Consortium for Algal Biofuels Commercialization (CAB-Comm)

May 21, 2013

Biomass Program Algae Peer Review

Principal Investigator: Stephen Mayfield

Organization: University of California San Diego

Presenters: Stephen Mayfield, Michael Burkart, Jon Shurin, UC San Diego

CAB-Comm Goal Statement

- The goal of this consortium is to address hurdles to algal biofuels commercialization in three research areas: 1) Crop Protection 2) Nutrient Utilization and Recycling and 3) Genetic Tool Development

- The research projects are collaborative efforts between 17 academic labs at UC San Diego, UC Davis, UN Lincoln, and Rutgers, and two industrial partners - Sapphire Energy and Life Technologies

Summary

Academic Partners: 4

University of California: San Diego		
Biology-Chemistry-Admin	6 PIs	$4.10 M
Scripps Oceanography	4 PIs	$1.77 M
University of Nebraska:	3 PIs,	$1.57 M
Rutgers University:	3 PIs,	$1.32 M
University of California, Davis:	1 PI,	$0.20 M
Total:	**17 PIs**	**$9.00 M**

Cost-Share Partners: 2

Sapphire Energy,	$ 1.5 M
Life Technologies,	$ 1.2 M
Total:	**$11.7 M**

Publications	**Presentations**	**Patents:**
Crop Protection: 13	Crop Protection: 64	Crop Protection: 2
Nutrient Utilization: 4	Nutrient Utilization: 34	Nutrient Utilization: 0
Genetic Tools: 23	Genetic Tools: 60	Genetic Tools: 4
Total: 40	**Total: 158**	**Total: 6**

CAB-Comm
Academic Partners

UC San Diego SIO	U Nebraska Lincoln	Rutgers University	UC Davis
S. Mayfield	D. Weeks	P. Falkowski	A. Kendall
M. Burkart	J. Van Etten	C. Dismukes	
S. Golden	G. Oyler	D. Bhattacharya	
J. Golden			
S. Briggs			
J. Shurin			
B Palenik			
G. Mitchell			
M. Hildebrand			
B. Brahamsha			

Quad Chart Overview

Timeline

- Project start date April 2011
- Project end date May 2014
- Percent complete 75%

Barriers

- Barriers addressed
  - Crop Protection
  - Nutrient Utilization and Recycling
  - Improved Genetic Tool

Budget

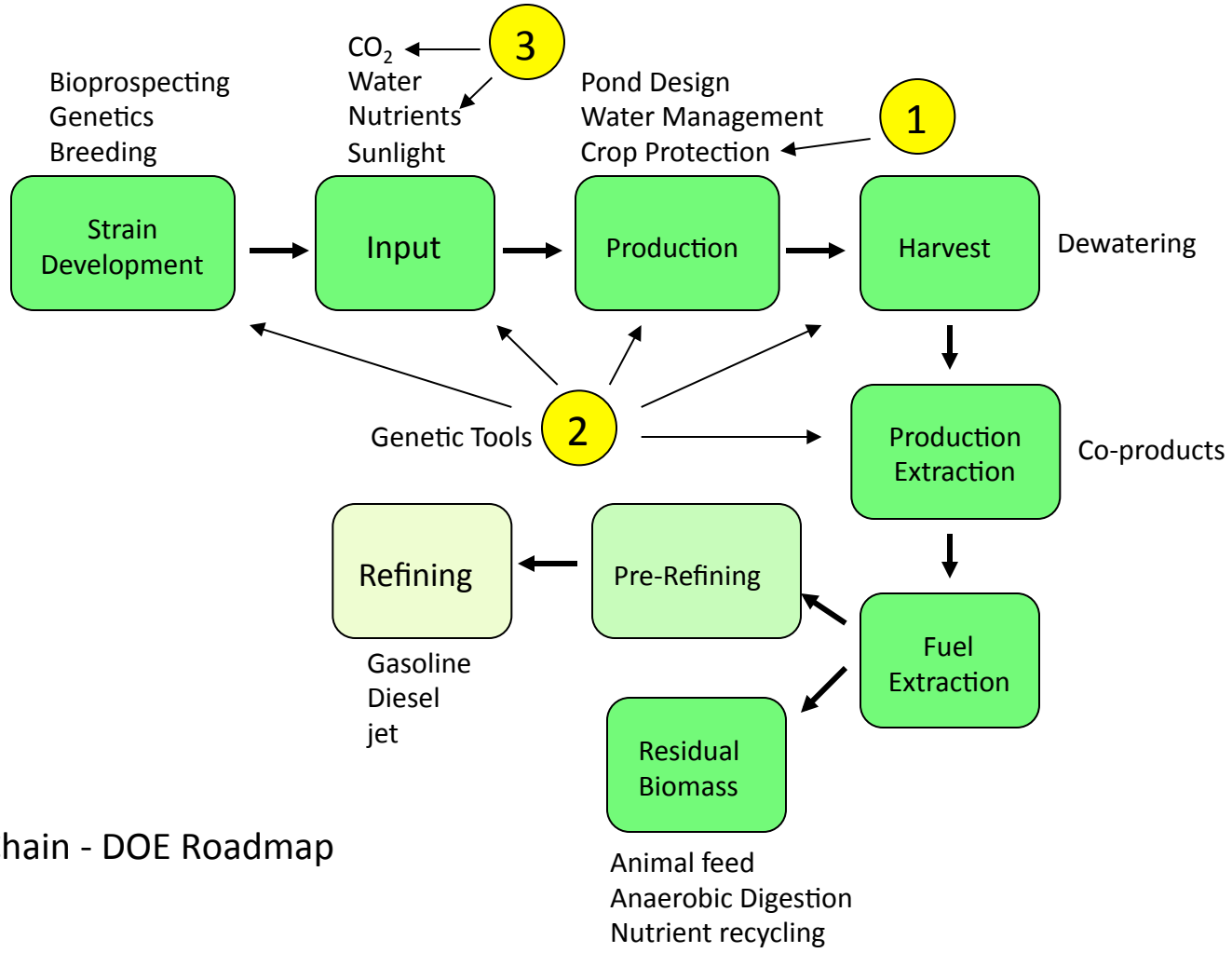
- Total project funding
  - DOE share $9 M
  - Cost share $3 M
- Funding for FY11 -$3 M
- Funding for 2012 - $3 M
- Funding for 2013 - $3 M
- ARRA Funding - $0

Partners

- Sapphire Energy
  - Crop Protection
  - Nutrient Utilization-Recycling
- Life Technologies
  - Genetic Tools

Project Overview

CAB-Comm was selected to work on a reduced set of the perceived hurdles to algal biofuels commercialization. From this set we chose three research areas - Crop Protection, Nutrient Utilization and Recycling, and Genetic Tool Development. We also reduced our Industrial Partners to the two most serious players, Sapphire Energy and Life Technologies, to ensure that significant progress was made in these critical areas of algal biofuels research.



Algal Biofuels Value Chain - DOE Roadmap

1 - Approach

- ***Overall Technical Approach***
  - ***In consultation with Commercial Partners Identify the most pressing research needs and assign a priority status***
  - ***Identify the research group(s) with the capabilities and expertise to address each priority***
  - ***Identify milestones and deliverables for specific projects***

- ***Structure of Research Projects and Milestone Review***
  - ***Majority of research projects are collaborations between multiple labs and one industrial partner***
  - ***Milestones and deliverable are reviewed quarterly, with annual stage gate review for continuation of funding***
  - ***Review panel includes both academic and industrial participants***
  - ***Two projects failed to pass 1st stage gate review***

2 - Technical Accomplishments/ Progress/Results

- ***Statement of work and budget were initially approved in July 2010***

- ***Contract negotiation from August 1, 2010 to April 2011 final funding authorized April 07, 2011 - No change in SOW or overall budget during this time.***

- Progress from the last 24 months will be presented
  - Crop Protection: Mike Burkart
  - Nutrient Utilization and Recycling: Jon Shurin
  - Genetic Tools Development: Steve Mayfield

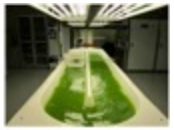
3 - Relevance

The three research focus areas all fall within critical stages of the algal biofuels production chain – as identified by DOE/NAS

Sapphire Energy™

life technologies™

GeneArt® Algae Engineering Kits



- The first commercially available standardized & complete system for algae metabolic engineering
- Tools for Chlamydomonas reinhardtii and Synechococcus elongatus PCC 7942

4 - Critical Success Factors

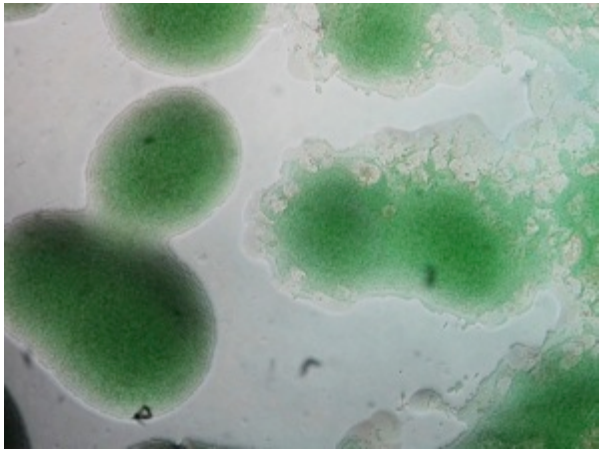
- *Critical success factors to technical and commercial viability:*
  - *Integration of research projects with **commercial partners***
  - *Achieve milestones and deliverable on time*
  - *Continuous review of projects for progress and commercial relevance*

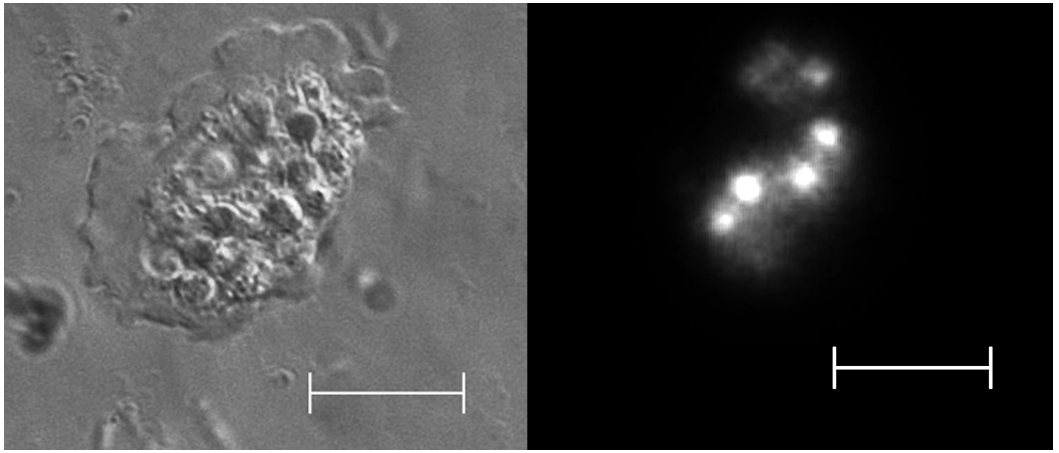
- *Potential Challenges to overcome to achieve project results:*
  - *Field is moving fast need to remain nimble*
  - *With 17 academic participants, communication between labs and commercial partners is essential*
  - *With only two industrial partners, commercial research focus will be much easier to manage*

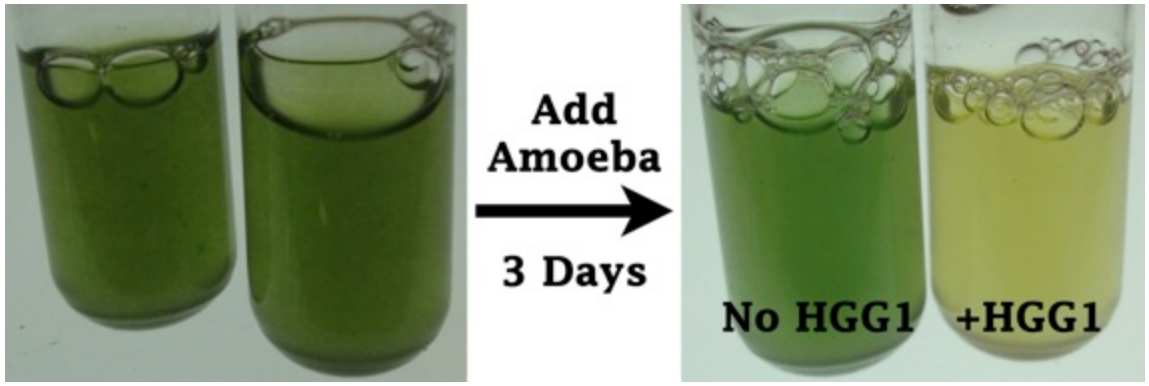
- *Demonstrate how project will advance the commercial viability of biomass:*
  - ***Sapphire projects are directly tied to viability of first integrated algal biorefinery – started operations in 2012***
  - ***Life Technology products entered the market as research tools in 2012***

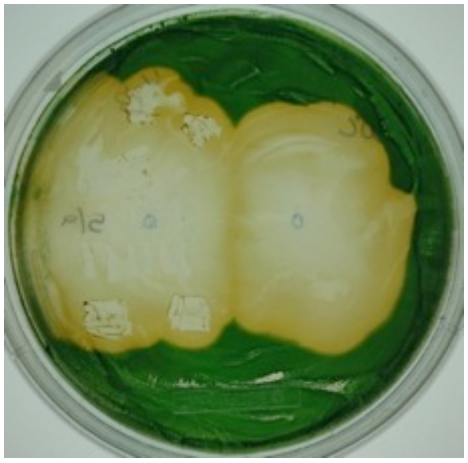
Crop Protection

Amoeba HGG1: A Model Grazer of Cyanobacteria





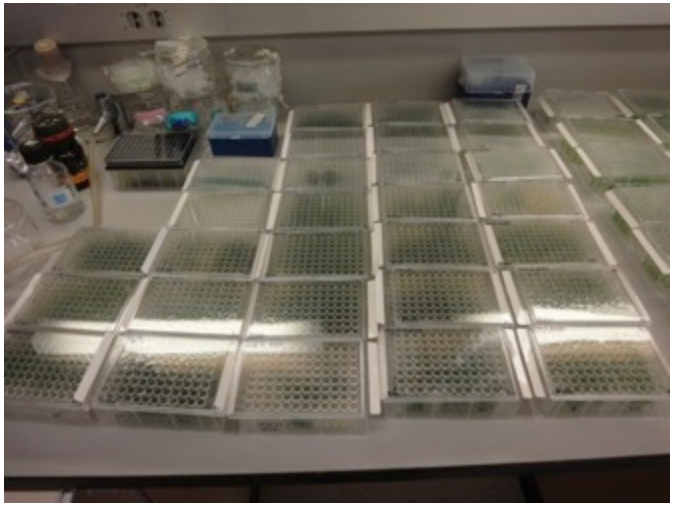


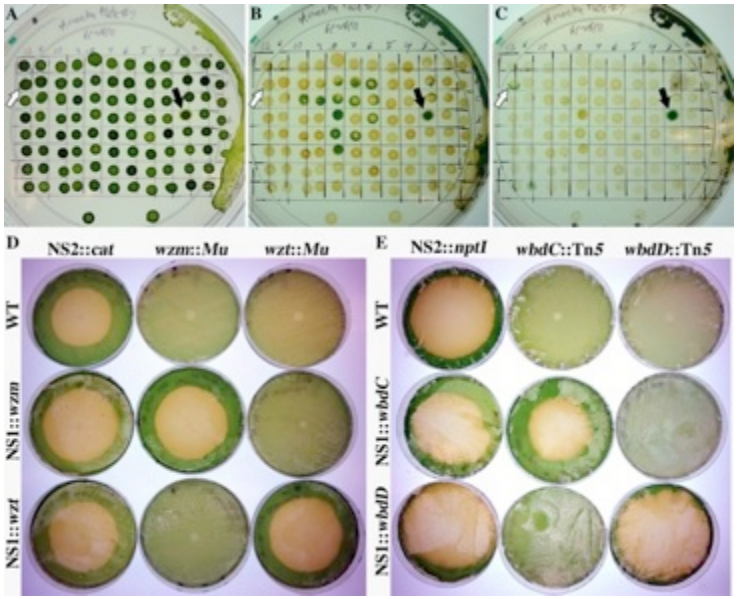


Grazing of PCC7120 in liquid culture

Grazing of PCC7942 on plate

Screening a KO Library for Resistance

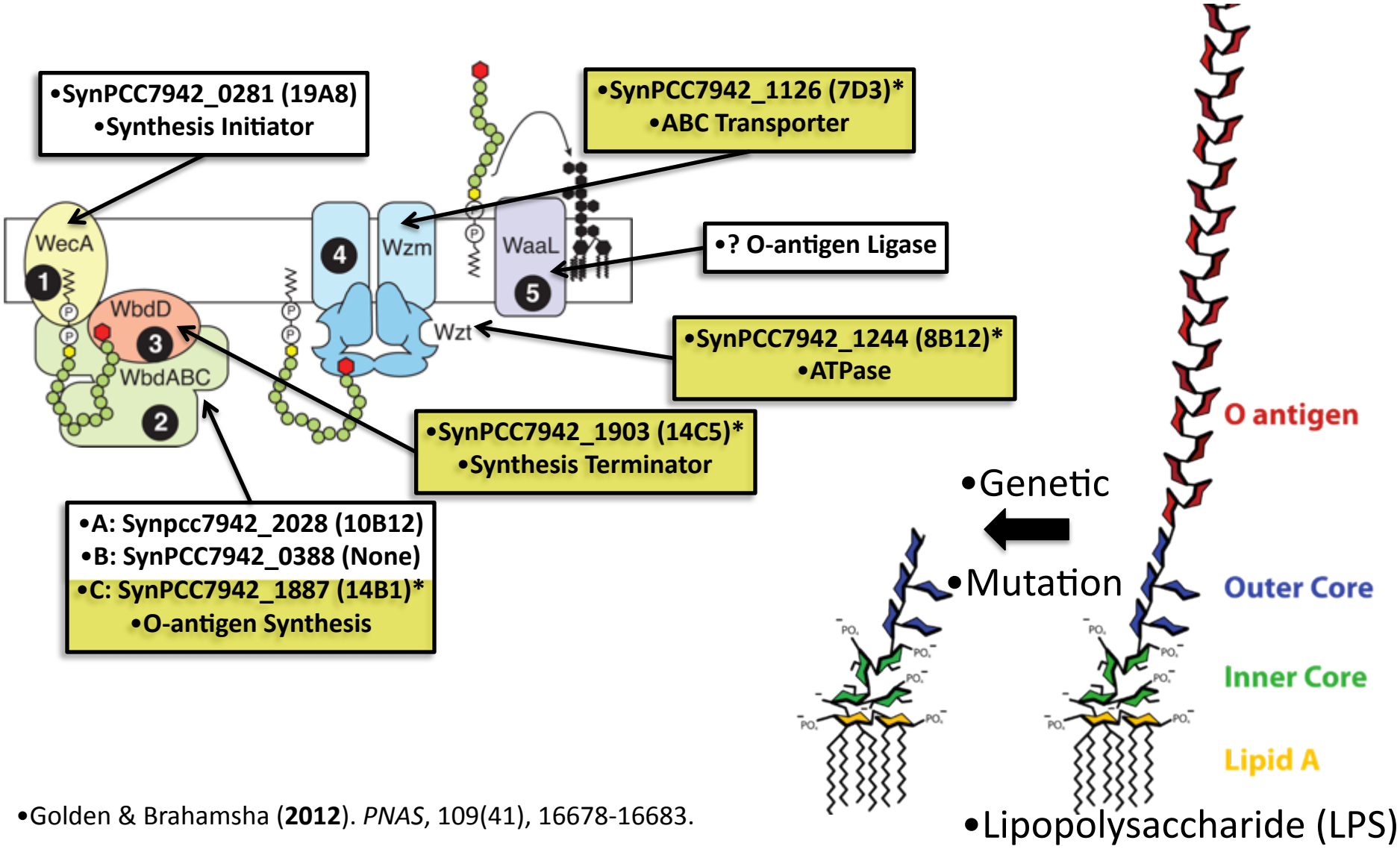




- Unigene Set of Insertional Mutants ~2,600 individual mutants,
- covering ~88% of the genome

- Plates of the mutant library were grown 13 days
- Amoebae were added, plates were monitored for 25 days for grazing, resistance, and colony survival.
- Discovered one resistant line: **7D3**

Impairment of O-antigen Confers Grazer Resistance



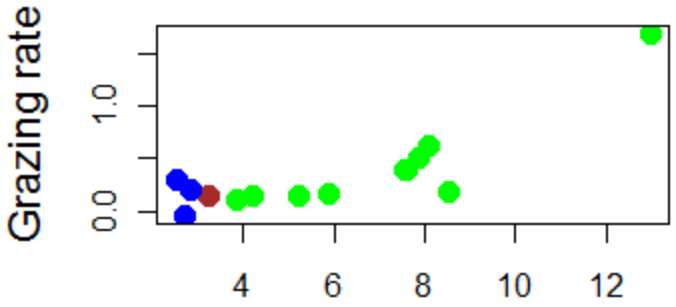
•Golden & Brahamsha (**2012**). *PNAS*, 109(41), 16678-16683.

