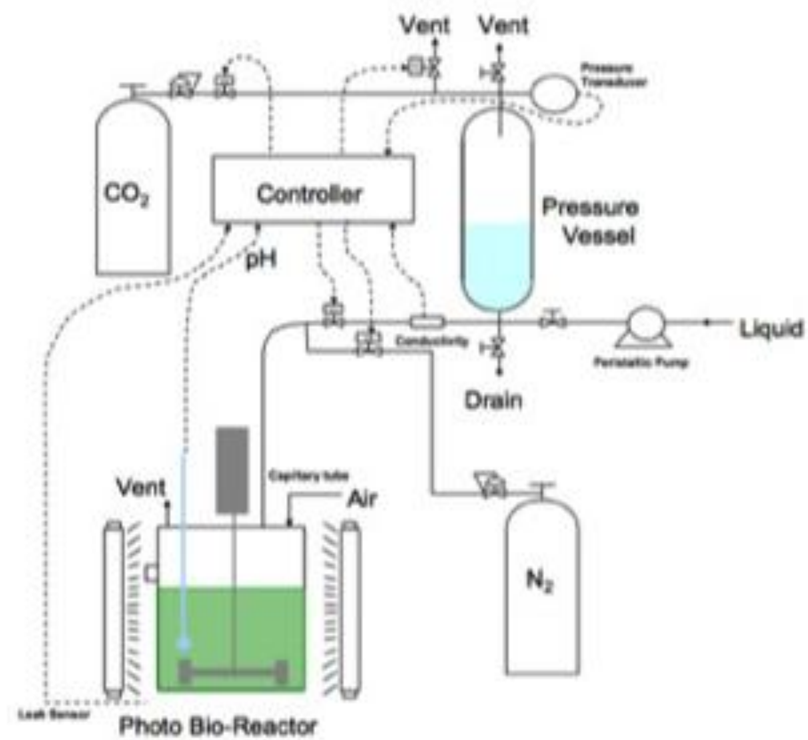
Improved CO_2 Delivery System

Carbonated Water $CO_{2\,(CW)}$ addition system



System Features

- Over pressure water with CO_2 to 50 ATM
- Measures the CO_2 pressure
- Carbonated water is added through a capillary
- Nitrogen purge of capillary to eliminate clogging
- Controller
  - Measures pH of growth medium
  - Controls the inlet valve to maintain pH
  - Uses PID to control pH
  - Logs pH, pressure, and addition time
- Safety features
  - Regulator
  - PRV
  - Liquid detection (conductivity)

Results in photobioreactor

- Growth rate is 20-30% faster then with CO_2 sparging
- 63-75% recovery of carbon input
- Isotope discrimination by RuBisCO suggests greater CO_2 saturation

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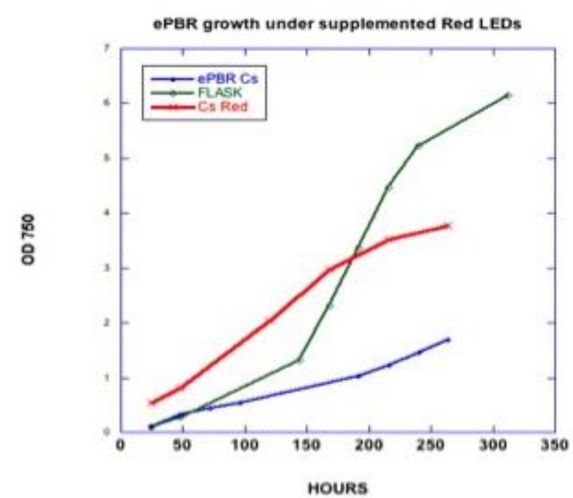
ePBR Array

Barrier addressed: AFt-B. Sustainable Algae Production

ePBR array

- Established a 30+ Phenometrics ePBR array, available for use
  - http://www.lanl.gov/science-innovation/capabilities/bioscience-biosecurity-health/bioenergy/bioreactors.php
- We have made many modifications to the ePBRs to get them to more closely mimic flask productivity and to decrease inter-ePBR variability, including:
  - aeration control, cell mixing dynamics, pH regulation, and red rich LEDs
- *CRADA in FY14 on spectroscopic approach to cultivation monitoring*

ePBR Growth of Chlorella



Conclusions and Future Steps

- *C. sorokiniana* transformants (different strains) are being transitioned to the NMSU ABY project (REAP)
- Synergistically combine the two CO_2 efficiency systems
  - Successful improvements in separate processes of CO_2 delivery system and CO_2 cellular uptake systems
  - Scale-up into raceways
- Flow sorted *Nanno* with 27% improved lipid production can be handed off to larger scale systems with environmental challenges. *(See additional slides)*
- Combine improvement strategies: Utilize flow sorted cells for further genetic enhancement-can we improve growth rate? Need to continue to push that barrier.
- ePBR array has been vetted and provides an important resource for evaluating scale-up potential of future improved strains.

Progress on development of low-cost, energy-efficient ultrasonic approaches: Harvesters, Separators, and Extractors and more

Barriers addressed:
Aft-D. Sustainable Harvesting and Aft-H. Integration & Scale-up

•Use of low-energy ultrasound delivers pressure waves to the sample to separate particles from surrounding media.

•Pressure intensity is related to frequency

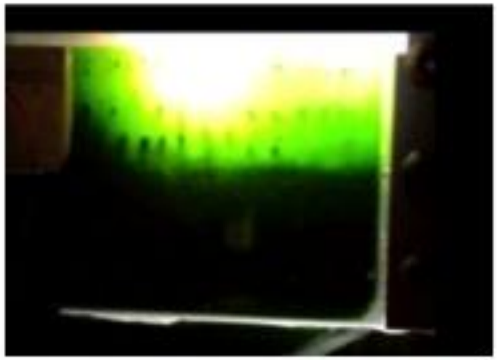
- Low pressure, high frequency: Harvesting and phase separations
- High pressure, low frequency: Cell lysis; solution mixing, streaming effects

•*Advantages*:

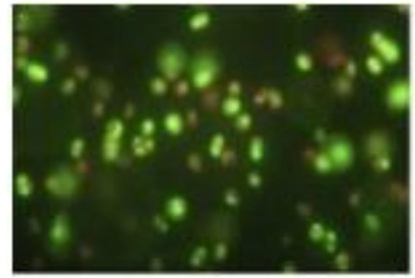
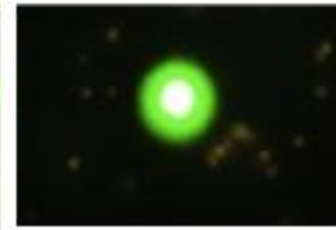
- Environmentally friendly
- Low cost, energy-efficient
- Fast-acting
- No chemical addition; Compatible with any conversion pathway—Lipid extraction, Whole biomass (e.g. HTL) and Bioproducts production