Algal diversity and crop protection

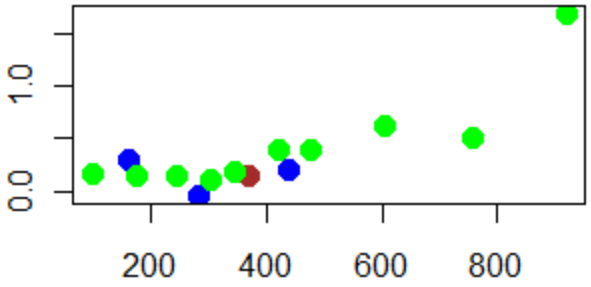
What species are most vulnerable to pests?

Are consortia of species more resistant than monocultures?

Low nutrient species get eaten the most

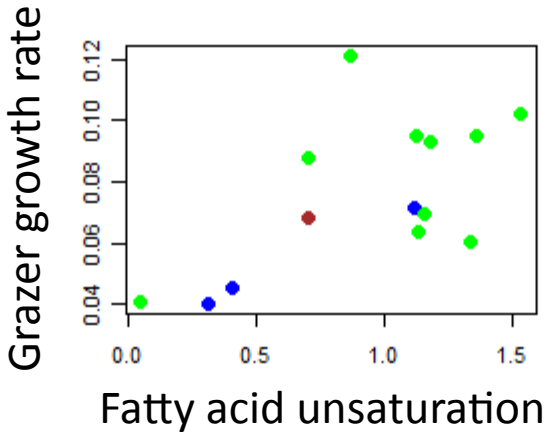


•Algal C:N

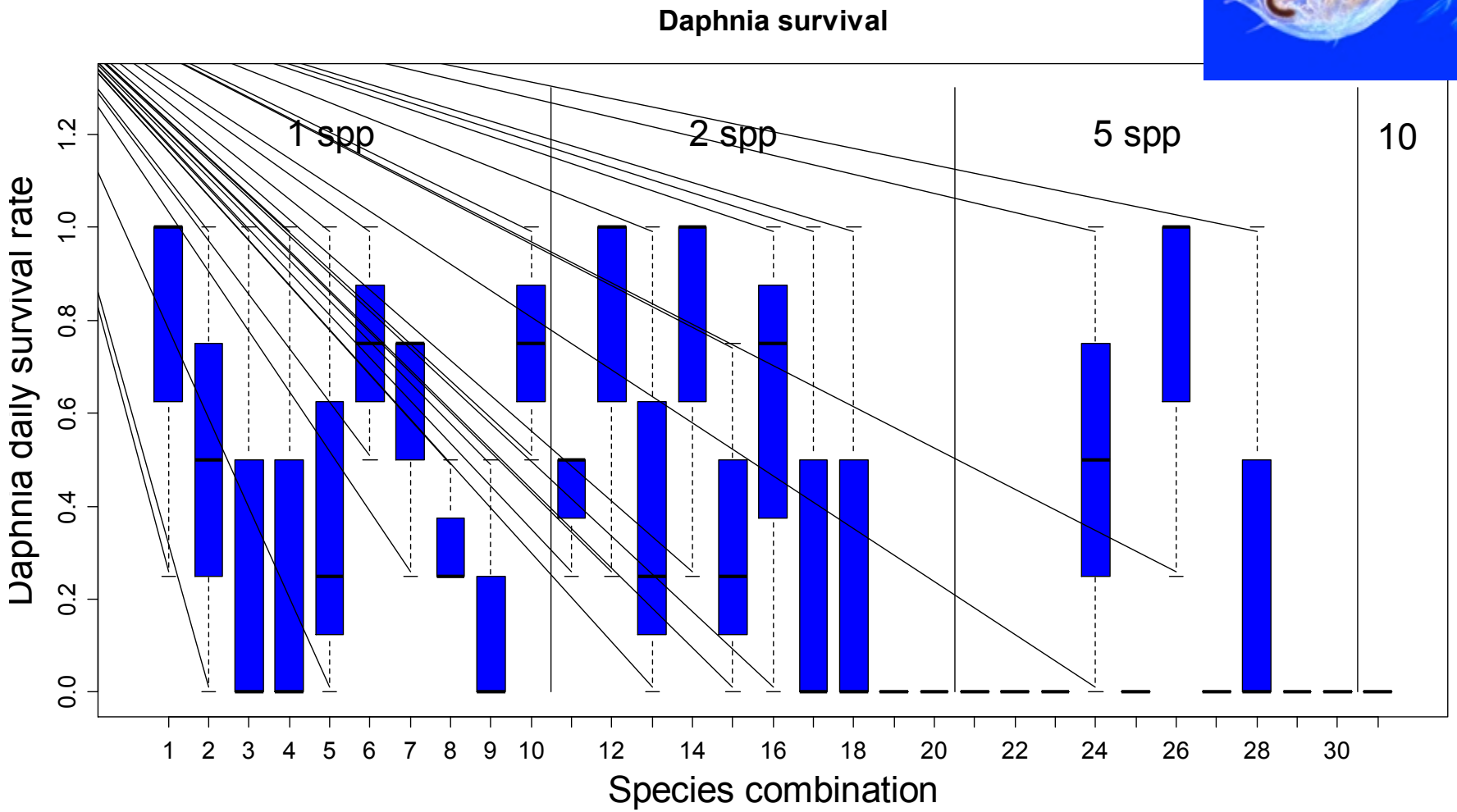


•Algal C:P

Highly unsaturated fatty acids increase grazer growth rate



Grazers more easily invade monocultures than mixtures of 5-10 species



Microscopy based tracking of pest detection

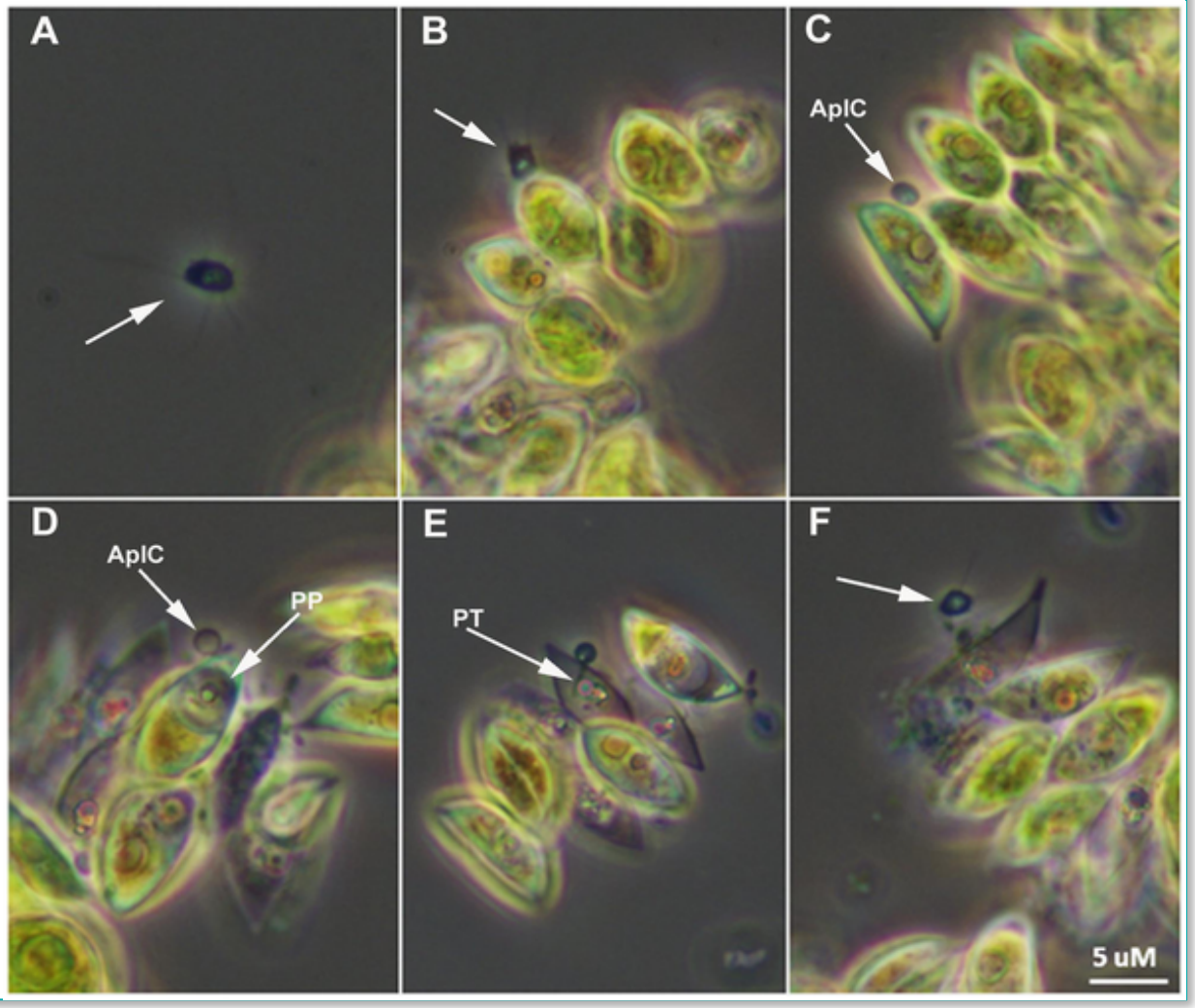
First step in detection & tracking of a pest

Example: *Amoeboaphelidium protococcarum*

Isolated by Sapphire in Las Cruces, NM in 2010

Identified and studied in collaboration with leading academics in the field

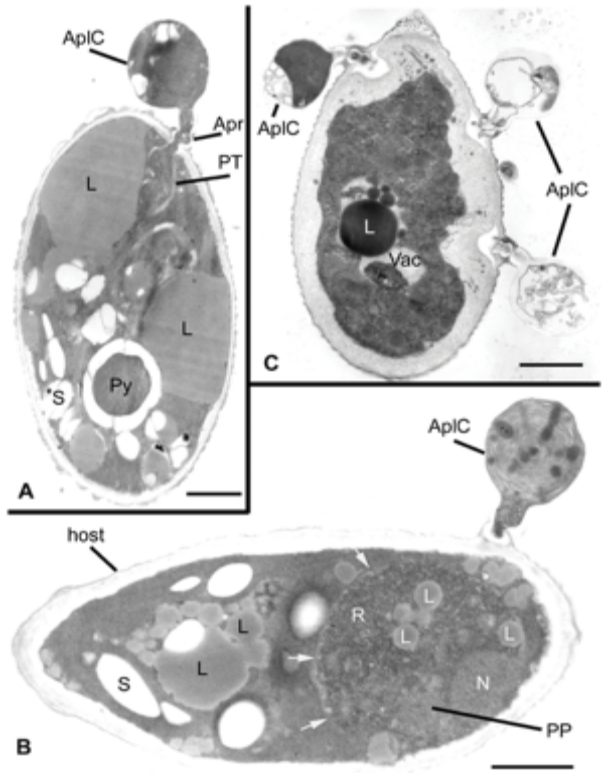
Published in PLOSone [Letcher et al. (2013) 8 (2):e56232]



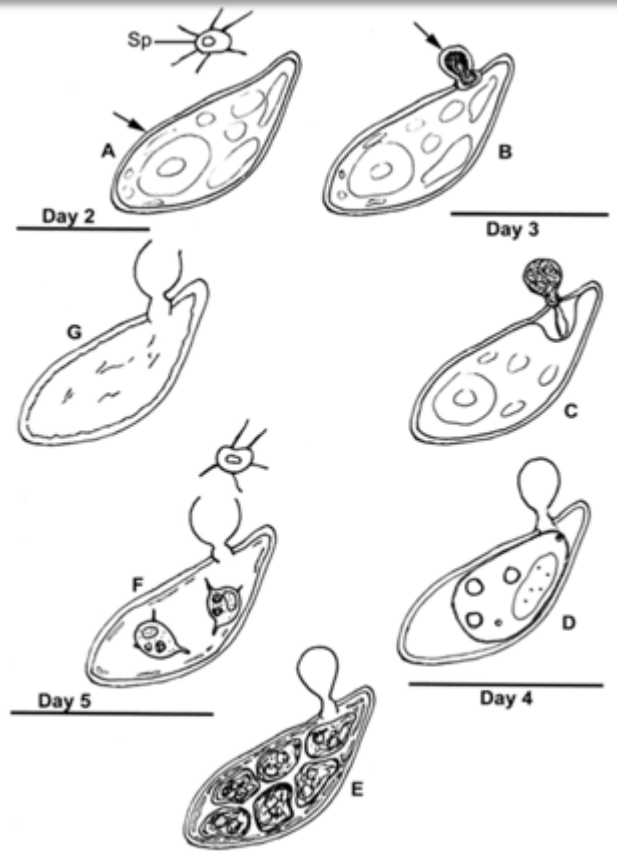
Sapphire Energy™

Understanding pest life history is important to detection and management

•Ultrastructural features of *Amoeboaphelidium protococcarum* parasitizing *Scenedesmus dimorphus*



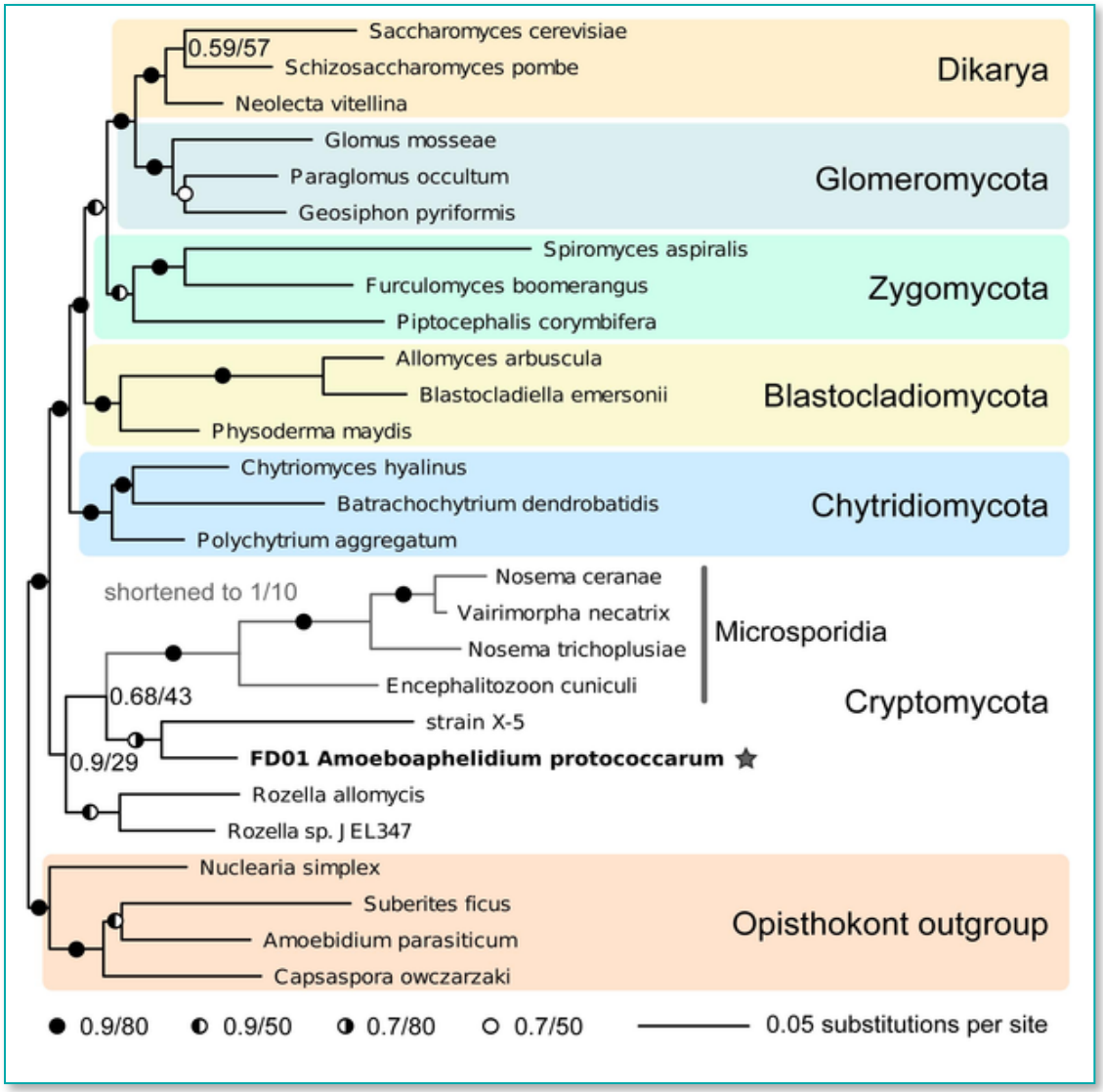
•Life cycle of *Amoeboaphelidium protococcarum* parasitizing *Scenedesmus dimorphus*



Sapphire Energy™

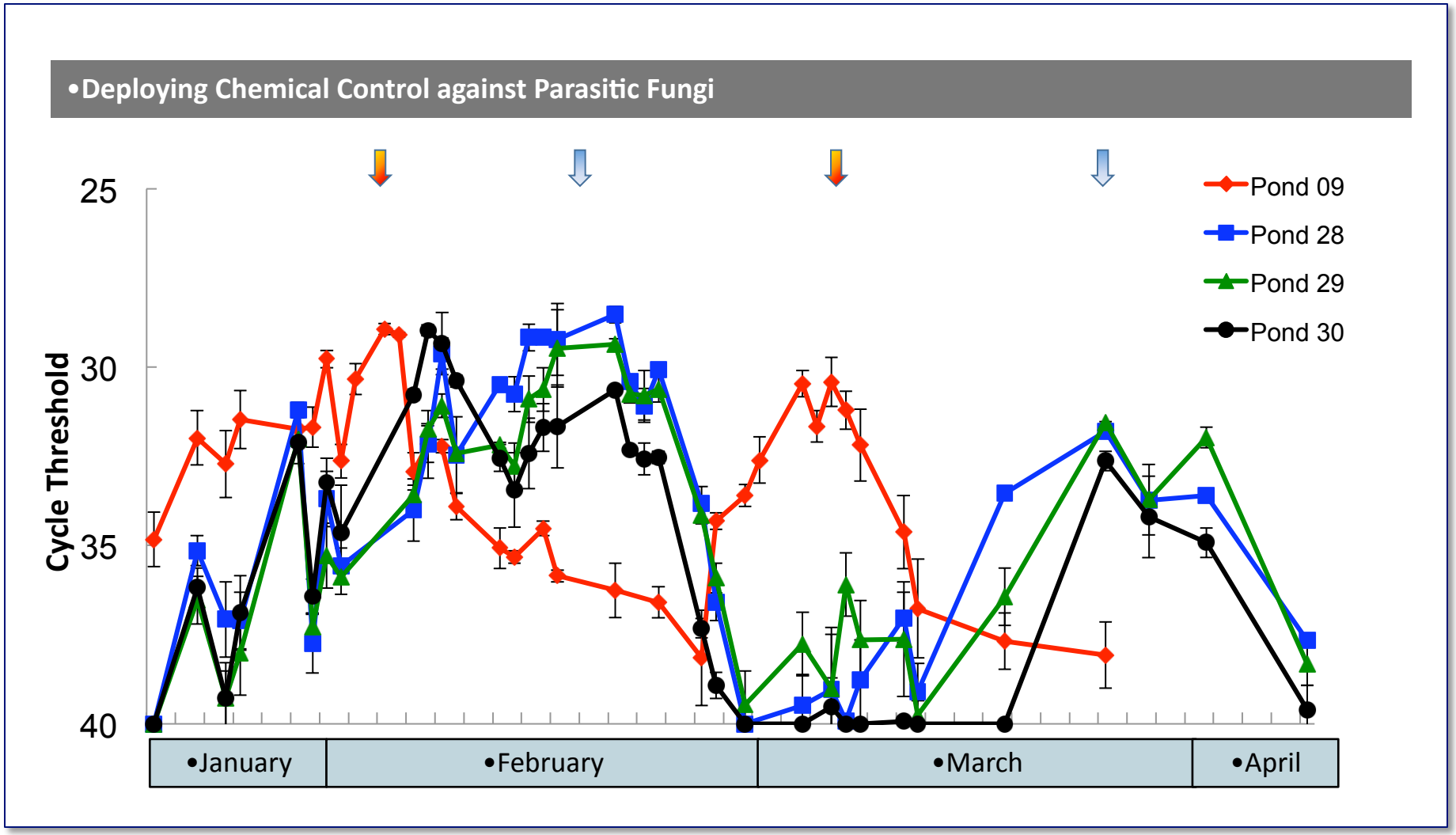
Molecular genetic tracking for pest monitoring

- Once identified, pests are tracked by molecular genetic tools
- ITS regions of rDNA sequenced
- qPCR primers developed
- Used for field tracking of pests
- Full rDNA gene cluster used for phylogenetic assignment



Sapphire Energy™

Sapphire uses targeted qPCR primers to track pests DNA in open ponds



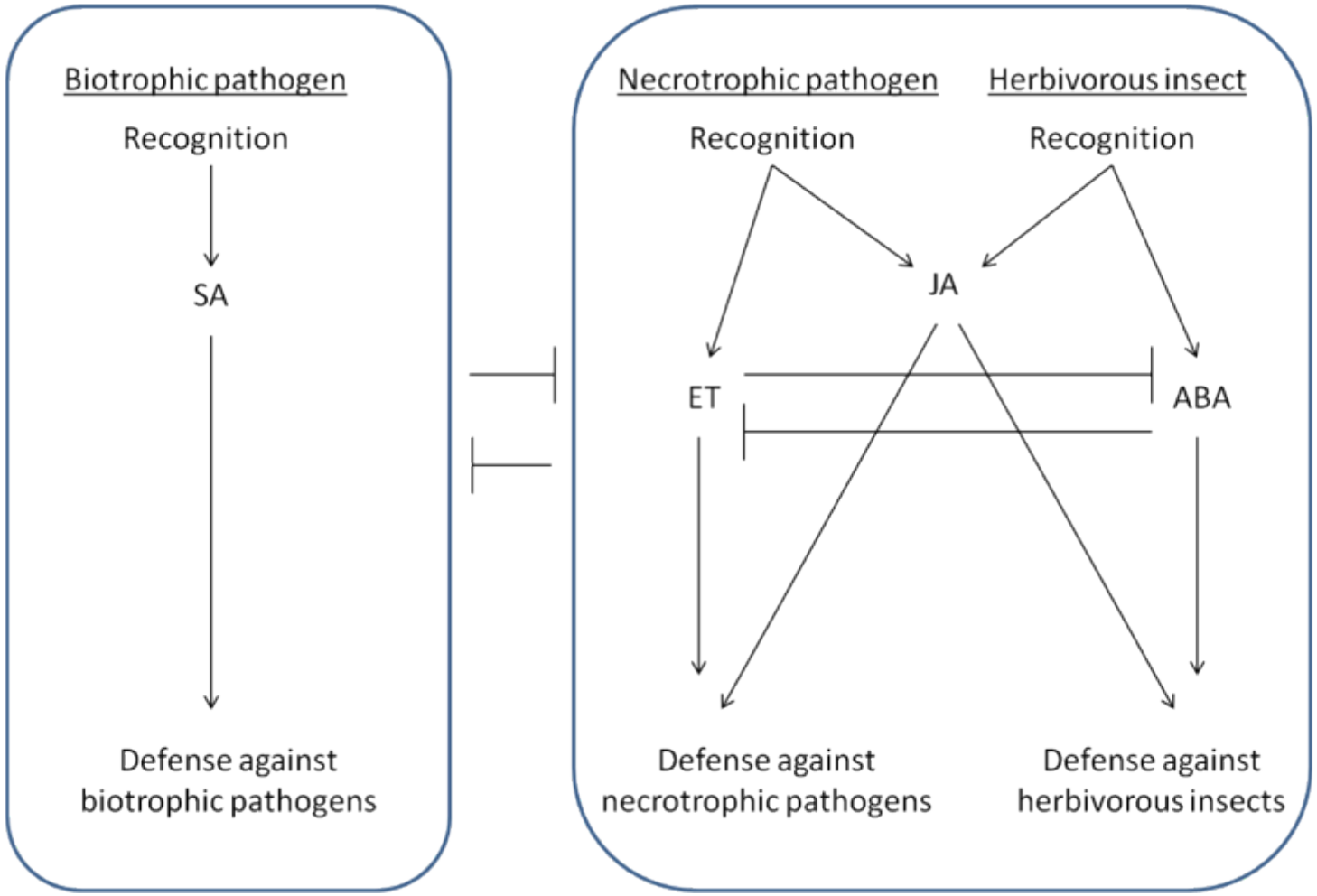
Do green microalgae have immunity?

- There is no published evidence that SA or JA play a role in green microalgae
- There are no published examples of innate immunity against cellular pathogens of single-celled hosts, including green microalgae

Algae ponds at Sapphire Energy in Las Cruces, NM

- Our proteomics data revealed that infection induces the same proteins in plants and in *Scenedesmus dimorphus*, including SA biosynthetic enzymes (e.g., chorismate synthase) and signaling proteins (e.g., calcium dependent protein kinase)
- Green microalgae gave rise to higher plants, thus we hypothesize that *S. dimorphus* possesses the evolutionary origins of the higher plant immune system
  - Hypothesis: *S. dimorphus* will respond predictably to stress hormones (SA and JA) that are known to modulate immunity in higher plants

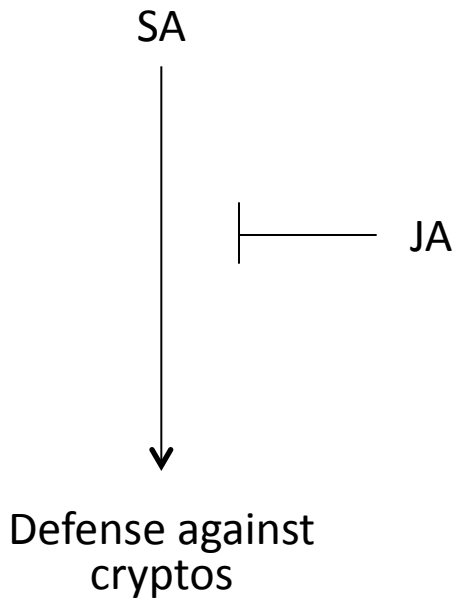
Stress hormones mediate plant immunity

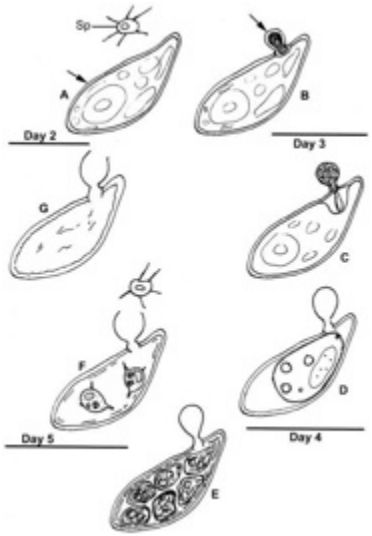


SA (salicylic acid); JA (jasmonic acid); ET (ethylene); ABA (abscisic acid)

Predictions based on plant responses

- SA will increase defense
- JA will decrease defense
- Each hormone will inhibit growth





(Crypto infection of *S. dimorphus*. Letcher *et al,* 2013)

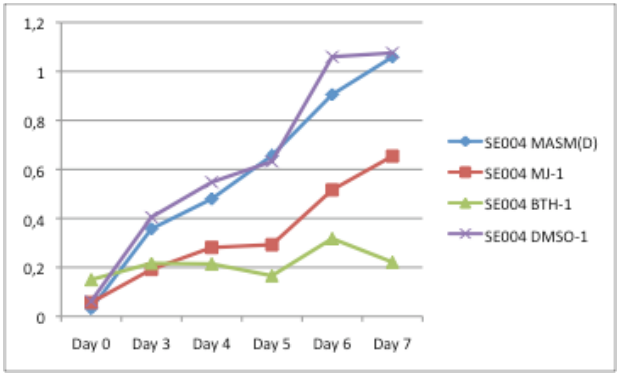
- Our predictions assume that the cryptos are biotrophic pathogens, as suggested by their obligate parasitism

Algae respond to plant stress hormones and alter fungal infection

Predictions are confirmed

- SA treatment increases defense
- JA treatment decreases defense
- Either hormone inhibits growth

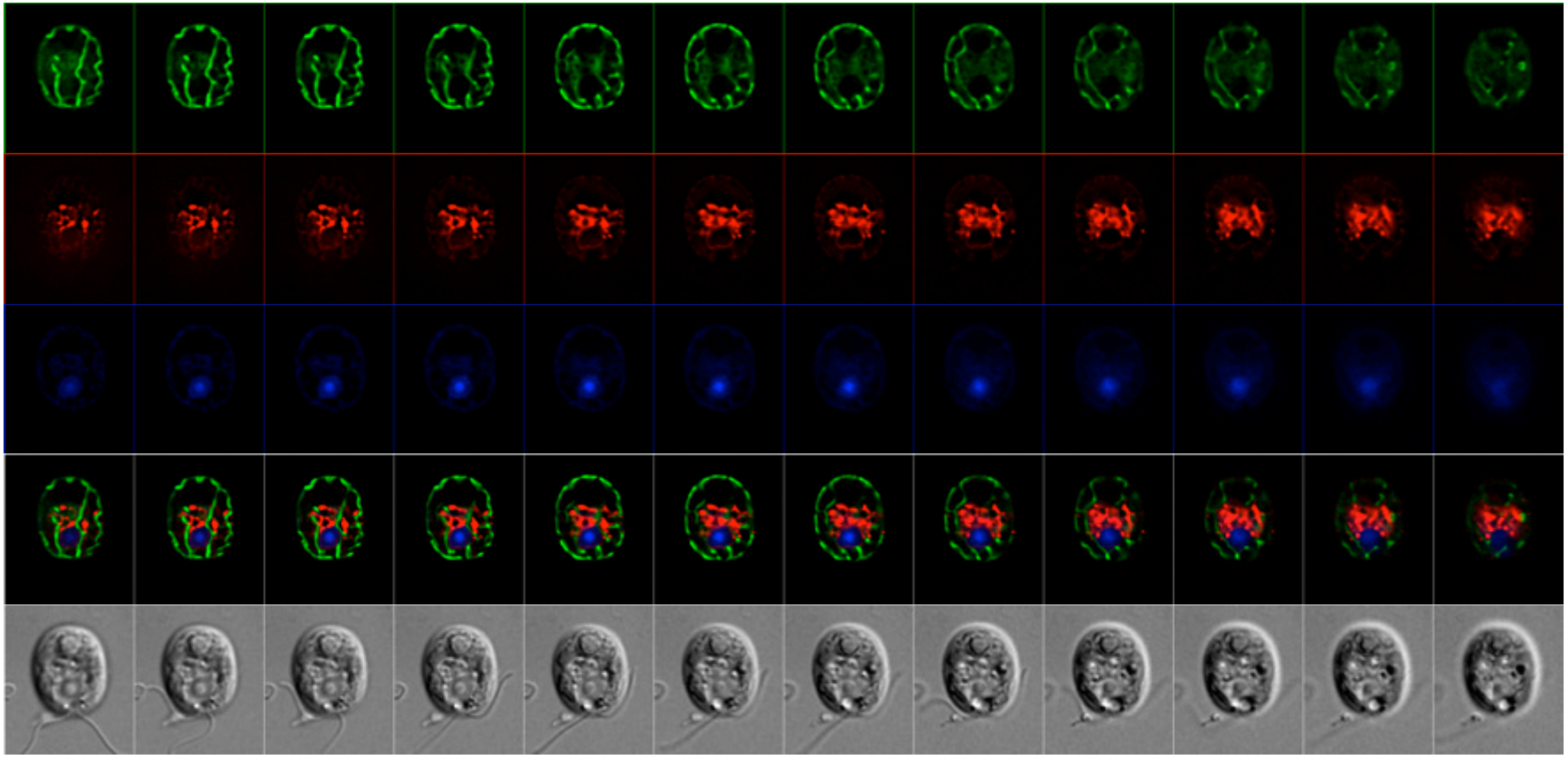
Both SA and JA inhibit growth of *S. dimorphus* in the absence of infection



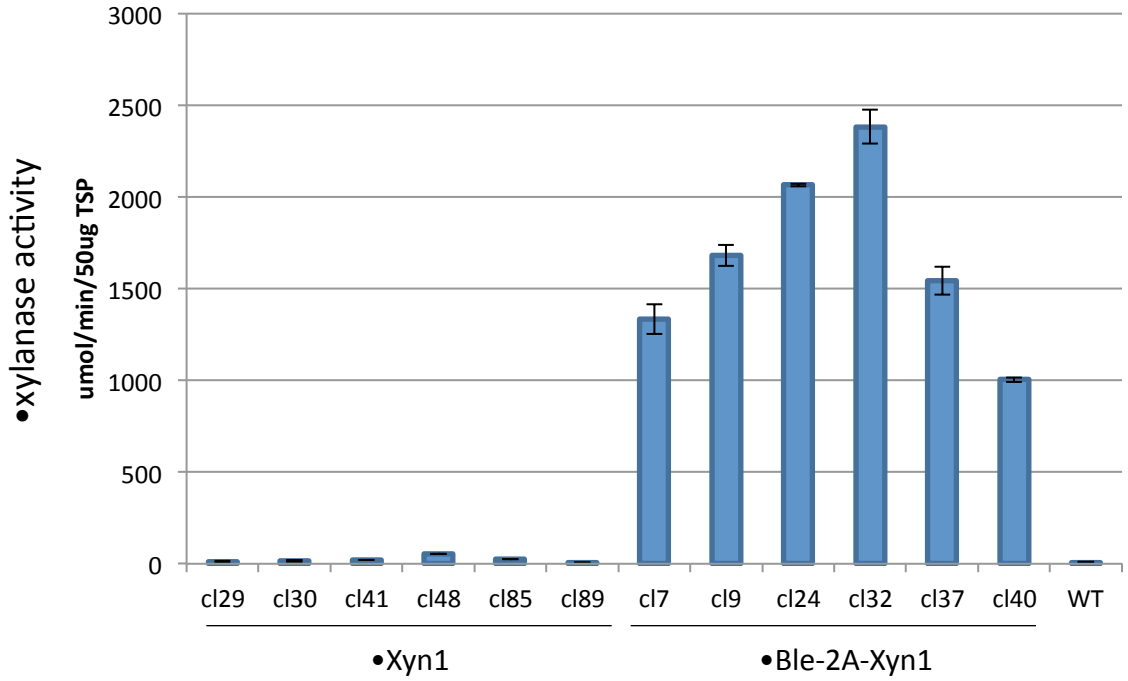
SE004 is *S. dimorphus*; FD01, FD61, and FD95 are cryptos

Treatment with the SA analog BTH **increases** defense!

Genetic Tools

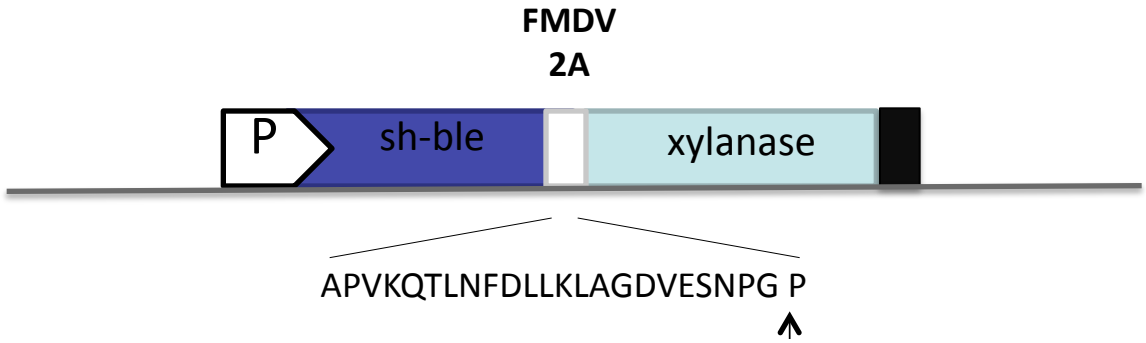


Co-expression of Xyn1 with Ble leads to the selection of transformants with higher xylanase activity



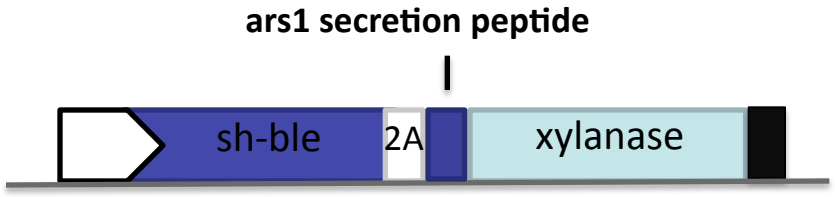
~ 100-fold higher activity

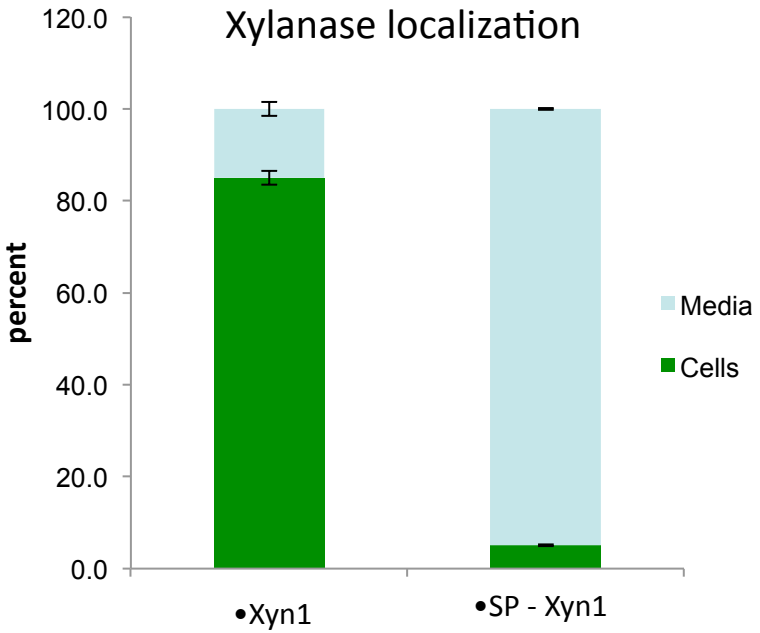
Linking heterologous gene expression to bleR via a viral self-cleaving sequence

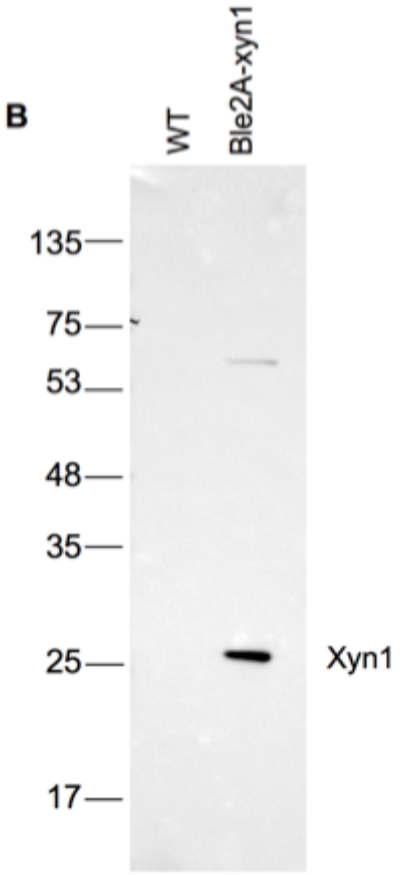


The foot-and-mouth disease virus (FMDV) 2A peptide is a cis-acting self-cleaving sequence.