Device Performance Assessment, Scale-up, and Integration

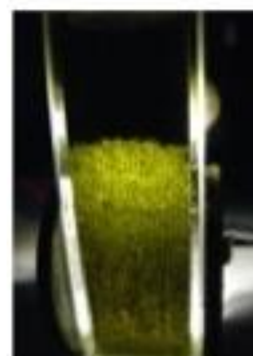
- Algae harvesting, lab scale to field scale and testing

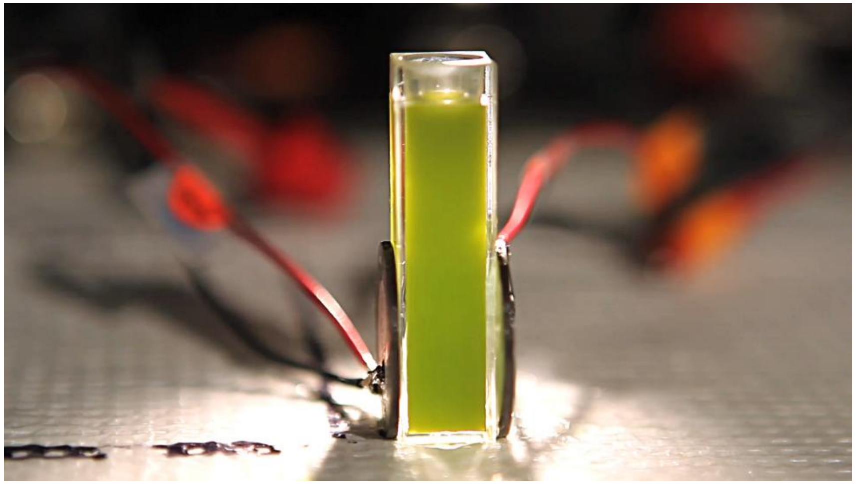

- Algae cell disruption/lysis, lab scale

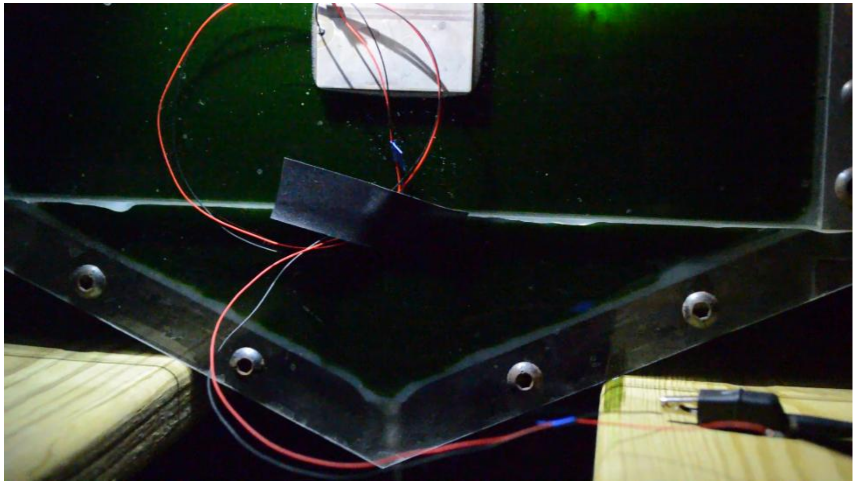



- Oil/water/emulsion separations, lab scale



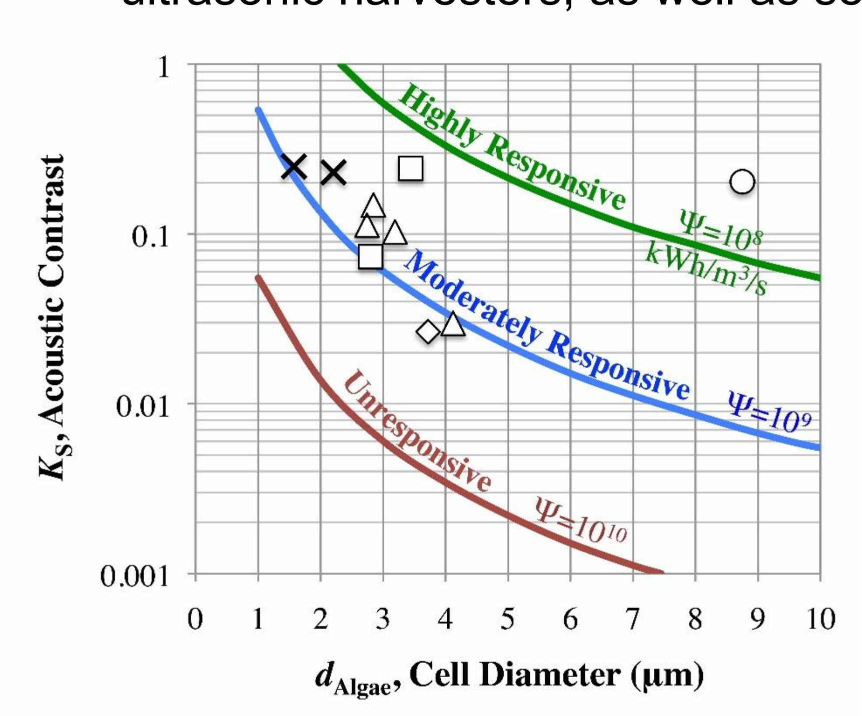
Videos





Accomplishments in Ultrasound Technologies

- Combined theoretical study of device properties and algae physical properties (buoyant density, size, speed of sound, etc.) to predict device performance and energy requirements. This is an *entirely new approach to guide scale-up!*
  - Enables prescreening of algae to predict performance in device.
  - Validated theory using a single ultrasonic harvester device.
  - Made progress toward energy efficiency factor (EEF) determination of ultrasonic harvesters, as well as separators, and extraction devices (FY15).



Properties of algae that affect harvester energy requirements and performance.

Harvester scale-up and field testing will transition to Cellana's ABY project in FY15-FY16.

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Conclusions and Future Steps

- A new scale-up model for predicting performance of specific algae strains in ultrasonic device performance was validated
  - *Ultrasonic harvesting scale-up is transitioning to Cellana's ABY project*
  - Field test in 2015
  - Revised patent application
- Re-direct harvesting R&D to extraction (lysis) and oil separation development and scale-up in FY15, FY16
- Continue to make progress on examination of other acoustic/ultrasonic technologies to lower the cost of algae biofuels *(additional slides)*
  - Progress on acoustic stimulation
  - Expand to acoustic mixing (e.g. paddlewheel replacement) in out years

Omics Integration and Website

Barriers addressed: AFt-C. Biomass Genetics and Development and AFt-B. Sustainable Algae Production

Objective:

Create a website, supporting informatics tools, and omics resources for a range of algae biotechnology efforts

Motivation:

- Enable strain improvement strategies
  - Inform genetic engineering strategies to maximize biomass production rates
- Support crop protection and cultivation diagnostics
  - Monitor feedstock stability
  - Contamination/predator detection
- Facilitate data storage, dissemination, and standardization

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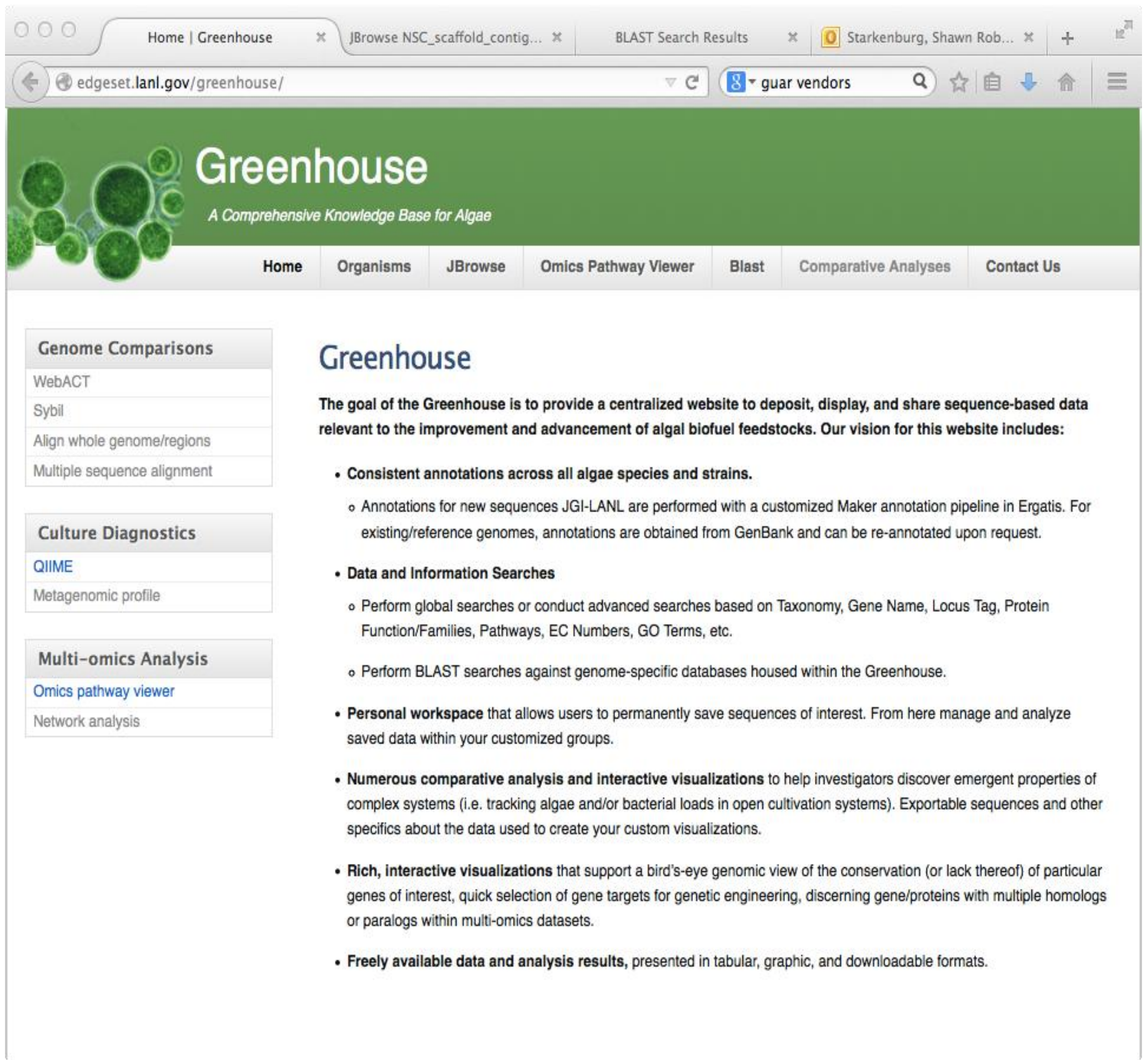
https://greenhouse.lanl.gov/