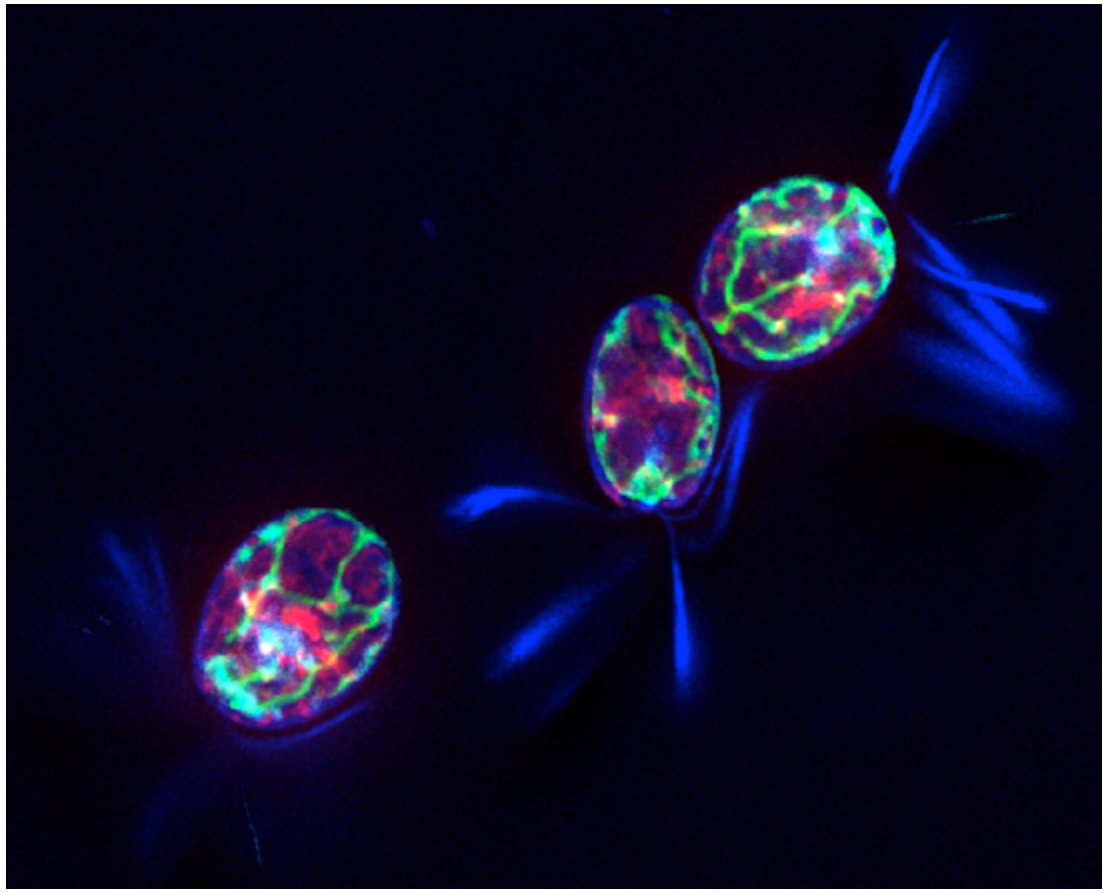
We can now secrete significant amount of industrial enzymes from algae





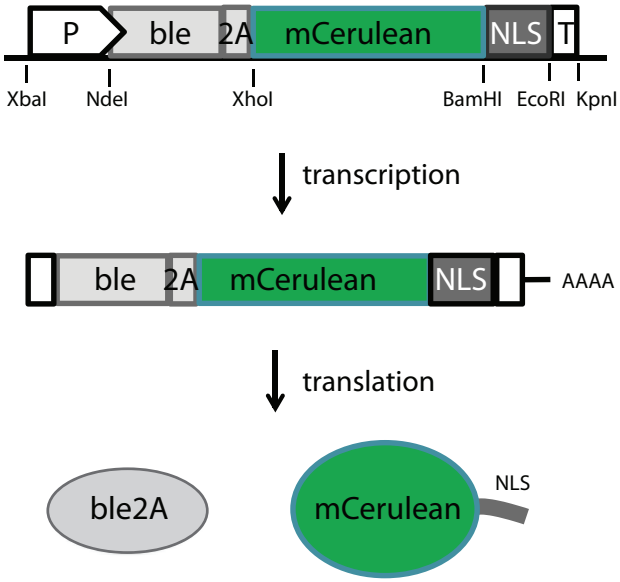


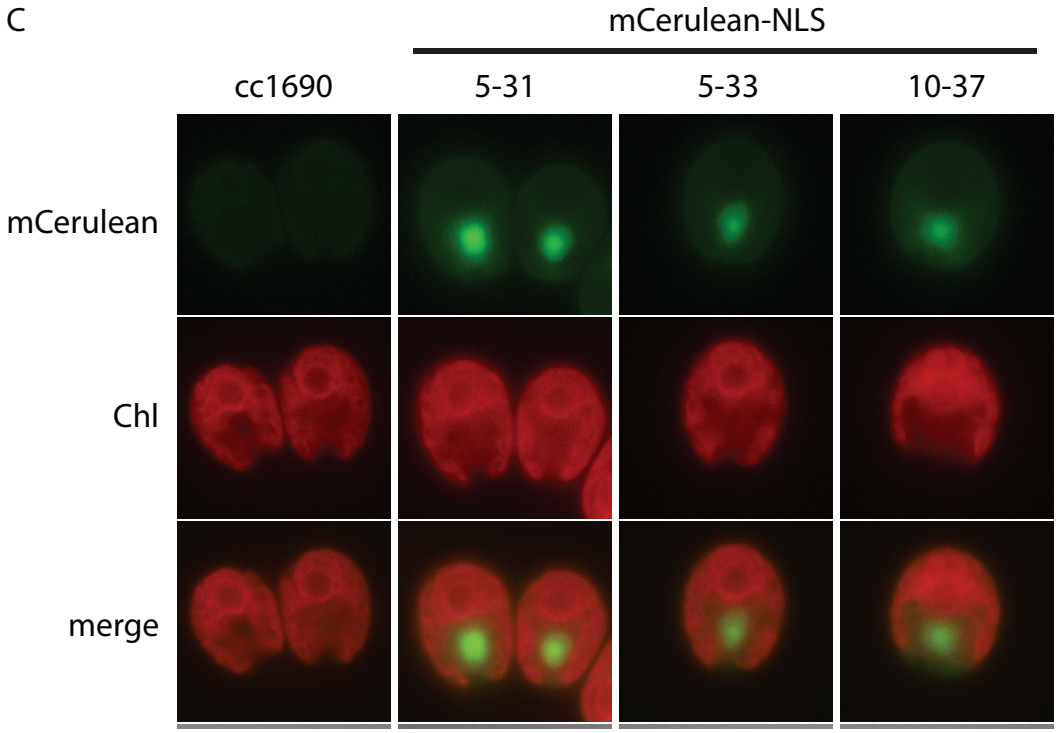
Production of Rainbow Colored Algae



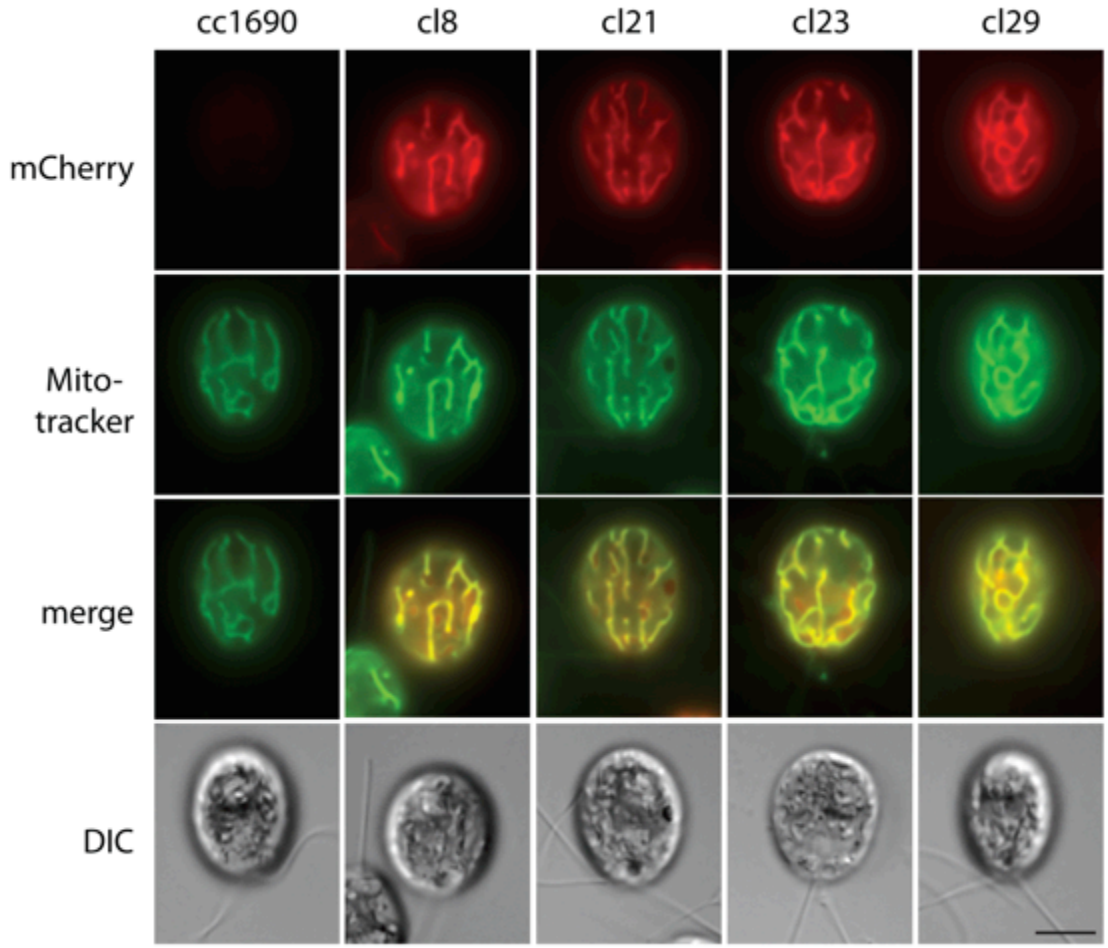
Live cell microscopy of algae expressing fluorescent proteins

Nuclear localization of mCerulean using ble2A

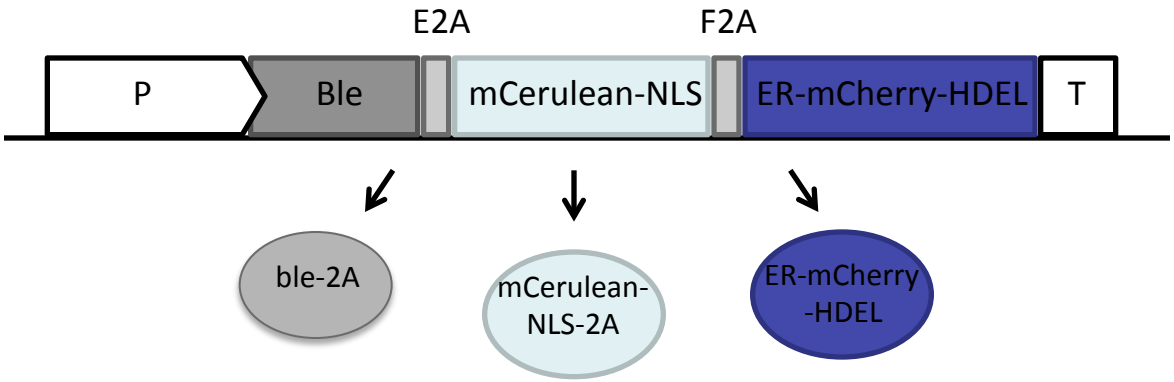


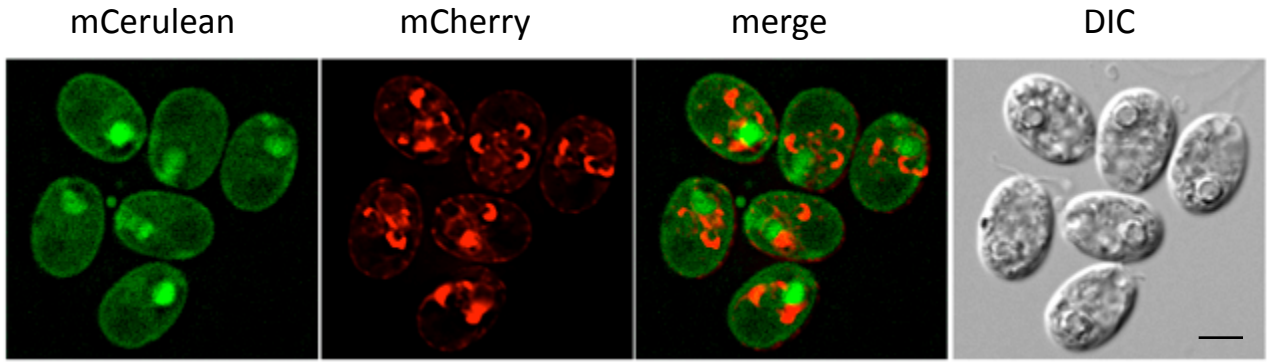


Mitochondria targeting of mCherry using ble2A

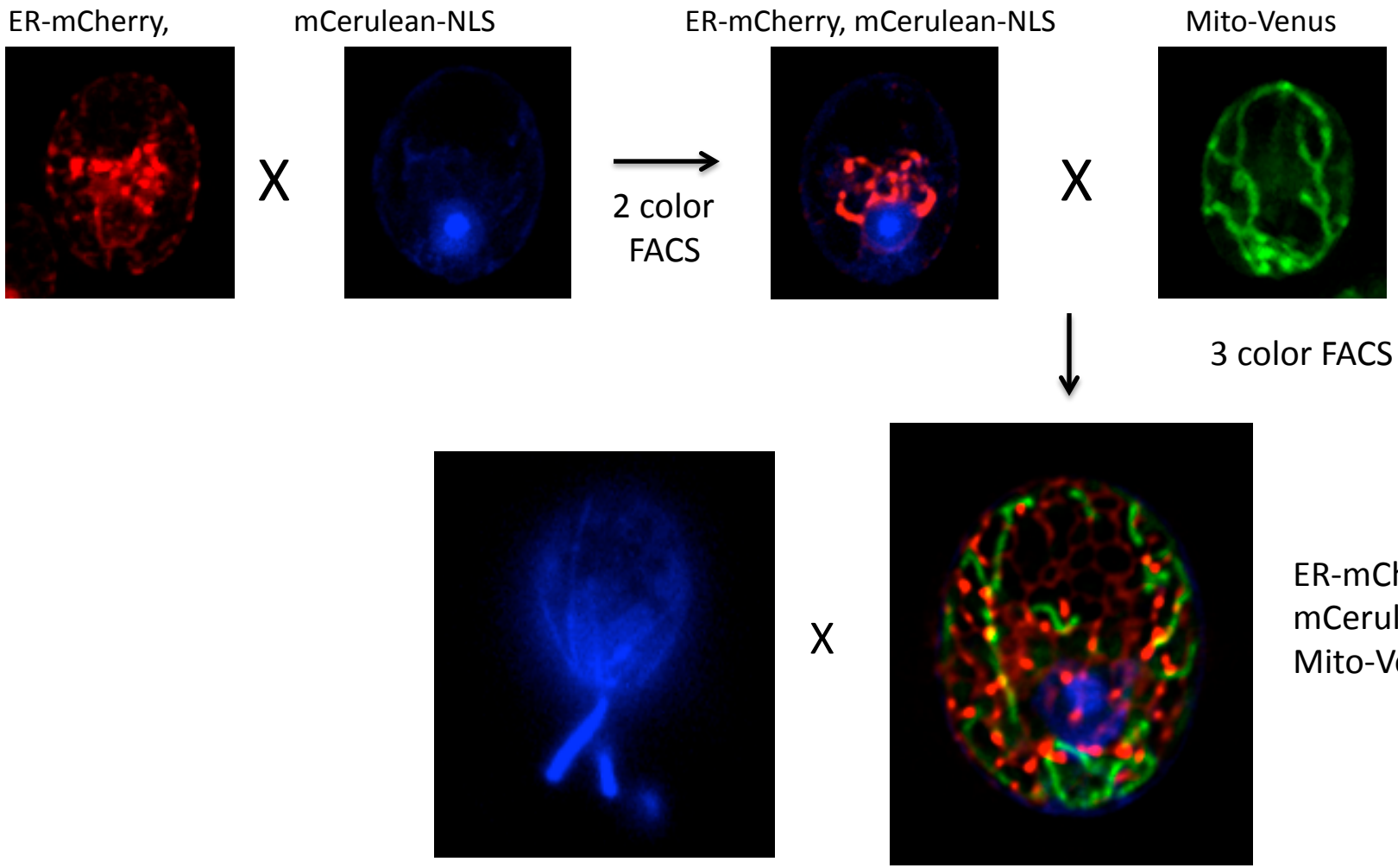


Gene stacking using a single poly protein gene

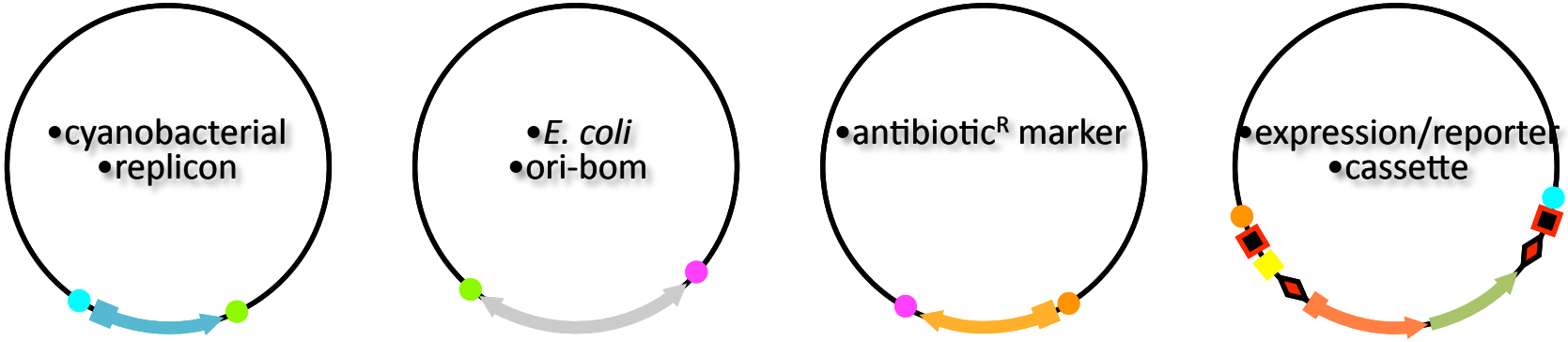




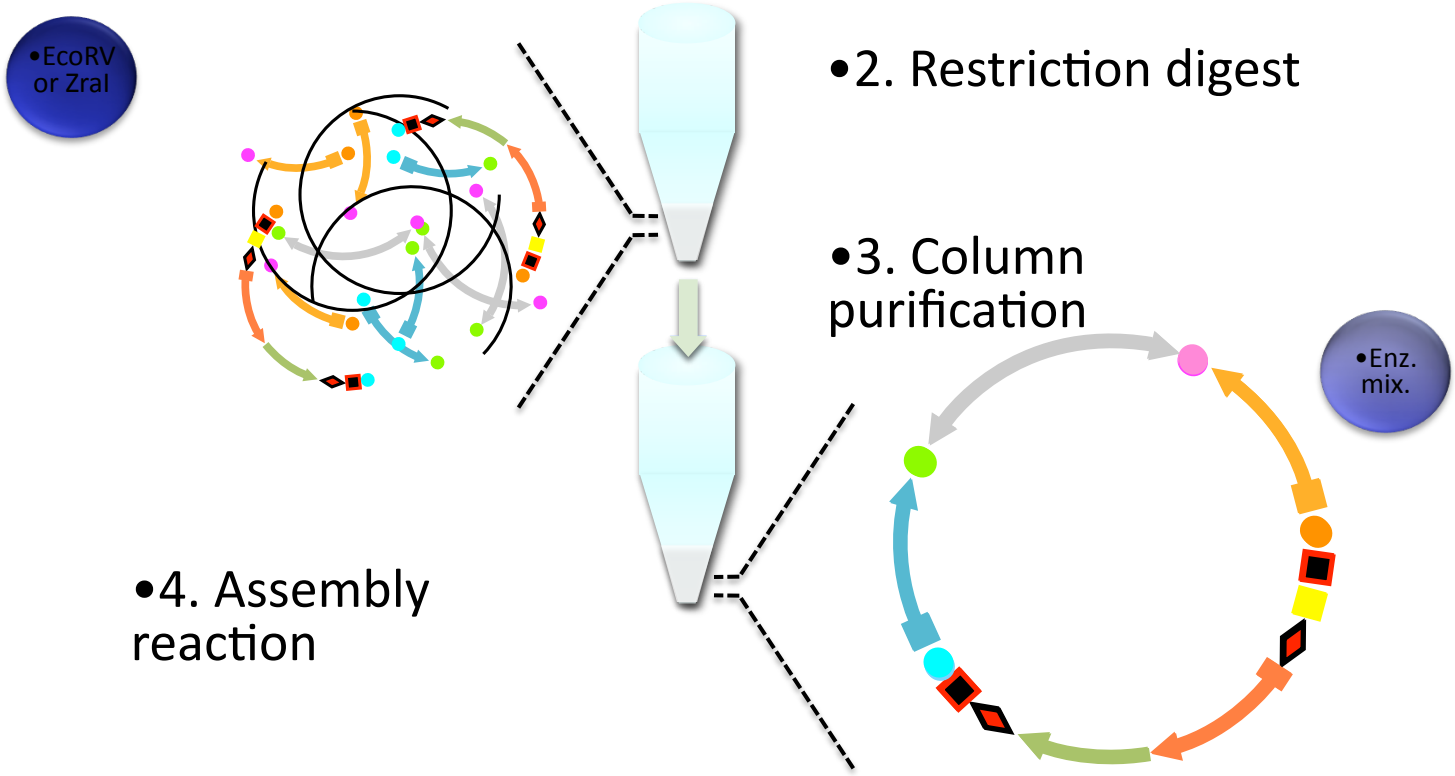
Gene stacking through successive rounds of mating



Assembly strategy for the modular construction of vector systems

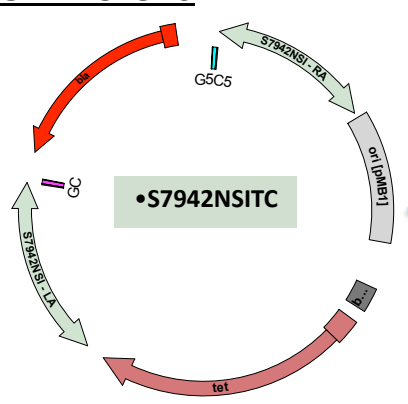


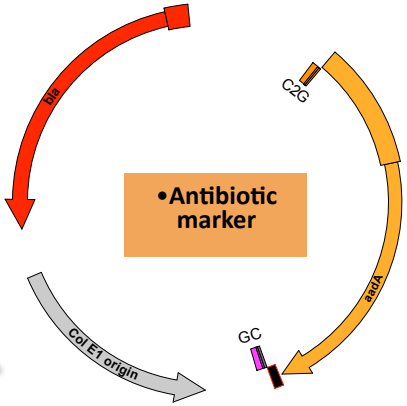
•1. Selection donors vectors

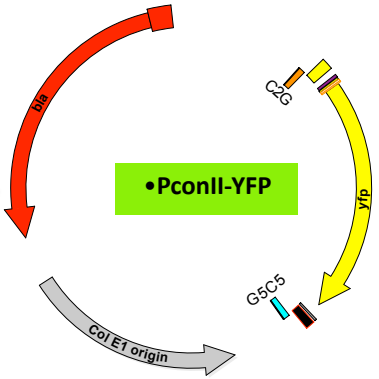


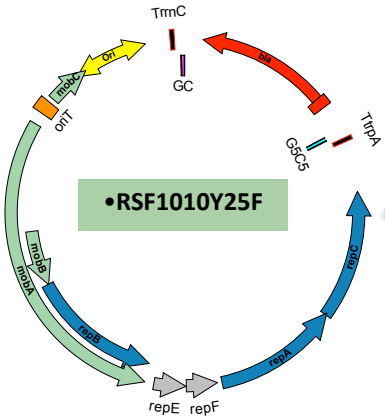
Antibiotic resistance markers - Pipeline

- **DONOR VECTORS**

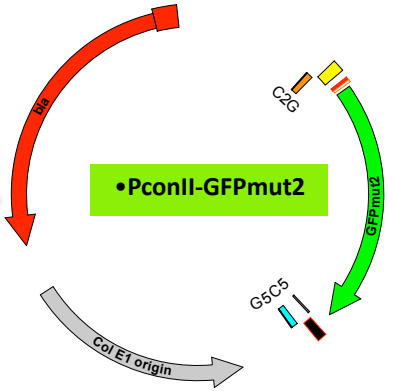








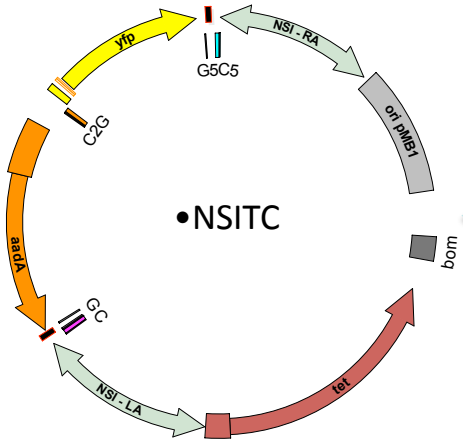
1. *aadA* (SpSm^R)
2. *aacC1* (Gm^R)
3. *aphI* (Km^R/Nm^R)
4. *bla* (Ap^R) [co.A7120]*
5. *bla* (Ap^R) [co.S7942]*
6. *cat* (Cm^R) [co.A7120]*
7. *cat* (Cm^R) [co.S7942]*
8. *natI* (Nr^R) [co.S7942]*
9. *natI* (Nt^R) [co.A7120]*

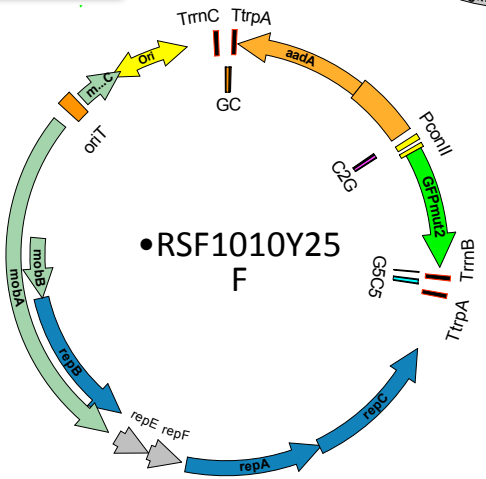


- **SHUTTLE VECTORS**

•Transformation

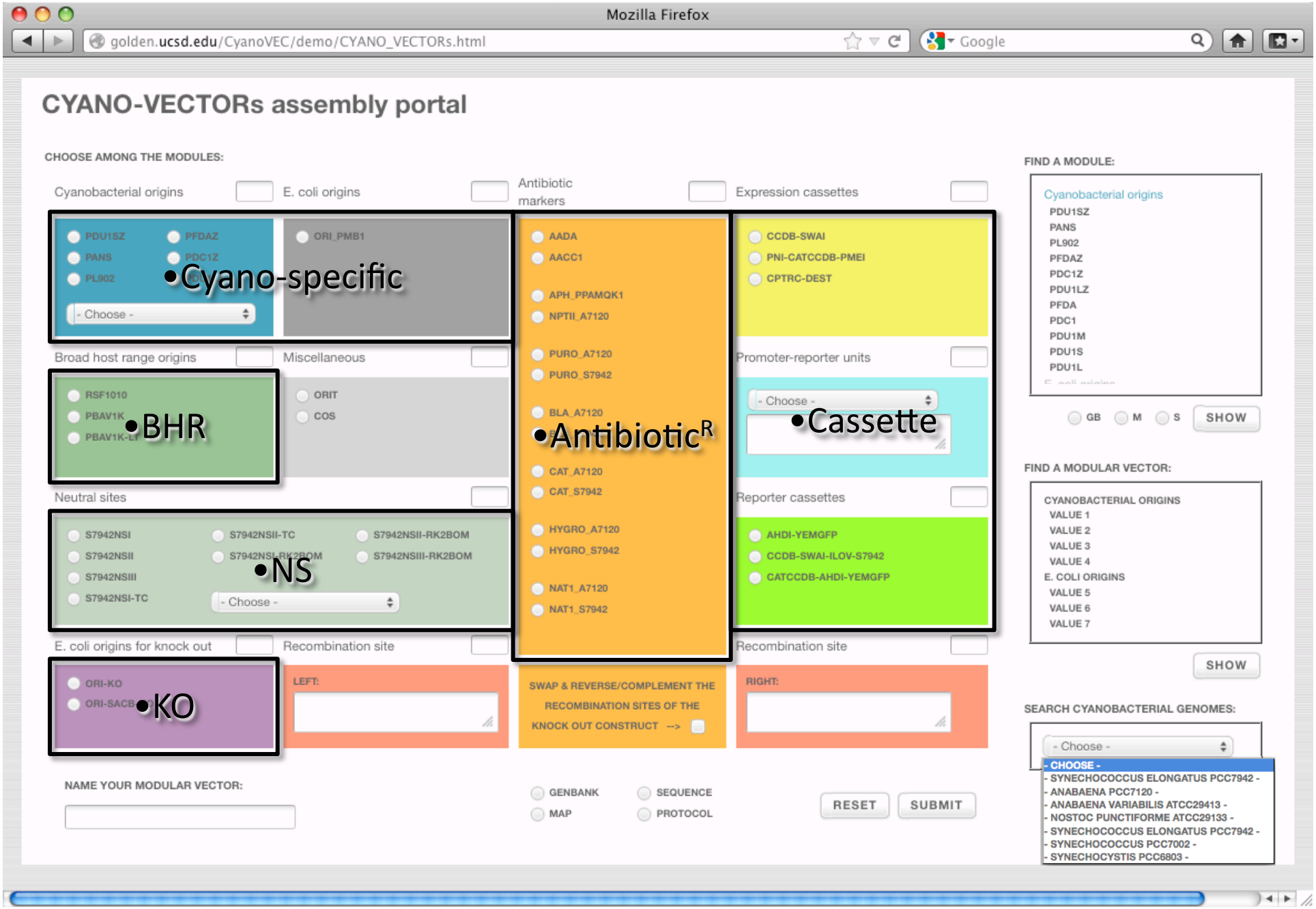
•*S. elongatus* PCC7942





•Conjugation

In silico assembly of cyanobacterial vectors



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life technologies™

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- Constructs of up to 40 kb
- Tools for Horizontal Transfer included

Do-it-yourself gene synthesis with Correctase™ Enzyme

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CorrectASE™ Enzyme

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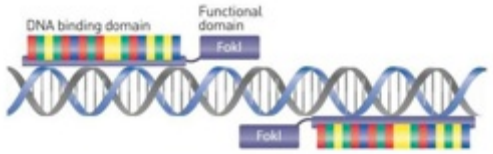
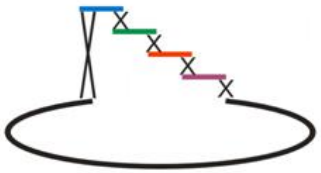
Custom DNA binding proteins for precision DNA targeting

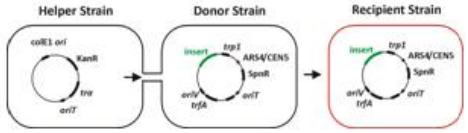


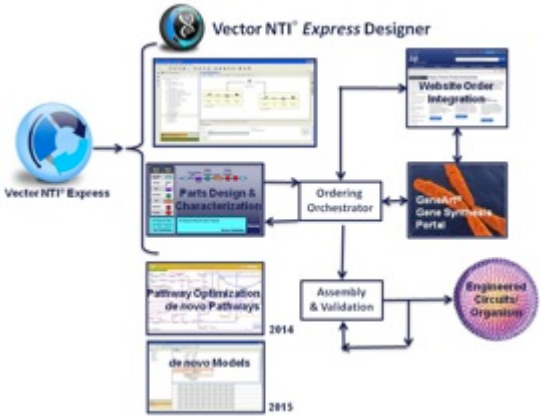
GeneArt® High-Order Assembly System



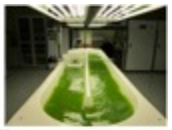
- Yeast based, >100 kb constructs
- Oligo stitching (no end-homology required)

Interspecies Horizontal Transfer



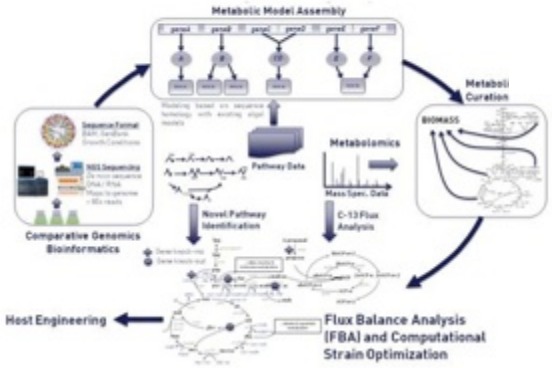


GeneArt® Algae Engineering Kits

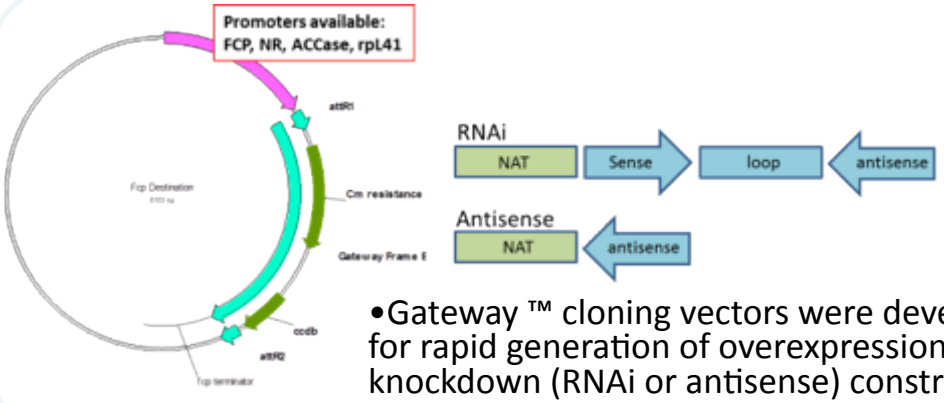


- The first commercially available standardized & complete system for algae metabolic engineering
- Tools for Chlamydomonas reinhardtii and Synechococcus elongatus PCC 7942

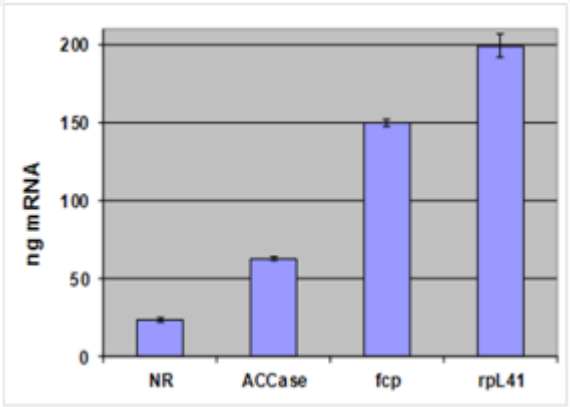
Algal Systems Biology & *in silico* Model



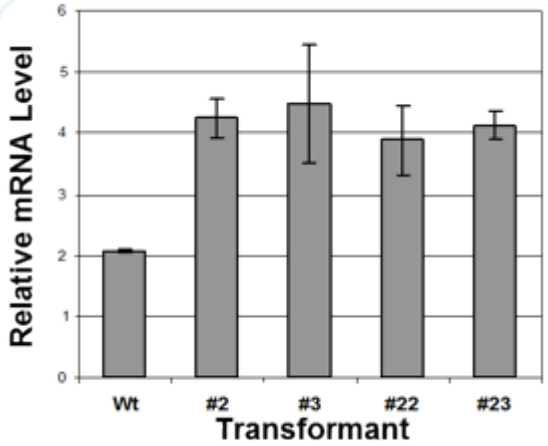
Development of Diatom Genetic Manipulation Tools: Overexpression and Knockdowns



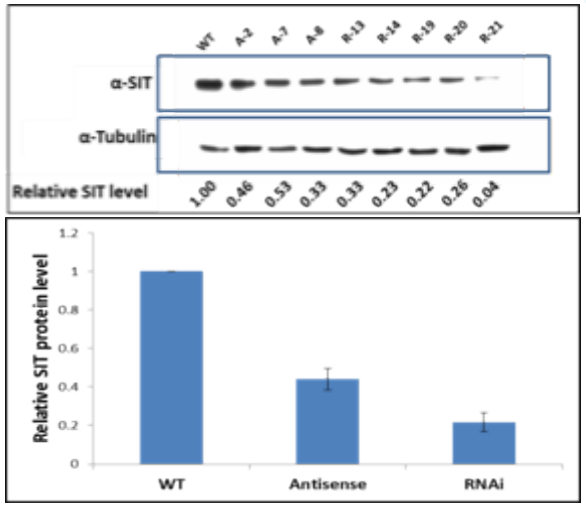
•Gateway ™ cloning vectors were developed for rapid generation of overexpression and knockdown (RNAi or antisense) constructs.



•Four different promoters were identified that drive different levels of mRNA accumulation.



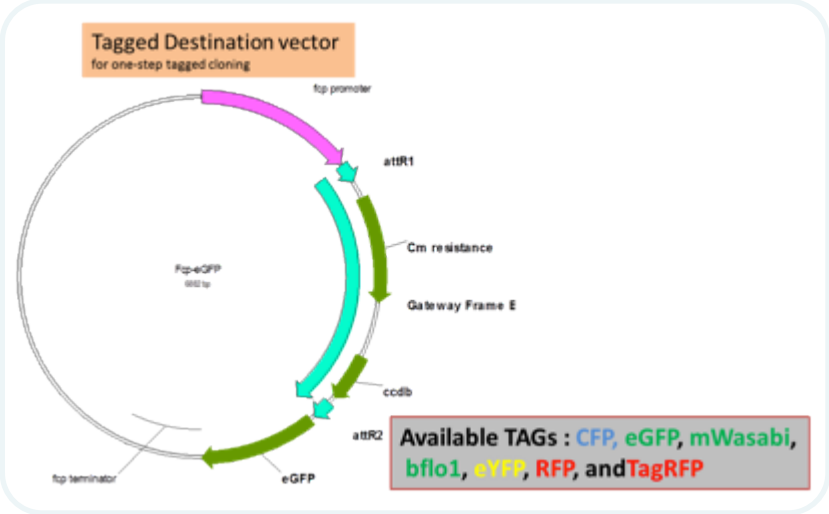
•A selected gene was over-expressed using the fcp promoter. Four transgenic clones showed consistent 2-fold increases in mRNA level.



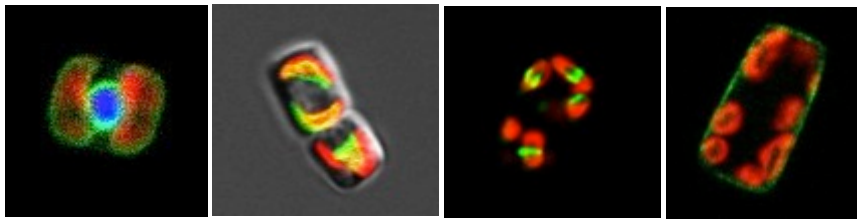
•Silicon transporters (SIT) were knocked down using antisense or RNAi. On average RNAi worked better, and consistent levels of 25% expression compared to wild type were achieved, with an exceptional knockdown achieving 4%. Phenotypic effects consistent with knockdown have been observed.

•Roshan Shrestha, Aubrey Davis, Luciano Frigeri

Development of Diatom Genetic Manipulation Tools: Fluorescent Tagging and New Selectable Markers



Intracellular Localization with Different Fluorescent Proteins



Localization to the cytoplasm, chloroplast, chloroplast membrane, periplastid membrane, ER, plasma membrane, pyrenoid, mitochondria, cell wall, and possibly peroxisome have been demonstrated.

Previous to this project only one selectable marker (nourseothricin resistance) was available for *T. pseudonana*. We have developed two additional markers, for zeocin resistance (generated by codon optimization), and glyphosate resistance (interaction with the Weeks lab at UNL).

Summary: A versatile set of genetic manipulation tools has been developed, bringing the technology for diatoms on a par with other model organisms.

•Aubrey Davis, Roshan Shrestha

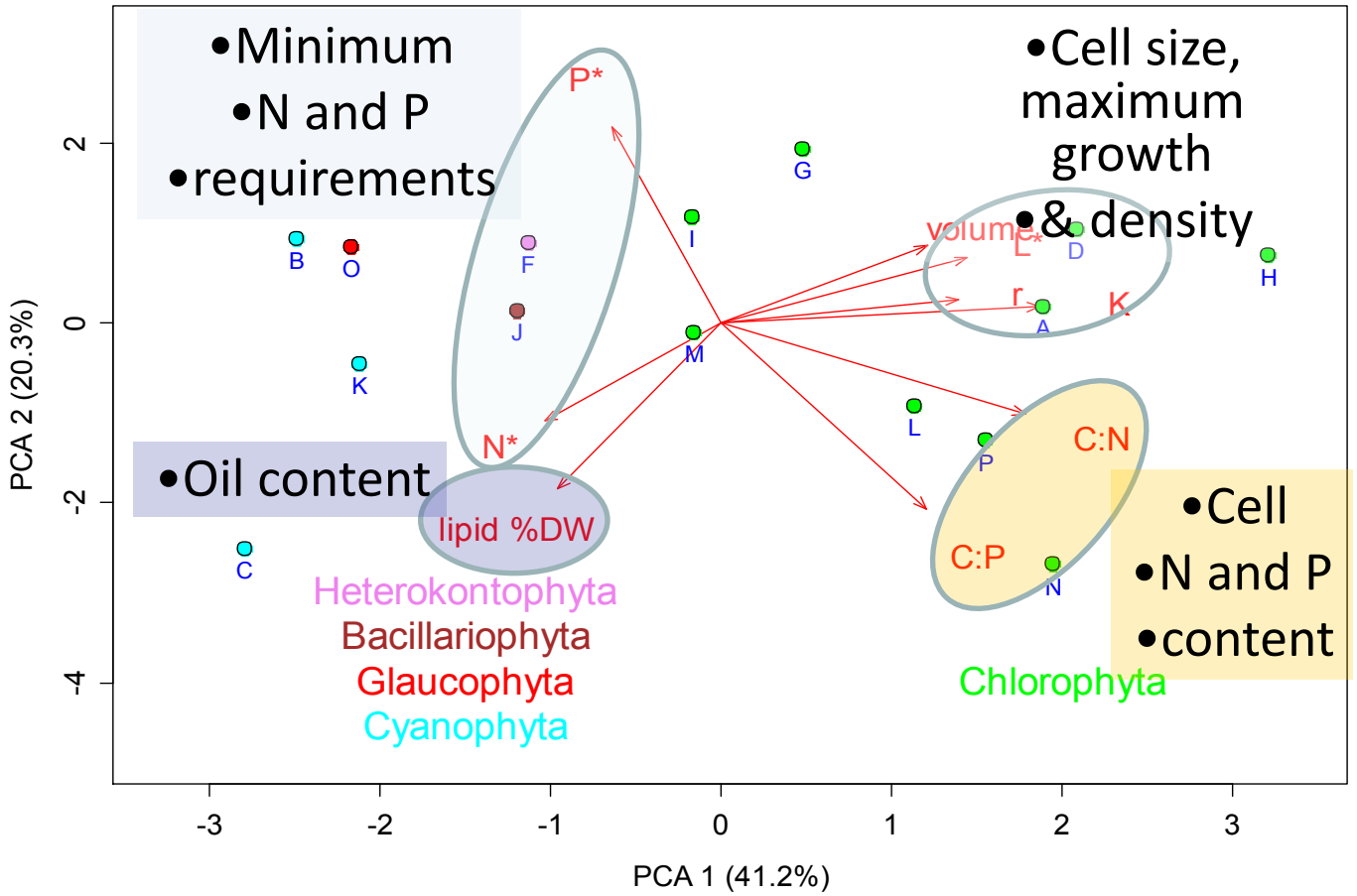
Nutrient use and recycling- a multi-functional approach

- **What limits algal productivity?**
  - **Resources**: N, P, CO_2, light
  - **Enemies**: Pathogens, grazers, competitors
  - Downstream issues: pesticide and nutrient use
  - Can we use "free" (recycled or waste) nutrients?