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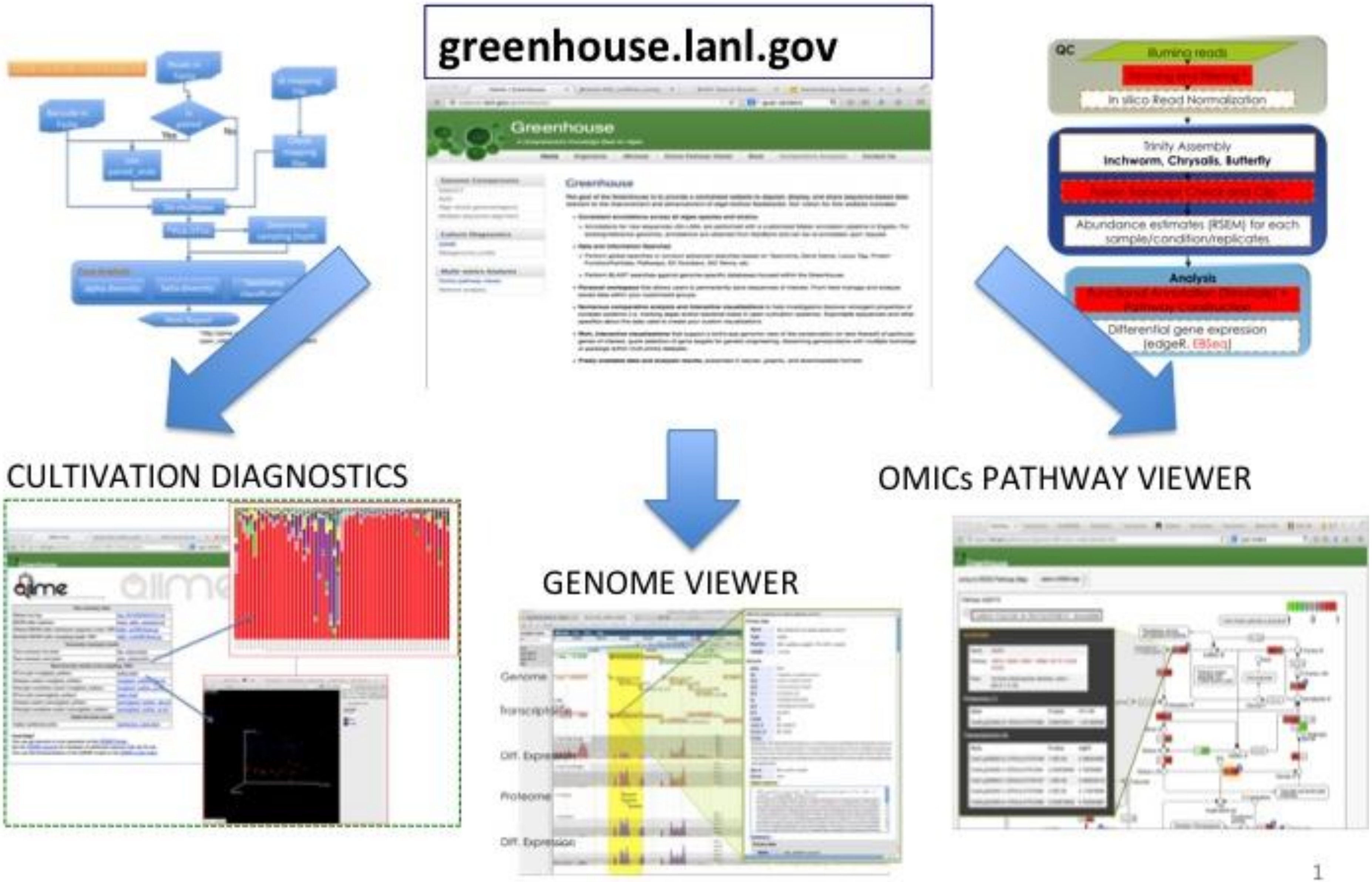
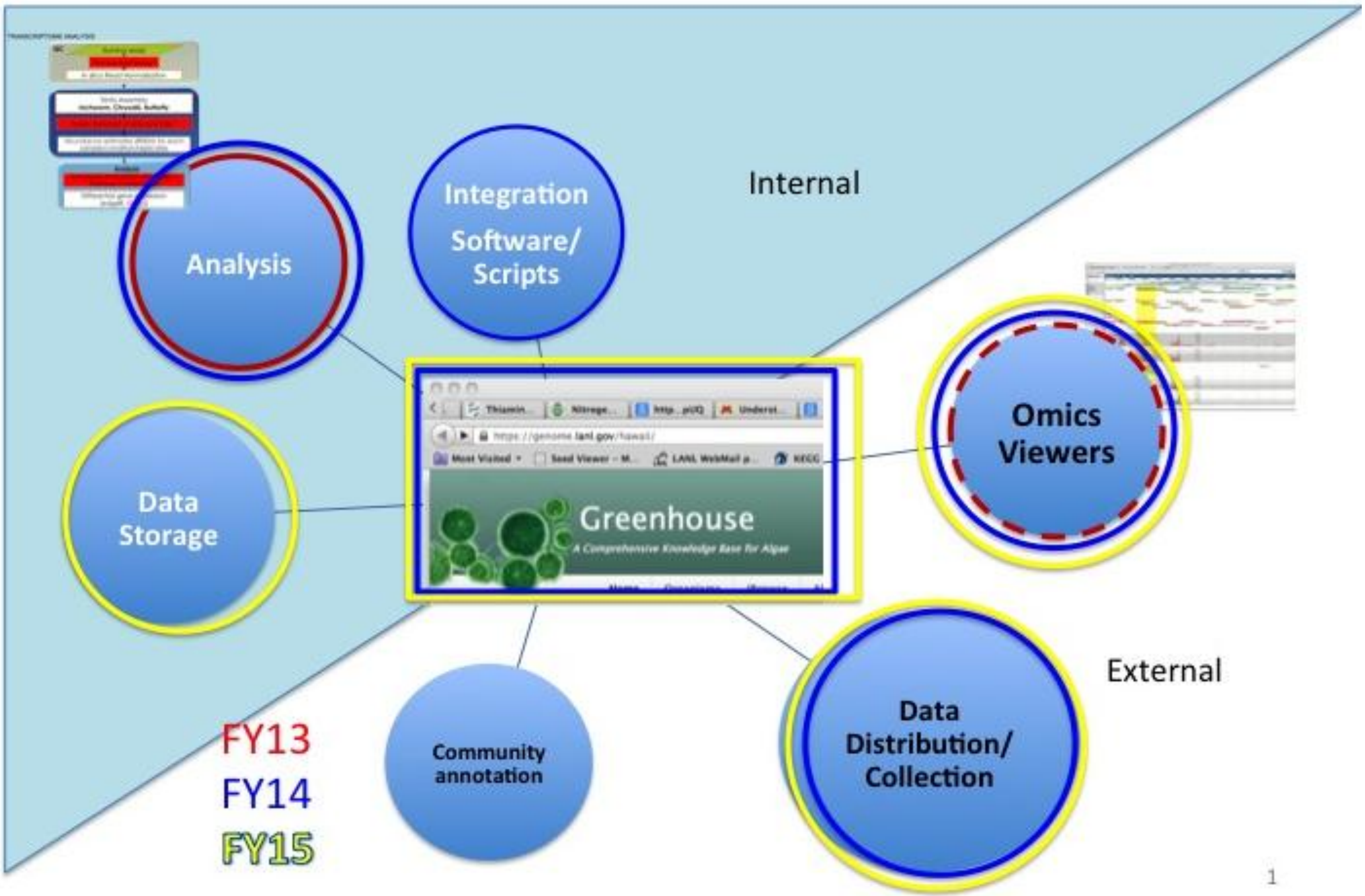
Algal Omics Platform



In silico Framework of -Omics Platform



Conclusions and Next Steps

- The Greenhouse website is active

- *Planned*: GREENHOUSE UPGRADES:
  - Build display pages for *Scenedesmus*
  - Add User Control/ Group Control Module
  - User Driven Analysis Module(s)
  - Meta-data storage capability
  - Add Metabolomics to Pathway Omics Viewer

- *Planned*: CULTIVATION DIAGNOSTICS/ PATHOGEN CONTROL
  - Sequence Based Pathogen Detection Assays
  - Enable Algal species monitoring via GOTTCHA

FY15: Algae Biotechnology Hub with LANL, NREL, PNNL

Algae Biotechnology Hub

'Omics resources

Transformation tools

Environmental screening

"Industrialized" strains

Biotechnology services

Data Management System

Deployment, Scale-up, and Integration

NREL

Strain repository

Halotolerant strain development tools

SAGE reactors

(Colorado School Of Mines)

LANL

Omics website/bioinformatics tools

Transformation pipeline

ePBRs array

Flow cytometry and sorting

Non-GMO strain improvement

Logistics technologies

PNNL

Cold tolerant strain development tools

Sexual crossing

Climate-simulation ponds and photobioreactors

Biomass growth modeling

BAT scripts

(Brooklyn College)

Envisioned Future: Expanded Effort

Coordinated 3-part, multi-lab effort to develop improved strains for robust performance in specific environmental and industrial challenges

Algae Biotechnology

Strategies for strain improvement, customization, and increased productivity

- Lab scale development
- Strain improvement tools:
  - GMO
  - Flow sorting
  - Breeding
  - Adaptive evolution
- Logistics tools

Multi-scale Flask2Farm

Strategies for strain down-selection & transition to outdoors

- Multi-scale development
- Cultivation with environmental challenges
  - Indoor PBRs and ePBRs with BAT scripts
  - Environmental ponds
  - Greenhouse
  - Outdoors with regional testbeds

Data Management System

Data integration at all levels

"Greenhouse" Website and Database

- Omics integration
- Bioinformatics tools
- Cultivation performance at multi-scales
- Metadata

4 – Relevance

Relevance to BETO goals:

- Strain improvements support increased biomass and lipid productivity.
- Strain improvements to enhance outdoor robustness will support extended growing seasons.
- Improvements to algae cultivation efficiency will help to lower the cost of producing algae biofuels by providing more efficient use of nutrients.
- Ultrasonic harvesting is energy-efficient and will help to lower the costs of producing algae biofuels.

Relevance to industry:

- These outcomes will help lower technical and financial barriers to algae biofuels production by reducing risks of technology transition and scale-up from lab to industrial scale operations.
- Technical readiness levels were advanced for specific technologies
  - Improved strains are closer to deployment
  - Ultrasonic harvesting is further along the path to commercial integration
- An expanded Hub approach will facilitate technology transfer

5 – Future Work

- Make Greenhouse website available and populate with new data streams, in coordination with AB Hub and other BETO-sponsored projects.
- Transition improved strains to larger scale or industry.
- Align existing project with expanded Algae Biotechnology Hub concept
  - Determine size, scope in time for Merit Review in FY15

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Summary

1. Innovative technologies developed in NAABB were further advanced in this project.
2. Our approach is to conduct precompetitive R&D to:1) facilitate transition of strains with improved performance to industry and 2) demonstrate scale-up feasibility of innovative logistics operations (cultivation, harvesting). We have formed the Algae Biotechnology Hub in FY15 to coordinate R&D activities in algae biotechnology and bioengineering at the national labs (LANL, NREL, and PNNL).
3. We accomplished: 1) improved productivity in genetically modified *C. sorokiniana* and *N. salina* strains and a flow-sorted, high lipid *N. salina* strain; 2) a more efficient CO_2 delivery system; 3) scale-up feasibility of an energy-efficient ultrasonic harvesting system; and 4) a unique website (Greenhouse) and analytical tools for algae strain omics integration and management of cultivation data.
4. The outcome of this project is directly relevant to BETO's mission to increase the yield of fuel and fuel-enabling bioproducts from algae, while lowering the costs of production.
5. Future work will focus on expanded coordination of R&D activities in algae biotechnology and bioengineering through the Hub; and collection and dissemination of strain information and cultivation performance through the Greenhouse website.

Project Team

- *Chlorella* sp.:
  - Dick Sayre, Angela Tonon, Amanda Barry
- *N. salina*:
  - Scott Twary, Cliff Unkefer, Hiro Teshima
- ePBR array:
  - Amanda Barry
- Ultrasound technologies:
  - Jim Coons, Dan Kalb, Taraka Dale
- Omics integration and website:
  - Shawn Starkenburg

Additional Slides

Publications

- Henley, W. J., R. W. Litaker, L. Novoveská, C.S. Duke, H. D. Quemada, and R. T. Sayre. Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. *Algal Research* 2: 66-77, 2013.
- Subramanian, S., A. N. Barry, S. Pieris, and R. T. Sayre. Comparative energetics and kinetics of autotrophic lipid and starch metabolism in chlorophytic microalgae: Implications for biomass and biofuel production. *Biotechnology for Biofuels* 6:150, 2013.
- Nesterov, A. I., G. P. Berman, J. M. Sánchez Martínez, and R. T. Sayre. Noise-assisted quantum electron transfer in photosynthetic complexes. *J. Math. Chem.* 51: 2514–2541, 2013.
- Rajamani, S. , M. Torres, V. Falcao, J. E. Gray, D. A. Coury, P. Colepicolo, and R. T. Sayre. Noninvasive evaluation of heavy metal uptake and storage in microalgae using a Fluorescence Resonance Energy Transfer-based heavy metal biosensor. *Plant Physiology* 164: 1059-1067, 2014.
- Starkenburg, S. R., K. J. Kwon, R. K. Jha, C. McKay, M. Jacobs, O. Chertov, S. Twary, G. Rocap, and R. A. Cattoloci. A pangenomic analysis of the *Nannochloropsis* organellar genomes reveals novel genetic variations in key metabolic genes. *BMC Genomics* 15: 212, 2014.
- Hovde BT, Starkenburg SR, Hunsperger HM, Mercer LD, Deodato CR, Jha RK, Chertkov O, Monnat RJ Jr, Cattolico RA. The mitochondrial and chloroplast genomes of the haptophyte *Chrysochromulina tobin* contain unique repeat structures and gene profiles. *BMC Genomics*. 15:604, 2014.
- Coons, J. E., D. M. Kalb, T. Dale, and B. L. Marrone. Getting to low-cost algal biofuels: A monograph on conventional and cutting-edge harvesting and extraction technologies. *Algal Research* 6:250-270, 2014.
- Marrone, B. L. Guest Editor: Virtual Special Issue on NAABB research. http://www.journals.elsevier.com/algal-research/virtual-special-issue/virtual-special-issue-the-national-alliance-for-advanced-bio/
- Barry, A. N., S. R. Starkenburg, and R. T. Sayre. 2015. Strategies for optimizing algal biology for enhanced biomass production. Mini Review Article. *Frontiers in Energy Research* **3**:1. doi: 10.3389/fenrg.2015.00001

Patents:

- Marrone, B. L., J. E. Coons, D. Kalb, and T. Dale. METHOD AND APPARATUS FOR ACOUSTICALLY MANIPULATING BIOLOGICAL PARTICLES. U.S. Patent Application No. 13/652,296. Published May 2013.