- **How can we overcome these constraints?**
  - A combination of genetic and ecological approaches

Nutrient utilization and recycling

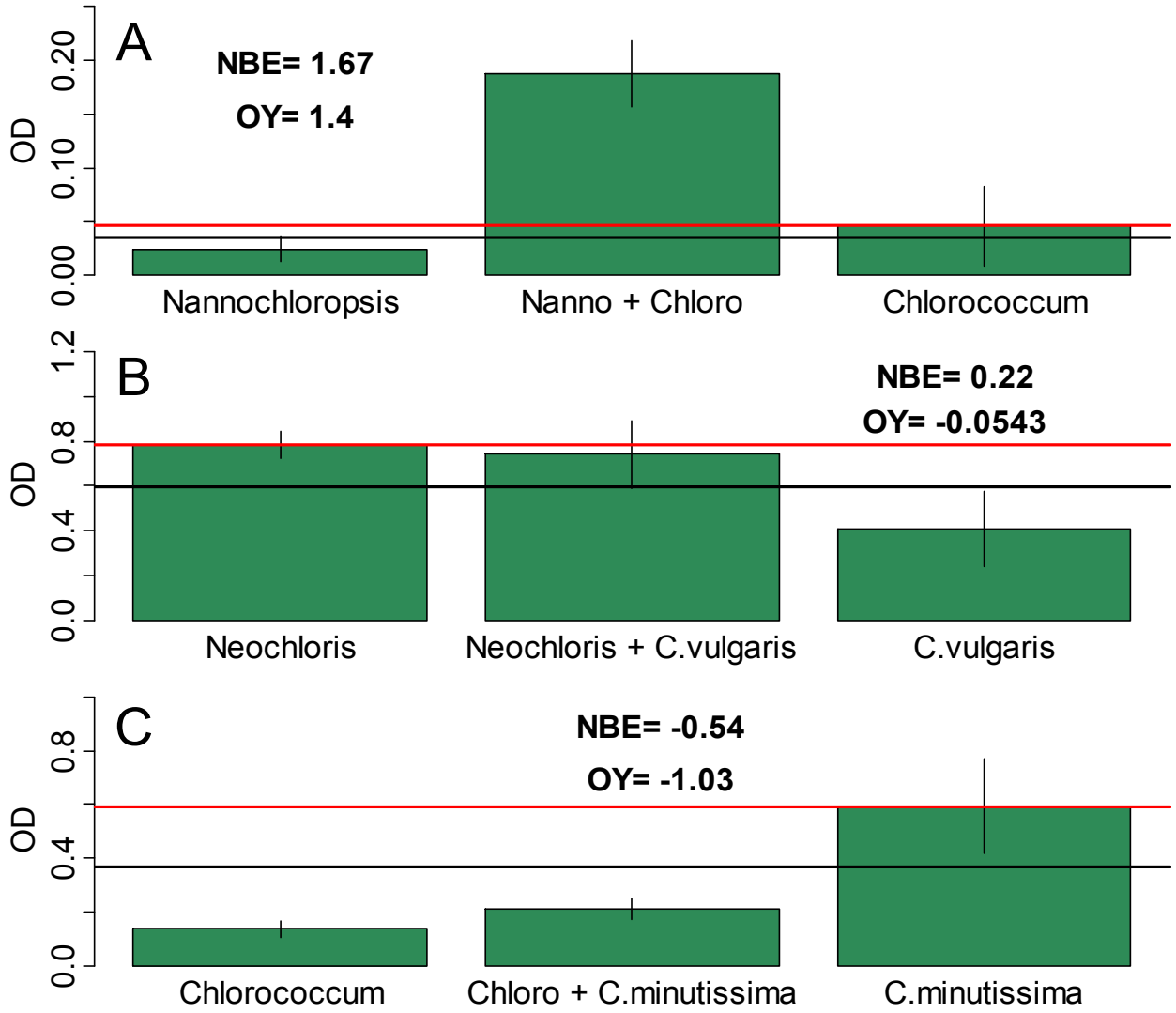
Trait correlations across diverse species of algae



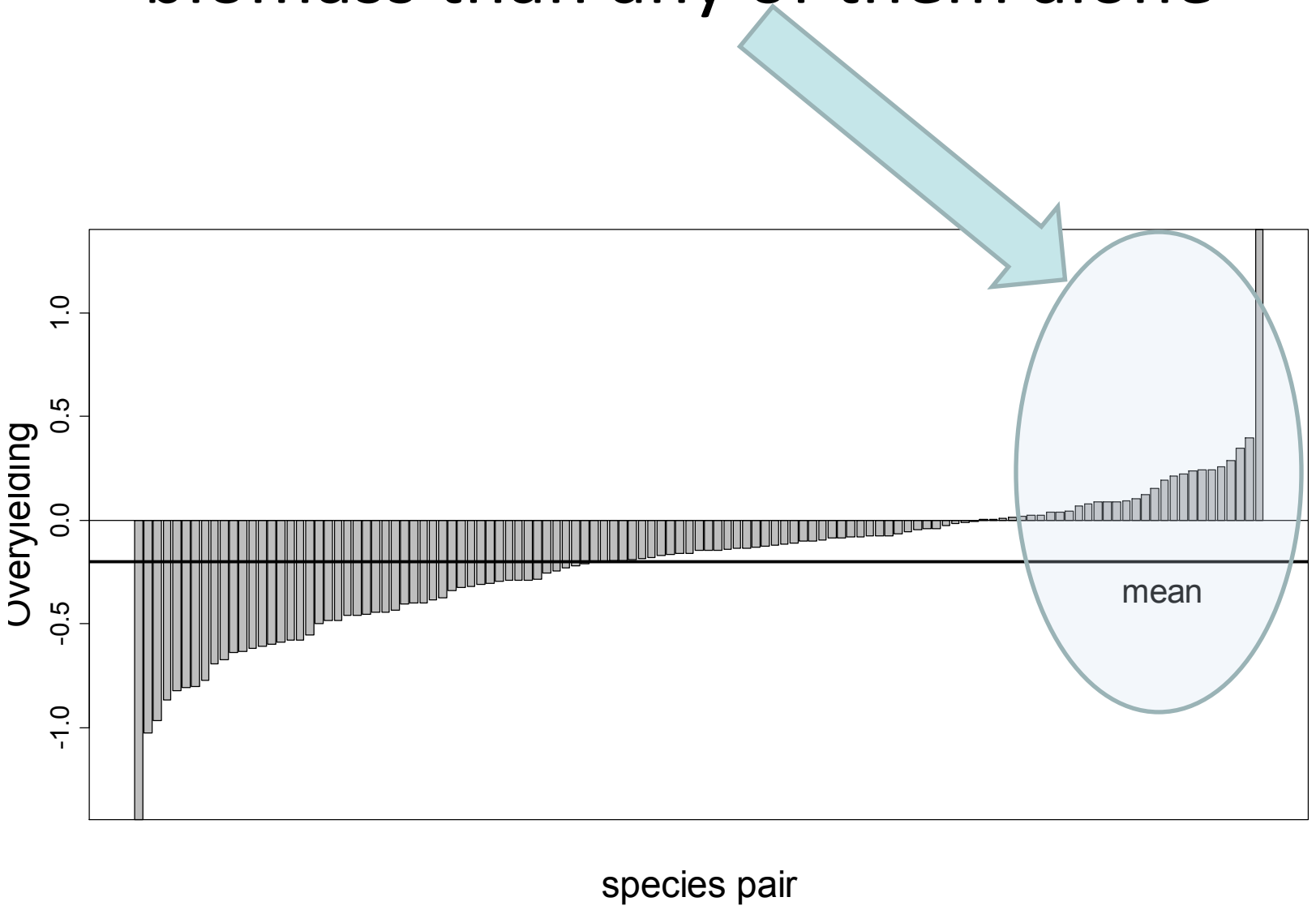
Nutrient utilization and recycling

- **How does nutrient use relate to other traits?**
  - Big species grow fast, use nutrients efficiently, produce a lot of biomass but not a lot of lipids, and get eaten a lot
  - Tradeoffs between light and nutrient demands, limitation by nutrients vs. grazers

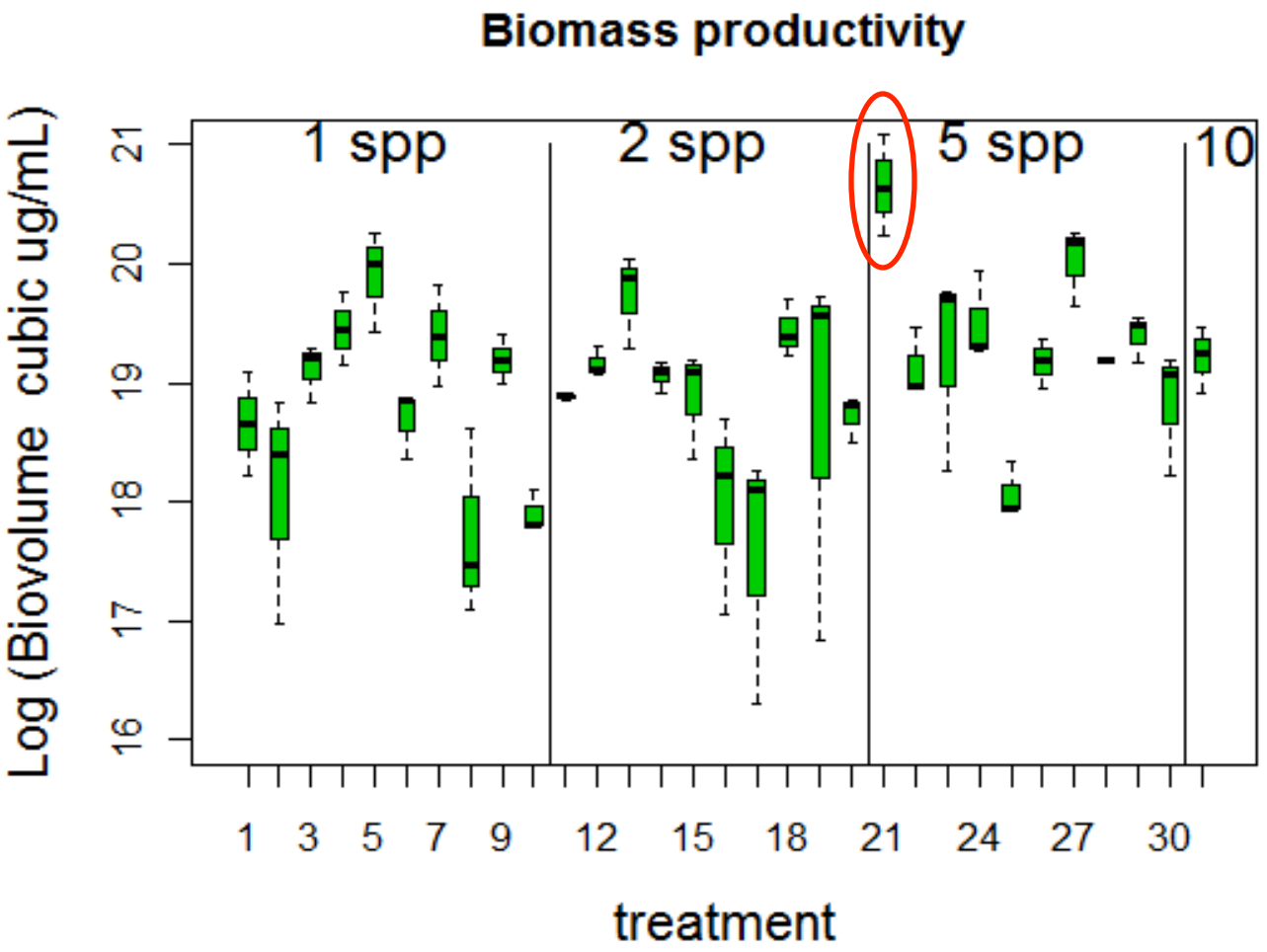
Can diversity overcome tradeoffs?



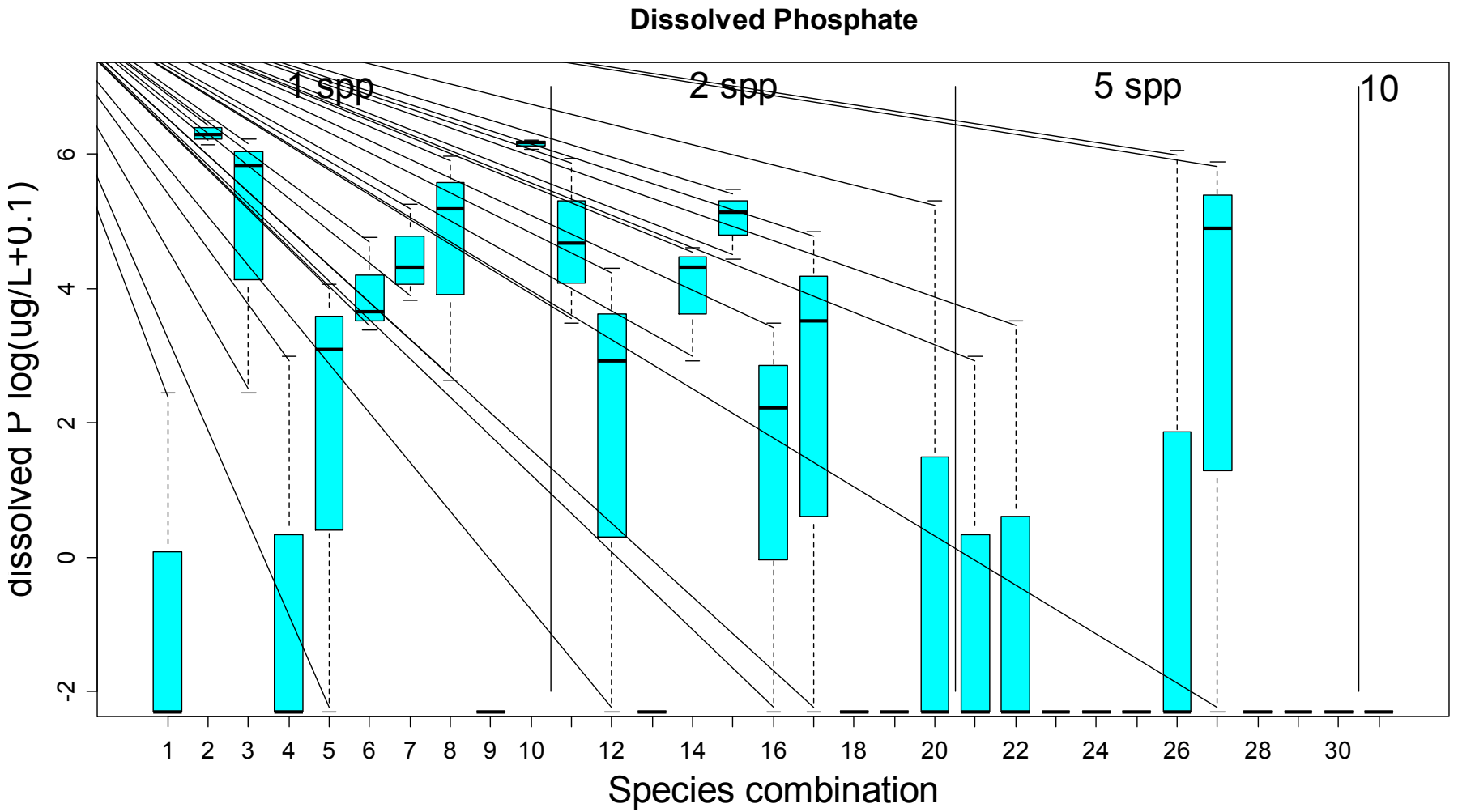
30/119 species pairs produce more biomass than any of them alone



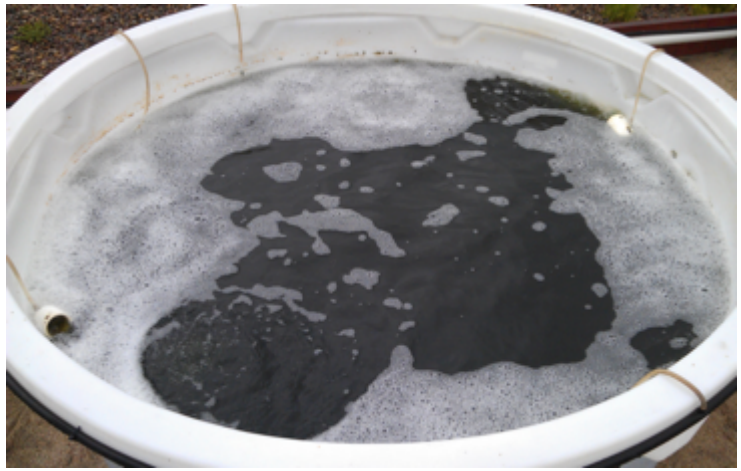
Adding more species can increase the yield



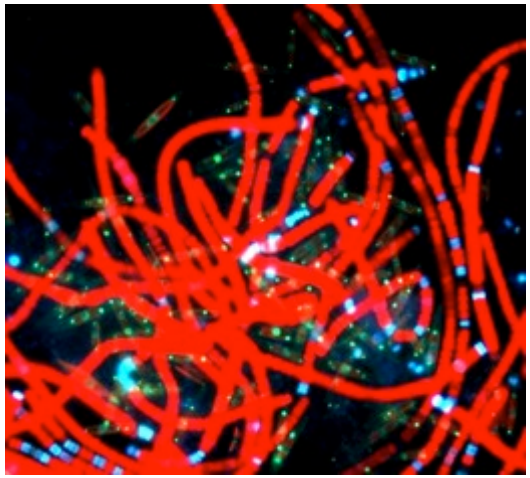
Adding more species increases nutrient use efficiency



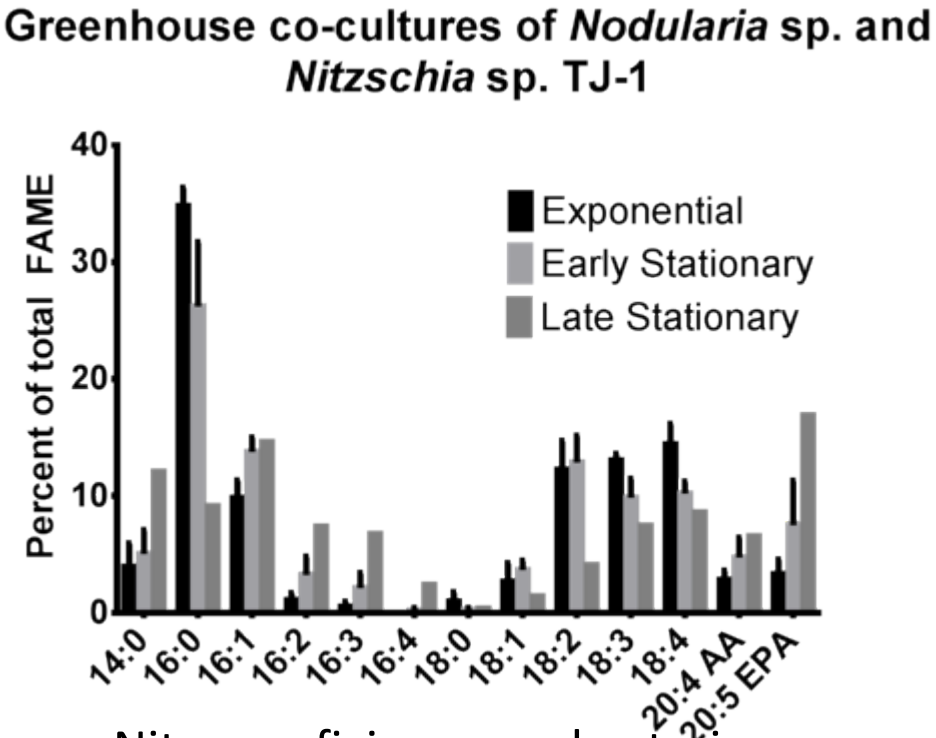
How do we provide a cheap nitrogen source?
Use nitrogen-fixing cyanobacteria/diatom co-cultures



•800L open pond culture



•Epifluorescence image of a *cyanobacterium*-diatom coculture. Green fluorescence is from BODIPY 493 (neutral lipid dye).