Webinars:

- Richard Sayre, October 22, 2014—Genetically Modified Algae: A Risk-Benefit Assessment

Manuscripts in review and in preparation

Submitted/In Revision:

- Blake Tyler Hovde; Chloe R Deodato; Heather M Hunsperger; Scott A. Ryken; Will Yost; Ramesh K Jha; Johnathan Patterson; Raymond J Monnat Jr.; Steven B Barlow; **Shawn R Starkenburg**; Rose Ann Cattolico**.** Genome sequence and transcriptome analyses of *Chrysochromulina*: metabolic tools for enhanced algal fitness in the prominent order Prymnesiales (Haptophyceae). *PLOS Genetics. submitted*
- Negi S, Barry AN, Friedland N, Sudasinghe N, Subramanian S, Pieris S, Holguin FO, Dungan B, Schaub T and Sayre RT (2015) Impact of nitrogen limitation on biomass, photosynthesis, and lipid accumulation in *Chlorella sorokiniana* using a pond-simulated environment. *J. Appl. Phycol*. (submitted).
- S.H. Park, **S.R. Starkenburg,** J. Kyndt, A. Angelova, O. Chertkov, and J. K. Brown. 2015. Chloroplast Sequencing and Analysis of the Green Alga, *Auxenochlorella protothecoides*. *BMC Genomics*. In Revision

In Preparation

- Coons, J.E. et al. Microalgae Passivity and its Relation to Ultrasonic and Centrifuge Harvester Performance
- S. Twary, P. Tiasse-Yoder, and C. Unkefer, Enhancing photosynthetic productivity of *Nannochloropsis salina* through engineered bicarbonate uptake systems
- S. Twary, M. Alvarez, S. Starkenburg, M. Teshima, P. Tiasse-Yoder, and C. Unkefer. Over-expression of acetyl coA-carboxylase increases triacylglycerol concentration in *Nannochloropsis salina*
- Dale, T. et al. Multiparameter Flow Cytometry Analysis of Lipid and Biomass Accumulation in *Picochlorum sp*
- Marrone, B. L. and T. Dale. Flow Cytometry in Algae Biofuels and Bioproducts Research (Review article)

Additional Slides: Technical Accomplishments

Molecular tool box for *Chlorella sorokiniana* is complete

- Nuclear transformation vectors were designed and built following standard practices using endogenous gene promoters and terminators obtained from the *C. sorokiniana* genome 1230 and 1412 genomes.
- We screened *C. sorokiniana for a*ntibiotic sensitivity to identify potential antibiotic resistance genes for use as selectable markers (Hygro, Par, Chlor).
- Successful transformation of *C. sorokiniana* was achieved using *E. coli* replicating plasmids containing a unique multi-cloning site plus one of two different (par^R , Hyg^R) antibiotic resistance, selectable marker genes driven by either the *actin* or *psaD* promoter/terminator pair.
- Codon-optimized transgenes have been successfully expressed in *C. sorokiniana.*
- Chlorophyll a oxygenase RNAi constructs were successfully expressed in *C. sorokiniana* resulting in elevated Chl a/b ratios (3.3) but now need to target both *Cao* (nonidentical) genes for improvements.

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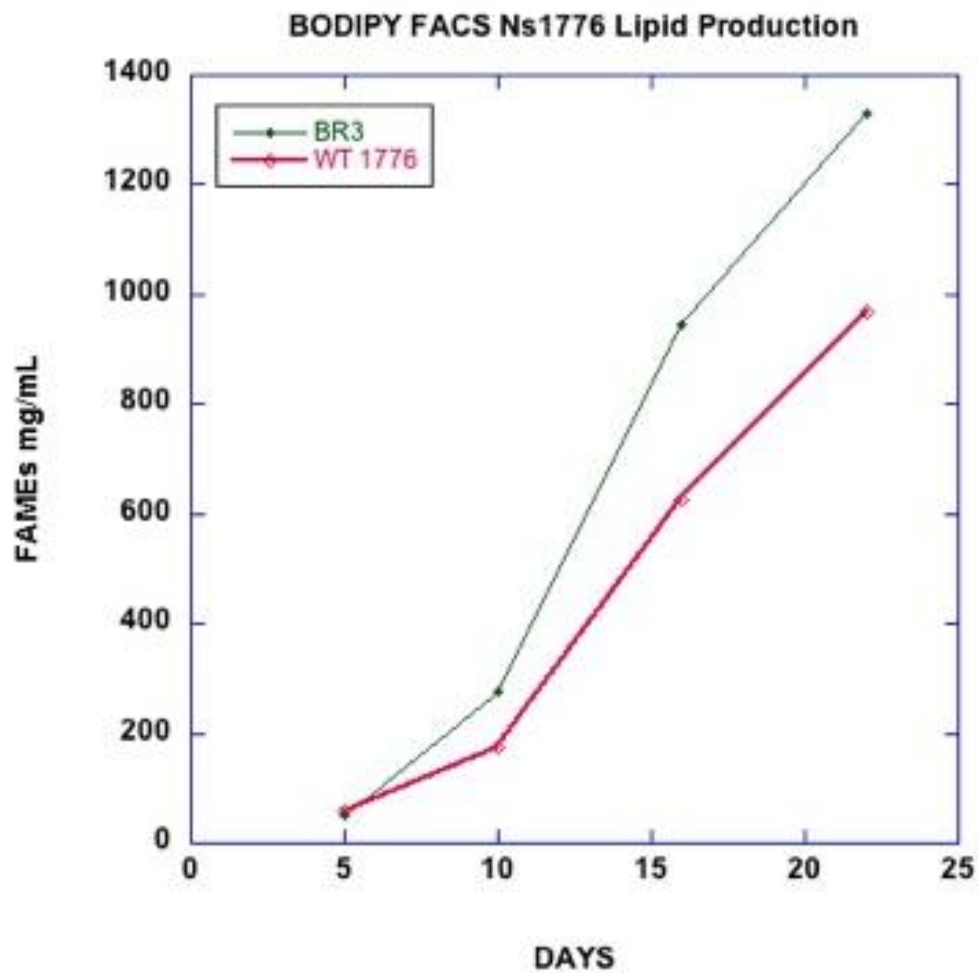
Non-GMO Population Improvements

- Select increased lipid accumulation phenotypes for non-GMO population improvement
  – BODIPY FAC sorting increased lipid accumulation (FAME analysis) by 27% after 4 generations of non-GMO selection of *N. salina*



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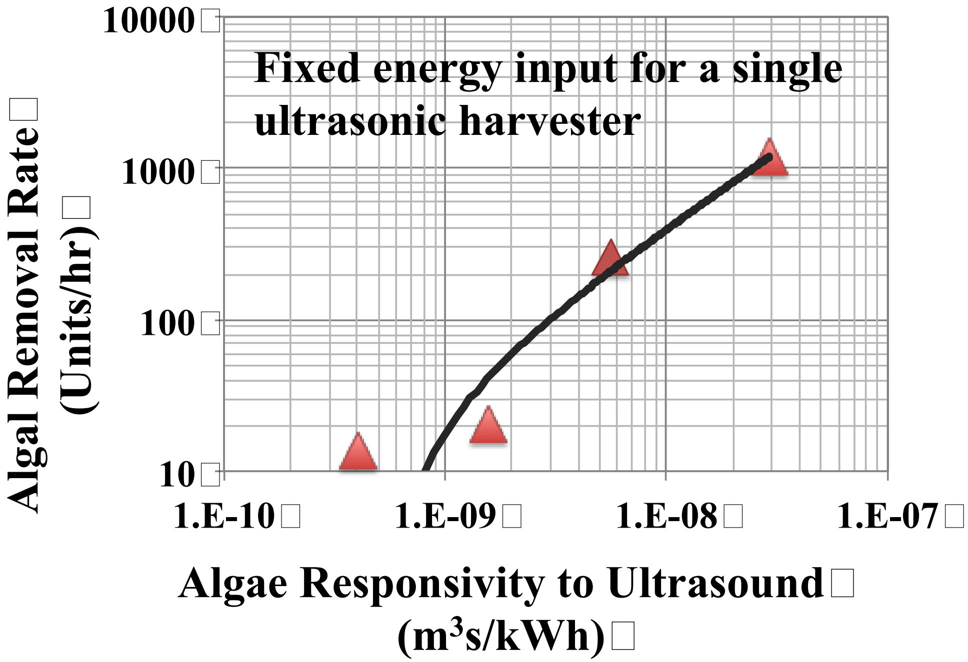
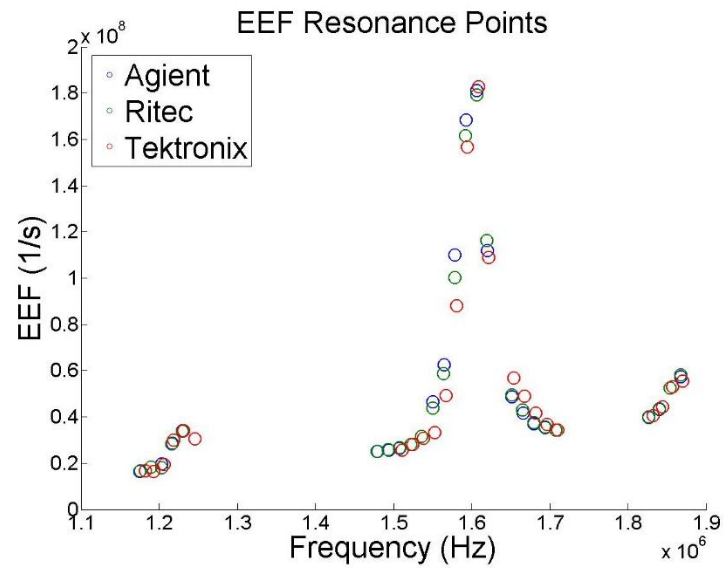
Accomplishments in Ultrasound Technologies, cont.

- Validated theory using a single ultrasonic harvester device.



The experimental results (above) show the rate of algae removal in an ultrasonic harvester is proportional to algae responsivity.

- Made progress toward energy efficiency factor (EEF) determination of ultrasonic harvesters, separators, and extraction devices.



Preliminary results (above) show consistent EEF measurement for a harvester vessel using different approaches. The maximum EEF is the most favorable operating condition.

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Acoustic Stimulation for Improved Algae Growth

Goal: Examine the feasibility of using audible and ultrasound as growth stimulants during algae cultivation

Barrier Addressed: Aft-B Sustainable algae production

Approach:

– Design and build cultivation systems in which audible and ultrasound treatments can be made

– Alter treatment conditions (frequency range, intervals, duration, nutrient regime)

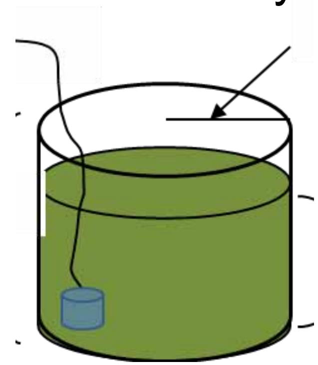
– Characterize algae biomass and lipid accumulation

– Examine molecular mechanisms underlying observed response

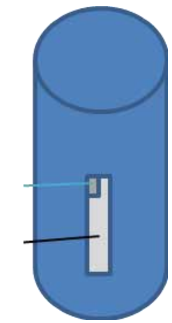
Results

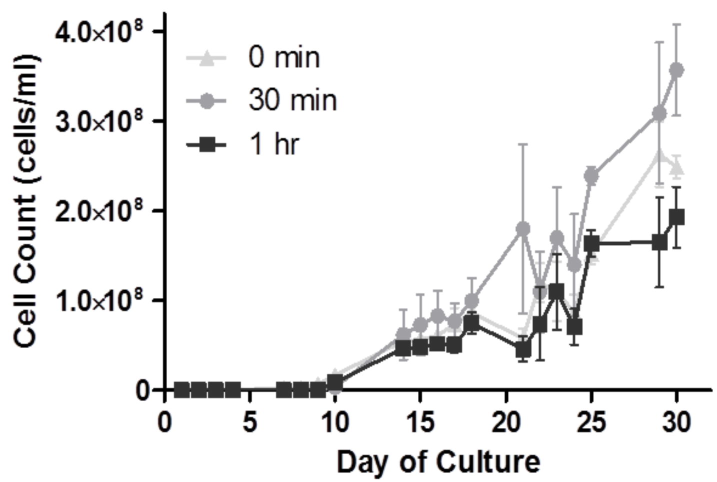
– Designed and built two different acoustic treatment cultivation systems

- Greenhouse environment (natural light)
- 9 L
- 2 conditions in triplicate
- Audible sound treatment



- PBRs (simulated outdoors)
- 0.5 L
- 3 conditions in triplicate
- Ultrasound treatment





Observed an increase in biomass accumulation with audible treatment in the greenhouse.