•Nitrogen fixing cyanobacterium provides lipids and nitrogen while diatom provides additional lipids, like commercially valuable EPA, especially towards stationary phase.

•Palenik Lab: W. Lambert, MS in prep.

Extraction breakthrough: Sapphire has substantially increased the amount of oil which can be recovered from biomass

- Sapphire uses a proprietary, innovative, solvent-based extraction system

Strain	Species	Extracted oil yield (% ash free dry weight)	
		Dry Hexane (soy bean oil extraction)	**Sapphire Process v2** (proprietary treatment and solvent extraction)
Strain A	*Nannochloropsis salina*	15%	50%
Strain B	*Nannochloropsis salina*	15%	43%
Strain C	*Desmodesmus sp.*	10%	48%
Strain D	*Scenedesmus dimorphus*	10%	38%
Strain E	*Spirulina sp. (Arthrospira)*	5%	38%
Strain F	*Spirulina platensis*	5%	36%
Strain G	*Spirulina maxima*	5%	41%
Strain H	*Spirulina maxima*	5%	39%

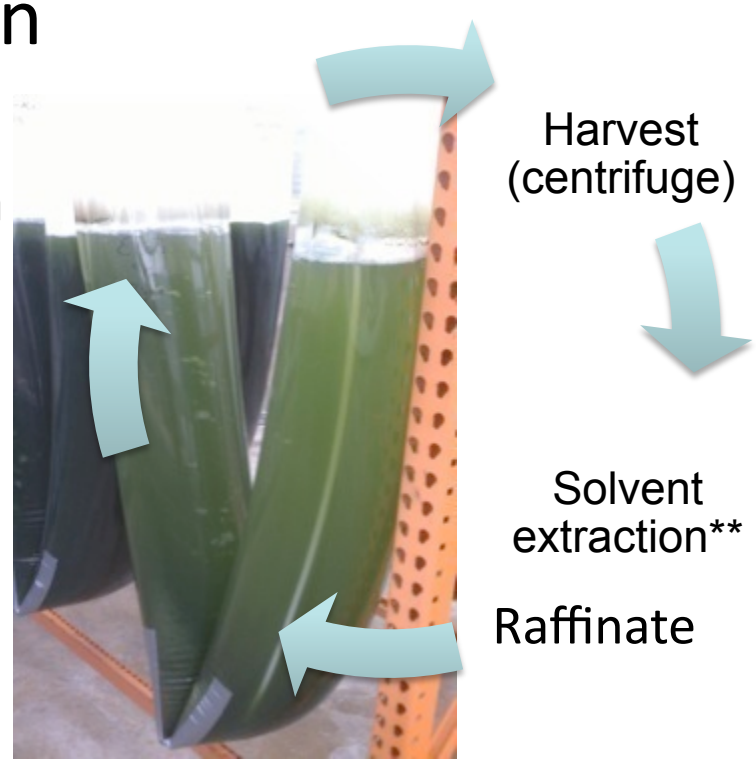
- Advantages:
  - Algae can be processed wet
  - High yield boost
  - Enables broad range of algae strains

Sapphire Energy™

How do we provide a cheap nitrogen source? Use recycled nitrogen

- Screen strains (small scale) for growth on amino acids, protein, and raffinate

- Screen best strains (green house scale) on raffinates in collaboration with Sapphire.



Best candidate strains to date :
Haematococcus (10% SW)
Picochlorum (100% SW)

Palenik Lab: Shuyi Wang, MS in prep.

*Medium: High Salt Medium (HSM) (Sueoka, N., 1960)

** Extraction was performed and raffinate data was provided by Sapphire Energy

Additional Slides

Responses to Previous Reviewers' Comments

- This is the first opportunity we have had to present our research findings. Our project had just started the week of the last review, so there were no comments from that review directed at our research.

Publications

Task A. Crop Protection

- Wulfmeyer, T., C. Polzer, G. Hiepler, K. Hamacher, R. Shoeman, D.D. Dunigan, J.L.Van Etten, M. Lolicato, A. Moroni, G. Thiel, and T. Meckel. (2012). Structural organization of DNA in chlorella viruses. PloS One 7, e30133
- Van Etten, J.L. and Dunigan, DD. (2012). Chloroviruses: not your everyday plant virus. Trends Plant Sci. 17, 1-8. (cover photo).
- Dunigan, D.D., Cerny, R.L., Bauman, A.T., Roach, J.C., Lane, L.C., Agarkova, I.V., Wulser, K., Yanai-Balser, G.M., Gurnon, J.R., Vitek, J.C., Kronschnabel, B.J., Jeannard, A., Blanc, G., Upton, C., Duncan, G.A., McClung, O.W., Ma, F., Van Etten, J.L. (2012). *Paramecium bursaria* chlorella virus 1 proteome reveals novel architectural and regulatory features of a giant virus. J. Virol. 86, 8821-8834. [highlighted article and cover photo]
- Jeanniard, A., Dunigan, D.D., Gurnon, J.R., Agarkova, I.V., Kang, M., Vitek, J., Duncan, G. McClung, O.W., Larsen, M., Claverie, J.M., Van Etten, J.L., Blanc, G. (20xx). Towards defining the chloroviruses: a genomic journey through a genus of large DNA viruses. (submitted for publication).
- Rowe, J.M., J.R. Gurnon, L.C. Lane, and J.L. Van Etten. (2010). Analysis of PBCV-1 resistance in *Chlorella* NC64A. Viruses of Microbes, Paris, France.
- Rowe, J. M., J. R. Gurnon, L. C. Lane, I. V. Agarkova, and J. L. Van Etten. (2010). Isolation and characterization of PBCV-1-resistant *Chlorella* NC64A. International Society for Microbial Ecology, 13th Symposium.
- Rowe, J.M., Dunigan, D.D., Blanc, G., Gurnon, J.R., Xia, Y., Van Etten, J.L. (2013). Evaluation of higher plant virus resistance genes in the green alga, *Chlorella variabilis* NC64A, during the early phase of infection with *Paramecium bursaria* chlorella virus-1. Virology (in press).
- Blanc, G., Agarkova, I., Grinwood, J., Kuo, A., Brueggeman, A., Dunigan, DD., Gurnon, J., Ladunga, I., Lindquist, E., Lucas, S., Pangilinan, J., Proschold, T., Salamov, A., Schumtz, J., Weeks, D., Yamada, T., Claverie, JM., Grigoriev, I.V., Van Etten, J.L. (2012). The genome of the polar eukaryotic microalga *Coccomyxa subellipsoidea* reveals traits of cold adaptation. Genome Biology 13, R39.

Publications

Task A. Crop Protection *(continued)*

- Rowe, J.R., Dunigan, D.D., Blanc, G., Gurnon, J.R., Xia, Y., Van Etten, J.L. Evaluation of higher plant virus resistance genes in the green alga, *Chlorella variabilis* NC64A, the host for *Paramecium bursaria* chlorella virus (in preparation).
- Blatti JL, Beld J, Behnke CA, Mendez M, Mayfield SP, Burkart MD. 2012 Manipulating Fatty Acid Biosynthesis in Microalgae for Biofuel through Protein-Protein Interactions. PLoS One. 2012;7(9):e42949. doi: 10.1371/journal.pone.0042949. Epub 2012 Sep 13.
- Blatti JL, Michaud J, Burkart MD Engineering fatty acid biosynthesis in microalgae for sustainable biodiesel. *Submitted*
- R. Simkovsky,, E. F. Daniels, K. Tang, S. C. Huynh, S. S. Golden, and B. Brahamsha. 2012. Impairment of O-antigen production confers resistance to grazing in a model amoeba–cyanobacterium predator–prey system. Proc. Natl. Acad. Sci. 109:16678-16683 DOI:10.1073/pnas.1214904109.
- Yang JY, Phelan VV, Simkovsky R, Watrous JD, Trial RM, Fleming TC, Wenter R, Moore BS, Golden SS, Pogliano K, Dorrestein PC.2012. A primer on agar-based microbial imaging mass spectrometry. J Bacteriol. 2012 Jul 20. [Epub ahead of print]

Publications

Task B. Nutrient Utilization

- Mass Balance Closure of the Major Cellular Carbon Pools of the marine diatom *Thalassiosira pseudonana* as regulated by irradiance, CO_2 and N-starvation" [1]Shang, F., [2]L.T. Carney, [1]P. Abelin, [1]E. Weiss, [1]M. Vernet, [1]D. Mendola and [1]B.G. Mitchell. [1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093.[2]Sandia National Laboratory, Livermore, CA 94551. To be submitted to the Journal of Applied Phycology.
- "A Model for Light-regulated Net Growth Rate of the Marine Diatom *Thalassiosira pseudonana*." Authors: Daniel Yee[1], Niu Du[1], Egil Sakshaug[2], Maria Vernet[1], Osmund Holm-Hansen[1], Satoru Taguchi[3], B. Greg Mitchell[1]. Collaborating Institutions: [1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093; [2] Trondhjem Biological Station, The Museum, University of Trondheim, Bynesueien 46, N-7018 Trondheim, Norway; [3] Soka University, 1-236 Tngi-cho, Hachioji-shi, Tokyo 192-8577, Japan. To be submitted to the Journal of Applied Phycology.
- Kendall, A., Yuan, J. (In Press) Comparing Life Cycle Assessments of Different Biofuel Options. *Current Opinion in Chemical Biology* DOI: 10.1016/j.cbpa.2013.02.020
- Dynamics of Lipid Biosynthesis and Redistribution in the Marine Diatom Phaeodactylum tricornutum Under Nitrate Deprivation. Elizabeth H. Burrows & Nicholas B. Bennette & Damian Carrieri & Joseph L. Dixon & Anita Brinker & Miguel Frada & Steven N. Baldassano & Paul G. Falkowski & G. Charles Dismukes. Bioenerg. Res. DOI 10.1007/s12155-012-9201-7

Publications

Task C. Genetic Tools

- Li T, Liu B, Spalding MH, Weeks DP, Yang B. 2012. High-efficiency TALEN-based gene editing produces disease-resistant rice. Nature Biotechnology. 30:390-2
- Brueggeman AJ, Gangadharaiah DS, Cserhati MF, Casero D, Weeks DP, Ladunga I. 2012. Activation of the carbon concentrating mechanism by CO2 deprivation coincides with massive transcriptional restructuring in Chlamydomonas reinhardtii. Plant Cell 24:1860-75
- Weeks DP 20121 Homologous recombination in Nannochloropsis: a powerful tool in an industrially relevant alga. Proc Natl Acad Sci U S A. 108:20859-60
- Li T, Huang S, Zhao X, Wright DA, Carpenter S, Spalding MH, Weeks DP, Yang B. 2011. 2011. Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. Nucleic Acids Res. 39:6315-25
- Li T, Huang S, Jiang WZ, Wright D, Spalding MH, Weeks DP, Yang B. 2011. TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. Nucleic Acids Res. 39(1):359-72
- Jiang WZ, Rosenberg JN, Wauchope AD, Tremblay JM, Shoemaker CB, Weeks DP, Oyler GA. Generation of a single-chain antibody library for microalgae: applications in species-specific cell surface binding. *Algae Research* (In Preparation)
- Jiang WZ, Rosenberg JN, Wauchope AD, Tremblay JM, Shoemaker CB, Weeks DP, Oyler GA. Large-scale phage display libraries of single-chain antibodies directed against proteins from five species of microalgae. *Plant Physiology* or *The Plant Journal*.
- Shrestha, RP, Haerizadeh, F, and Hildebrand, M. Molecular genetic manipulation of microalgae: Principles and applications. In: Handbook of Algal Mass Culture, 2nd edition (Richmond A and Hu Q, eds.). In press.
- Smith, S.R., Abbriano, R.M. & Hildebrand, M. 2012. Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways. Algal Research, 1:2-16

Publications

Task C. Genetic Tools *(continued)*

- Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways. Algal Research, 1:2-16. Smith, S.R., Abbriano, R.M. & Hildebrand, M. 2012.
- Hildebrand M, Davis AK, Smith SR, Traller JC, Abbriano R. 2012. The place of diatoms in the biofuels industry. Biofuels, 3:221-240.
- Hildebrand M, Abbriano RM, Polle J, Traller JC, Trentacoste EM, Smith SR, Davis AK. 2013. Metabolic and cellular organization in evolutionarily diverse microalgae as related to biofuels production, Current Opinion in Chemical Biology, Available online 26 March 2013, ISSN 1367-5931, 10.1016/j.cbpa.2013.02.027.
- Taton, A., E. Lis, D. M. Adin, G. Dong, S. Cookson, S. A. Kay, S. S. Golden, and J. W. Golden. 2012. Gene transfer in *Leptolyngbya* sp. strain BL0902, a cyanobacterium suitable for production of biomass and bioproducts. PLoS ONE **7**: e30901. PMCID: PMC3265524.
- Exploiting diversity and synthetic biology for the production of algal biofuels. Georgianna DR, Mayfield SP. Nature. 2012 Aug 16;488(7411):329-35.
- Analysis of heterologous regulatory and coding regions in algal chloroplasts. Gimpel JA, Mayfield SP. Applied Microbiology and Biotechnology. Accepted 8 Nov 2012.
- Rasala BA, Lee PA, Shen Z, Briggs SP, Mendez M, Mayfield SP. Robust expression and secretion of xylanase1 in Chlamydomonas reinhardtii by fusion to a selection gene and processing with the FMDV 2A peptide. PLoS ONE. 2012;7 (8):e43349
- Rasala, B.A., Barrera, D., Ng, J., Plucinak, T.M., Rosenberg, J., Weeks, D., Oyler, G., Peterson, T.C., Haerizadeh, F., Mayfield, S.P. (2013) Expanding the spectral palette of fluorescent proteins for the green microalga Chlamydomonas reinhardtii. The Plant Journal (in press)
- Vinyard, D.J., Gimpel, J., Ananyev, G.M., Cornejo, M., Golden, S.S., Mayfield, S.P., Dismukes, C.G. (2013) Natural variants of Photosystem II subunit D1 tune photochemical fitness to solar intensity. Journal of Biological Chemistry 288 (8), 5451-5462

Publications

Task C. Genetic Tools *(continued)*

- Specht, E. A., Mayfield, S. P. (2013). Synthetic Oligonucleotide Libraries Reveal Novel Regulatory Elements in Chlamydomonas Chloroplast mRNAs. ACS Synthetic Biology 2(1):34-46
- Kendall, A., Yuan, J. (In Press) Comparing Life Cycle Assessments of Different Biofuel Options. *Current Opinion in Chemical Biology* DOI: 10.1016/j.cbpa.2013.02.020
- Achieving high throughput sequencing of cDNA library utilizing an alternative protocol for the bench top next-generation sequencing system. J Microbiol Methods.Online First. Wan M, Faruq J, Rosenberg JN, Xia J, Oyler GA, Betenbaugh MJ. (2012)
- Physiological and genetic evaluation of a new Chlorella sorokiniana isolate for its biomass production and lipid accumulation in photoautotrophic and heterotrophic cultures. Biotechnol Bioeng, 109:1958–1964. Wan M, Wang R, Xia J, Rosenberg JN, Nie Z, Kobayashi N, Oyler GA, Betenbaugh MJ. (2012)
- Modifications of the metabolic pathways of lipid and triacylglycerol production in microalgae. Wei-Luen Yu, William Ansari, Nathan G Schoepp, Michael J Hannon, Stephen P Mayfield and Michael D Burkart. Microbial Cell Factories 2011, 10:91

Patents

Provisional patent application LLC. PCT/US2012/032662 "**Single chain antibodies for photosynthetic microorganisms and method of use.**" Oyler GA, Rosenberg JN, Weeks DP. University of Nebraska-Lincoln // Synaptic Research, Filed April 7, 2011.

Provisional patent application UCSD064.001PR "**Cyanobacterial Strains Resistant to Grazers and Capable of Autoflocculation.**" S.S. Golden, B. Brahamsha, R. Simkovsky, E. Daniels, B. Palenik, J.W. Golden. UCSD. Filed 4/19/2012.

Provisional patent application UCSD062.001PR "**Targeted Delivery of Nutrients to Recipient Organisms.**" S.S. Golden, J.W. Golden, A. Daulo. UCSD. Filed 4/19/2012.

Provisional patent application US201/2028267681 "**Compositions and Methods for Enhancing Lipid Production in Marine Microalgae.**" Paul Falkowski, Miguel Frada, Kevin Wyman, James Gibson. Rutgers State University of New Jersey. Filed 4/25/2012.

Provisional patent application 2012/036010 "**Production of P. falciparum surface proteins in algae as transmission blocking vaccine candidates.**" Stephen P. Mayfield. The Regents of the University of California, San Diego. Filed 03/10/2012.

U.S. Provisional Application Serial No. 61/775,283, filed March 8, 2013. "**Regulation of gene expression in cyanobacteria.**" James Golden and Amy Ma.

Presentations

Task A. Crop Protection

- Gross, J., Systemic genomics of Chlamydomonas for understanding salt tolerance mechanisms in biofuel algae. Annual Meeting, Consortium for Algal Biofuels Commercialization, San Diego CA, May 13, 2012.
- Gross, J., Wajid, S., Price, D., Chan, C. X., and Bhattacharya, D. sRNAs of Cyanophora paradoxa and Chlamydomonas reinhardtii, a system genomics approach to understand algal evolution. Phycological Society of America Annual Meeting, Charleston SC, June 20-23, 2012.
- Mueller, C.M., Dunigan, D.D., Gurnon, J. R., and Van Etten, J.L. (2012) Flyswat 2012 in Nebraska City, Nebraska. Title: An evaluation of Nebraska River Systems for chloroviruses
- Dunigan, D.D., and Van Etten, J.L. (2012). Algal Biofuels Symposium 2012 in La Jolla, California. Title: Algal – virus interactions: A proteomic approach to host range specificity.
- Dunigan, D.D. (2012). Plant Virus Ecology Network Workshop 5 in Lawrence, Kansas, USA. Title: A two year comparison of chloroviruses in Nebraska river systems.
- Van Etten, J.L. Invited talk at Algal Biofuels Symposium 2012 held in La Jolla, CA. May 2012. Title: Chlorella viruses: possible infectious agents of humans and experimental animals.
- Van Etten, J.L. Invited talk at the American Society of Microbiology annual meeting. June, 2012. Title: Giant viruses change the perception of viruses.
- Van Etten, J.L. Invited talk at the Second International Conference on Viruses of Microbes held in Brussels, Belgium. July 2012. Title: Chlorella viruses: possible infectious agents of humans and experimental animals.
- Van Etten, J.L. Invited talk at the annual meeting of the Mediterranee Infestion held in Gordes, France. Oct. 2012. Title: Chloroviruses: giant algal viruses
- Van Etten, J.L. Invited seminar at the University of California – Riverside. Riverside, CA. Feb, 2012. Title: Unusual life-style of giant algal viruses.
- Van Etten, J.L. Invited seminar at Centro de Investigación Científica y de Educación Superior de Ensenada Ensenada, Mexico. March, 2012. Title: Unusual life-style of giant algal viruses.