Further Work: Follow up on this result & ePBR experiments. Continue treatments, examine mechanisms.

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Genome Projects since NAABB

	Chlorella sorokiniana 1230	*Chlorella sorokiniana 1228*	*Scenedesmus sp. DOE152Z*
Quality	Improved HQ Draft	Improved HQ Draft	Draft
Size	58.6 Mb	61Mb	210
Scaffolds/ Chromosomes	-/12	13/12	N.D.
Contigs	22	64	2812
N50	3818 kb	2395 kb	152 kb
Max	5.1 Mb	4.56 Mb	2.33 Mb

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Genome-based Culture Diagnostics

		1230			1228			1412		
Sample	Description	% Mapped	% Genome	Cov. Depth	% Mapped	% Genome	Cov. Depth	% Mapped	% Genome	Coverage Depth
CSI-V1	1 ABD2	0	1	0	55	98	6	0	0	0
CSI-V2	2 AD3	0	0	0	51	98	5	0	0	0
CSI-V3	3 BR3	0	1	0	47	99	8	0	0	0
CSI-V4	4 WT B	0	1	0	76	99	10	0	0	0
CSI-V5	5 BD3	0	1	0	52	98	5	0	0	0
CSI-V6	6 WT A	0	1	0	69	99	8	0	0	0
CSI-V7	7 OLD CS	0	1	0	93	99	10	0	1	0
CSI-V8A	9 YULIYA 1228	1	3	0	91	99	7	0	1	0
CSI-V8C	8 NEW CS	91	99	8	1	2	0	0	1	0
CSI-V10	10 JO MIX	0	1	0	88	99	11	0	0	0
CSI2012	2012 DNA	81	99	25	0	4	1			

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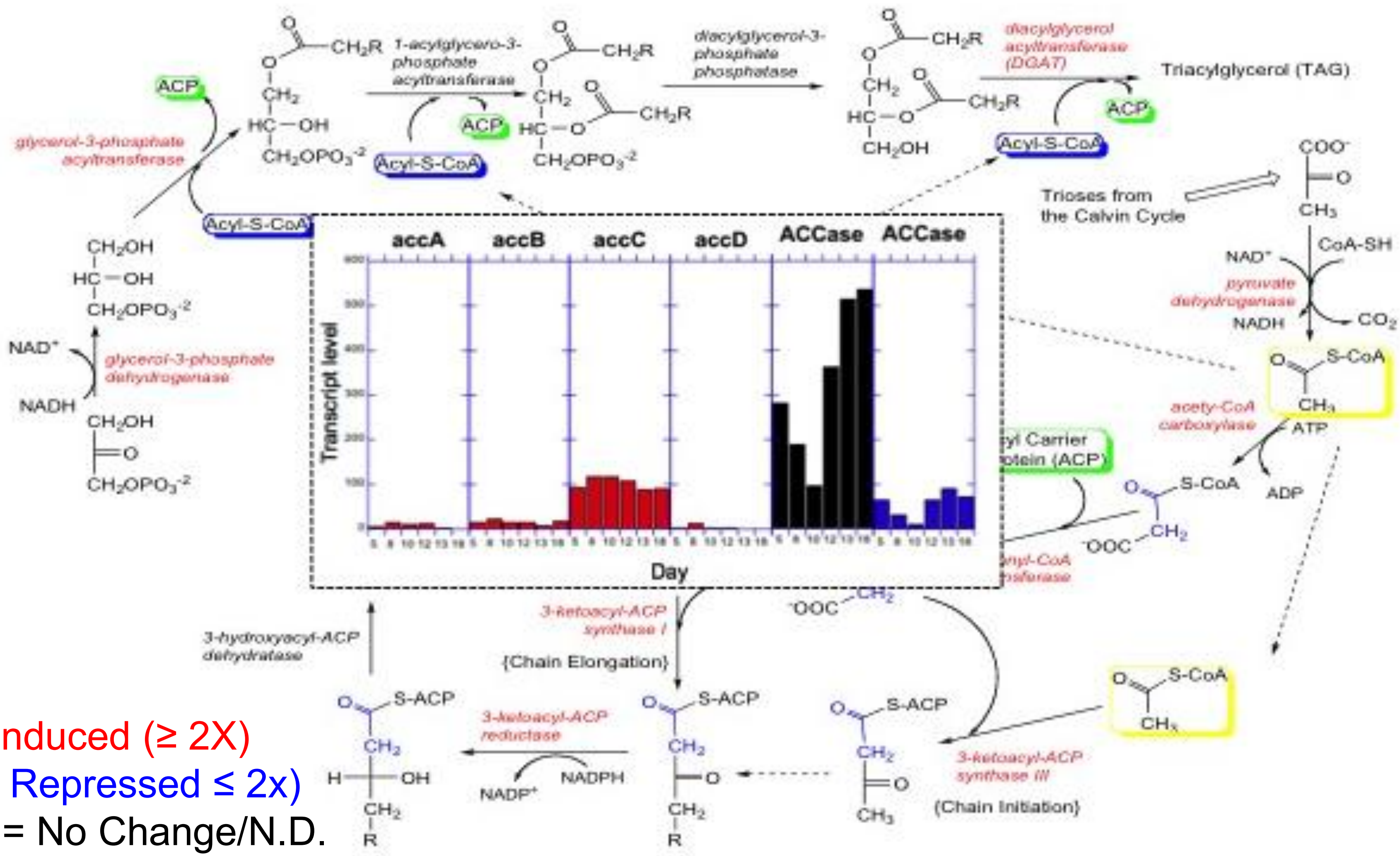
Induction of lipid and TAG biosynthesis (Pathway Maps)



RED = Induced (≥ 2X)

BLUE = Repressed ≤ 2x)

BLACK = No Change/N.D.

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