Presentations

Task A. Crop Protection *(continued)*

- Van Etten, J.L. Invited seminar at the University of Naples, Naples, Italy. July, 2012: Title: Chlorella viruses have a sweet tooth.
- Van Etten, J.L. Invited seminar at the University of Genova, Genova, Italy. July, 2012. Title. Chlorella viruses have a sweet tooth.
- Van Etten, J.L. Invited seminar at Nebraska Wesleyan University. Lincoln, NE. Oct. 2012. Title: A fascinating journey with giant chlorella viruses.
- Van Etten, J.L. Talk. The genetic potential of the chlorella viruses is huge. Food and Fuel for the 21st Century – Expanding the Opportunities. April, 2013.
- Dunigan: 11th Annual Symposium in Virology, held in Lincoln, Nebraska, September 2011. Title (Poster presentation): An evaluation of Nebraska River Systems for chloroviruses; Authors: Claire Mueller, David Dunigan, James Gurnon, James Van Etten.
- Dunigan: Aquatic Virus Workshop 6, held in Texel, The Netherlands, October 2011. Title (Oral presentation): The evolving view of chlorovirus structure; Authors: Dunigan, D.D., Duncan, G.A., Lane, L.C., Zhang, X., Rossmann, M., Van Etten, J.L.
- Dunigan: Aquatic Virus Workshop 6, held in Texel, The Netherlands, October 2011. Title (Poster presentation): An evaluation of Nebraska Streams for Chloroviruses; Authors: Mueller, C.M., Dunigan, D.D., Gurnon, J. R., Van Etten, J.L.
- Dunigan: 2011 Flyswat, held in Nebraska City, Nebraska, March 2011. Title: Chlorovirus major capsid proteins. Authors: D. D. Dunigan, L. C. Lane, G. L. Lewis, G. A. Duncan, G. M. Yanai-Balser, J. C. Vitek, R. L. Cerny, J. L. Van Etten.
- Dunigan: Algal Biofuels Symposium 2011, held in La Jolla, California; April 2011. Title: Future crop protection; viruses of eukaryotic algae. Authors: D. D. Dunigan, J. L. Van Etten.

Presentations

Task A. Crop Protection *(continued)*

- Dunigan, D.D. Talk. Algal-virus interactions: A transcriptomic Evaluation of the inate immune response of Chlorella variabilis to PBCV-1 infection. Food and Fuel for the 21st Century – Expanding the Opportunities. April, 2013.
- Van Etten. Aquatic Virus Workshop #6. Texel, Netherlands. Oct. 2011. Title: Chlorella viruses continue to surprise. Sole author.
- Van Etten. Invited keynote talk at the First International Conference on Viruses of Microbes held in Paris, France. June, 2010. Title: Early events associated with infection by chlorella virus PBCV-1.
- Van Etten. Invited seminar at Johns Hopkins University, Baltimore, MD. Dec. 2011. Title. Unusual life-style of giant algal viruses.
- Van Etten. Invited seminar at Temple University, Philadelphia, PA. April, 2011. Title: Unusual life-style of giant algal viruses.
- Van Etten. Invited seminar at the University of Mediterranean, Marseille, France. Feb. 2011. Title: Unusual life-style of giant algal viruses.
- Van Etten. Invited seminar at the University of Milano, Milano, Italy. June, 2010. Title: Unusual life-style of giant algal viruses.
- Van Etten. Invited seminar at the University of Minnesota, Minneapolis, MN. Dec. 2011. Title: Unusual life-style of giant algal viruses.
- Van Etten. Invited seminar at the University of Missouri – Kansas City. March, 2010. Title: Unusual life-style of giant algal viruses.
- Van Etten. Invited talk at a Food and Fuel meeting at the state capital in Lincoln, NE. Oct. 2011. Algal viruses – the good and the bad.
- Van Etten, J.L., L. Jones-Brando, E. Severance, M. Webster, S. Kim, J. Gurnon, D.D. Dunigan, F. Dickerson, R. Yolken. (2012). Chlorella viruses: potential infectious agents of humans and experimental animals. Viruses of Microbes 2012.

Presentations

Task A. Crop Protection *(continued)*

- De Castro, C., D. Garozzo, R. Lanzetta, A. Molinaro, M. Parrilli, L. Sturiale, J. Gurnon, J.L. Van Etten, M. Tonetti. (2013). A molecular modeling view into the complex N-linked oligosaccharide from A430L envelope protein isolated from *Paramecium bursaria* chlorella virus PBCV-1. Gordon Conference on Glycobiology.
- De Castro, C., D. Garozzo, L. Sturiale, A. Molinaro, F. Piacente, J. Gurnon, J.L. Van Etten, M. Tonetti. (2013). Structural characterization of the N-linked glycans associated with the major capsid protein of the giant DNA virus PBCV-1. Gordon Conference on Glycobiology.
- Duncan, G., D. Dunigan, J. Gurnon, and J.L. Van Etten. (2103). Antigenic mutants of the chlorovirus PBCV-1: Sequence analysis of the gene a064r. Abstracts of the American Society of Virology. p. xx.
- Dunigan, D.D., A. Jeanniard, J.R. Gurnon, I.V. Agarkova, M. Kang, J. Vitek, G. Duncan, O.W. McClung, M. Larsen, J.M. Claverie, J.L. Van Etten, and G. Blanc. (2013). A genomic analysis of giant viruses in the genus Chlorovirus. Abstracts of the American Society of Virology. p. xx.
- Agarkova, I., B. Hertel, X Zhang, D. Dunigan, M. Rossmann, G. Thiel, and J.L. Van Etten. (2013). Chlorovirus attachment to the host cell wall is reversible. Abstracts of the American Society of Virology. p. xx.
- Dunigan, D. D., Van Etten, J. L., Jeanniard, A. (2013). Exploration of Chloroviruses through genomics. Algal Biomass Summit.
- Blatti: Releasing stored solar energy within pond scum: Biodiesel from algal lipids, oral presentation, 243rd American Chemical Society National Meeting, San Diego, CA, March 29, 2012.
- Burkart: Role of protein-protein interactions in algal fatty acid synthase engineering, 243rd American Chemical Society National Meeting, San Diego, CA, March 28, 2012.
- Sonnenschein and Burkart: Metabolic engineering of microalgae towards the production of secondary metabolites, poster presentation at the Food and Fuel for the 21st Century, April 22-23, 2012, La Jolla, CA, USA

Presentations

Task A. Crop Protection *(continued)*

- Burkart: Modular synthase engineering in algae, oral presentation at the Food and Fuel for the 21st Century, April 22-23, 2012, La Jolla, CA, USA.
- Burkart: University of Maryland Center for Environmental Science (College Park, Maryland; October 2011)
- Sonnenschein and Burkart: Metabolic engineering of microalgae towards the production of secondary metabolites, poster presentation at the 15th International Conference on the Cell & Molecular Biology of Chlamydomonas, June 5-10, 2012, Potsdam, Germany.
- Burkart: Studies on Algal Fatty Acid Biosynthesis, oral presentation at the Food and Fuel for the 21st Century Symposium, April 19-20, 2013, La Jolla, CA.
- Sonnenschein and Burkart: Metabolic engineering of microalgae towards the production of secondary metabolites, poster presentation at the CEC Large Molecule Sustainable Fuels Roadmap Meeting - October 18, 2012, La Jolla, CA, USA.
- Joris Beld, Jen Michaud, Nathan Schoepp, Eva Sonnenschein, Michael Burkart: Towards a sustainable future - algae research in the Burkart lab, poster presentation at the Food and Fuel for the 21st Century, April 19-20, 2013, La Jolla, CA.
- Ryan Simkovsky, Emy Daniels, Karen Tang, Stacey Huynh, Susan Golden, and Bianca Brahamsha: Impairment of O-antigen production confers resistance to grazing in a model cyanobacterium-amoeba predator-prey system; poster presentation at the Food and Fuel for the 21st Century, May 11-12, 2012, La Jolla, CA, USA
- Amy Ma, Emy Daniels, Javier Paz Yepes, James Golden, and Bianca Brahamsha: Proteomic analysis of *Anabaena sp.* Strain PCC 7120 after co-culture with wild amoebal isolate HGG1; poster presentation at the Food and Fuel for the 21st Century, May 11-12, 2012, La Jolla, CA, USA
- Ryan Simkovsky, Emy Daniels, Karen Tang, Stacey Huynh, Susan Golden, and Bianca Brahamsha: O-antigen Impairment Confers Resistance to Grazing in a Model Cyanobacterium-Amoeba System.; poster presentation at the San Diego Microbiology Meeting, June 9, 2012, La Jolla, CA, USA

Presentations

Task A. Crop Protection *(continued)*

- Ryan Simkovsky: Protecting cyanobacteria from amoebal predators, San Diego Microbiology Group, April 17, 2013, La Jolla, CA, USA
- Amy T. Ma, Emy Daniels, Brian Knight, James W. Golden, Bianca Brahamsha: Characterizing Interactions Between *Anabaena* sp. PCC 7120 and Amoeba HGG1. poster presentation at the Food and Fuel for the 21st Century, April 19-20, 2013, La Jolla, CA.
- Amy T. Ma & James W. Golden: Riboswitch-Mediated Regulation of Gene Expression in Cyanobacteria. poster presentation at the Food and Fuel for the 21st Century, April 19-20, 2013, La Jolla, CA.
- Golden, S. Impairment of O-antigen production confers resistance to grazing in a model cyanobacterium-amoeba predator-prey system, 14th Intl Symposium on Phototrophic Prokaryotes, Porto, Portugal, 5 - 10 Aug 2012.
- Golden, J. July seminars on "Cyanobacterial Biotechnology" in China at the Hydrobiology Institute and Central Normal University (Wuhan), Qingdao Institute of Bioenergy and Bioprocess Technology (Qingdao), and the Shanghai.
- S. Briggs. Talk. "Reconstruction of Protein Networks from an Atlas of Proteotypes", FF21 Symposium La Jolla, CA. April 2013.
- F. Nohilly. Talk. "Algae chytrid interactions", SD-CAB Student and Post-doc symposium. La Jolla, CA. April 2011
- Z. Shen. Talk. "Chytrid pathogenesis programs the algal host proteotype", SD-CAB & FF21 Syposium, La Jolla, CA, May 2012.
- S. Wehrkamp-Richter. Talk. "Proteomics Analysis of Scenedesmus infection with Fungus", SD-CAB/Sapphire Collaborators Meeting, La Jolla, CA. March 2013.
- F. Nohilly. Poster. "Chytrid pathogensis programs the algal host proteotype". SD-CAB & FF21 Symposium, La Jolla, CA. May 2012.
- F. Nohilly. Poster. "Chytrid pathogensis programs the algal host proteotype", International Conference on Algal Biomass, Biofuels, and Bioproducts. San Diego, CA. June 2012

Presentations

Task A. Crop Protection *(continued)*

- F. Nohilly, Poster. "Chytrid pathogensis programs the algal host proteotype", UCSD Biological Sciences Research Symposium. La Jolla, CA. June 2012.
- F. Nohilly. Poster. "Chytrid pathogensis programs the algal host proteotype", California Energy Commission Roadmap Meeting, La Jolla, CA. October 2012.
- F. Nohilly. Poster. "Chytrid pathogensis programs the algal host proteotype", French BioBeach Symposium , La Jolla, CA. November 2012.
- S. Wehrkamp-Richter, F. Nohilly. Poster. "Conservation of plant stress hormone responses in the unicellular alga, Scenedesmus dimorphus". Food and Fuel for the 21st Century Symposium. La Jolla, CA. April 2013.

Presentations

Task B. Nutrient Utilization

- **James Golden:** July seminars on "Cyanobacterial Biotechnology" in China at the Hydrobiology Institute and Central Normal University (Wuhan), Qingdao Institute of Bioenergy and Bioprocess Technology (Qingdao), and the Shanghai Institute of Plant Physiology and Ecology (Shanghai).
- S. Wang, B. Palenik. Microalgal use of diverse nitrogen sources for growth. Poster presentation at the Food and fuel for the 21st Century, April 19-20, 2013, La Jolla, CA, USA.
- W. Lambert, B. Palenik. Co-culturing nitrogen fixing cyanobacteria and diatoms in nitrogen deplete media for biofuels production. Poster presentation at the Food and Fuel for the 21st Century, May 11-13, 2012, La Jolla, CA, USA.
- W. Lambert. **Greenhouse and Pond Cultivation of Nitrogen-fixing Cyanobacteria Co-cultured with Diatoms**. Poster presentation at the Food and Fuel for the 21st Century Symposium, April 19-20, 2013, La Jolla, CA.
- **Jonathan Shurin** presented on ecology of algae biofuel in following conferences/ seminars:
- 1. Departmental Seminar, University of Kansas, March 6, 2012
  2. Departmental Seminar, Missouri State University, March 9, 2012
- 3. Associations for the Sciences of Limnology and Oceanography, special symposium on Biodiversity, July 10, 2012.
- 4. Ecological Society of America, special invited symposium on Microbial Ecology, August 10, 2011.
- 5. Sapphire Energy, special seminar, August 21, 2012
- **B. G. Mitchell**, UCSD-SIO CAB-COMM PRESENTATION, 2012
- 1) National Alliance for Advanced Biofuels and Bioproducts, 2nd International Conference, June 10-13, San Diego, CA. Poster Presentation: "Effect of Light and Temperature on Photosynthetic Quantum Yield, Pigment, Lipid, Protein and Carbohydrate Content in the Marine Diatom *Thalassiosira pseudonana*" Danielli Matias Dantas[1], Frank Shang, Sitti Raehanah Muhamad Shaleh, Patricia Abelin, Niu Du, Elliot Weiss, Dominick Mendola and B. Greg Mitchell, Scripps Institution of Oceanography UCSD, La Jolla CA 92093.

Presentations

Task B. Nutrient Utilization *(continued)*

- 2) National Alliance for Advanced Biofuels and Bioproducts, 2nd International Conference, June 10-13, San Diego, CA. Oral Presentation: "Modeling gross production, net production, and respiration of photosynthetic microalgae", B. Greg Mitchell, Niu Du. Scripps Institution of Oceanography UCSD, La Jolla CA 92093.
- 3) 31sr IUBS General Assembly and Conferences on Biological Sciences and Bioindustry, July 5-9, 2012, Suzhou, China, Oral Presentation: "Modeling growth of photosynthetic microalgae and carbon partitioning between lipid, protein and carbohydrate", B. Greg Mitchell*, Niu Du, Scripps Institution of Oceanography UCSD, La Jolla CA 92093.
- 4) Algae Biomass Organization Summit Meeting. Sept. 24-27, 2012, Denver, CO, USA. Oral Presentation: "Photsynthetic Quantum Yield of Algae", B. Greg Mitchell*, Niu Du, Daniel Yee, Scripps Institution of Oceanography UCSD, La Jolla CA 92093.
- 5) Algae Biomass Organization Summit Meeting. Sept. 24-27, 2012, Denver, CO, USA. Poster Presentation: "Determination of water soluble carbohydrate for microalgae cultures using the TPTZ method and application to the study of cellular carbon partitioning", [1]Patricia Abelin, [2]Laura T. Carney, [1]Frank Shang and [1]B. Greg Mitchell; [1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093.[2]Sandia National Laboratory, Livermore, CA 94551.
- 6) Mendola, D. 2013. Micro-Algae Biofuels & Bio-Products Research & Pilot Commercial-Scale Projects Worldwide. Ningbo University, Ningbo, China, April, 2013.
- 7) Mendola, D. 2013. Algae Biofuels & Bio-Products - R&D. Ningbo University, Ningbo, China, April, 2013.
- Mitchell: Opportunities for Improving Environmental Quality and Enhancing Natural Resource Base Provided by Algal Biofuels, NAS-NRC Study, March 17, 2011, San Diego, CA . B. G. Mitchell
- Mitchell: Photosynthetic Quantum Efficiency Measurement and Modeling: How and Why, ABO Summit, October 25, 2011, Minneapolis, MN. B. G. Mitchell

Presentations

Task B. Nutrient Utilization *(continued)*

- Mitchell: Algal Biofuels: Because A Pig Won't Eat A Lump Of Coal, BIOCOM, September 15, 2010 San Diego, CA. B. G. Mitchell
- Mitchell: Are (Micro/Macro)Algae a Viable Option for Biofuel? G'Day USA Clean Tech Conference, January 12, 2010, San Diego, CA. B. G. Mitchell
- Mitchell: Consortium for Algal Biofuels Commercialization (CAB-Comm), DOE Webinar, September 8, 2010. B. G. Mitchell
- Mitchell: Growth Rates and Yields of Oil, Protein and Carbohydrate for Microalgae as Regulated by Growth Conditions, Algae World Summit, May 23, 2011, Del Mar, CA. B. G. Mitchell
- Mitchell: Imperial Valley, Salton Sea and Water: The Algae Alternative, California Independent Voter Project, November 15, 2010, Maui, Hawaii
- Mitchell: Photosynthetic Quantum Efficiency Measurement and Modeling: How and Why SDCAB, Algal Biofuels Symposium, April 30, 2011, La Jolla, CA B. Greg Mitchell
- Mitchell: Prediction of the Consequences of the Obvious, SD-CAB Symposium, April 23, 2010. B. G. Mitchell
- Mitchell: Program Committee Member and Session moderator; Algae Biomass Summit meeting, Denver, CO., 23-27 September, 2012.
- Mendola: Energy from Algae - Aquatic Biomass Conversion for the Coachella Valley, Coachella Valley Energy Summit, May 12, 2011.Dominick Mendola
- Mendola: The Future for Algae Crops in the Imperial Valley, Imperial Valley Renewable Energy Expo, March 17, 2011, Dominick Mendola
- Du/Woertz/Rhodes/Mendola/Mitchell/Lundquist/Benemann; Critical review on algal biofuel LCA articles' parameters and results, ABO Summit, October 25, 2011, Minneapolis, MN. Du Niu, Ian Woertz, James Rhodes, Dominick Mendola, Greg Mitchell, Tryg Lundquist, John Benemann

Presentations

Task B. Nutrient Utilization *(continued)*

- Neori/Abelin/Shaleh/Mendola/Mitchell: Spray Irrigation Culture of Macroalgae in CO2 Enriched Atmosphere using Recirculated Seawater, ABO Summit, October 25, 2011, Minneapolis, MN. Amir Neori, Patricia Abelin, Sitti Raehanah M.Shaleh, Dominick Mendola, B. G. Mitchell
- Shang/Carney/Abelin/Brookhart/Mitchell: Effect of Temperature and Light Variation of on Fatty Acids, Pigment and Carbohydrate Yields for Marine Diatom, Thalassiosira pseudonana, ABO Summit, September 9, 2010, Denver, CO. Frank Shang, Laura Carney, Patricia Abelin, Rex Brookhart, B. G. Mitchell
- Shurin, "Algal traits and diversity as components of productivity and stability for biofuels", oral presentation at the Food and Fuel for the 21st Century, April 22-23, 2012, La Jolla, CA, USA
- Shurin/Mitchell: Effect of irradiance and CO2 on the utilization of carbon in the marine diatom Thalassiosira pseudonana. SD-CAB Symposium, May 13, 2012, La Jolla, CA. Jonathan Shurin, B. G. Mitchell
- Shurin: NRC Committee on Sustainable Development of Algal Biofuel (UC Irvine, CA; Sept 2011)

Presentations

Task C. Genetic Tools

- Weeks: Transcriptome analysis of Chlamydomonas subjected to carbon dioxide deprivation, to USDA North Central 1168 Photosynthesis Conference, Michigan State University
- Weeks: Li-COR Corporation (Lincoln, NE; December 12, 2011)
- Weeks: Algal Biofuels Symposium 2012, held in La Jolla, California; May 2012. Title (Oral presentation): Massive changes in Chlmydomonas gene expression during activation of the CO2-concentration mechanism triggered by CO2 depletion.
- D. P. Weeks, Invited presentation: Massive changes in gene expression associated with activation of the CO_2-concentrating mechanism in *Chlamydomonas reinhardtii*. Don weeks, 15th International Chlamydomonas Conference, Potsdam, Germany, June 7, 2012
- D. P. Weeks, Oral Presentation: Developing Surface Nanobodies Specific to *Chlamydomonas reinhardtii*, ABO Algae Biomass Summit (Denver, CO), September 27, 2012.
- D. P. Weeks, Seminar: Lawrence Berkeley Laboratory, UC Berkeley. "Massive Transcriptome Changes During Activation of the CO_2-concentrating Mechanism (CCM) in Microalgae and Development of TAL Effector Nucleases (TALENs) for Targeted Gene Knockout for Yeast and Rice". December 18, 2012
- D. P. Weeks, Seminar: Nebraska LEAD Program. Algal and Agricultural Biotechnology: The Next Wave. October 25, 2012
- Drew Brueggeman, Poster: UNL Research Fair. "Massive changes in gene expression associated with CO_2 deprivation in *Chlamydomonas reinhardtii*. November 16, 2012.
- D. P. Weeks, Seminar: USDA NC-1200 Regional Meeting, Reno, NV. "Use of TAL Effector Nuclease (TALN) Technologies for Targeted Gene Knockout and Gene Replacement". November 17, 2012
- Drew Brueggeman, Seminar. Development and testing of herbicide resistance genes for *Chlamydomonas reinhardtii* and algae of potential commercial importance. Nebraska Coalition for Algal Biology and Biotechnology, February 25, 2013

Presentations

Task C. Genetic Tools *(continued)*

- Thomas Plucinak, Seminar. The power of FMDV 2A technology for production of multiple proteins from a single gene in *Chlamydomonas reinhardtii*. Nebraska Coalition for Algal Biology and Biotechnology, February 25, 2013
- D. P. Weeks, Seminar: UNL Center for Plant Science Innovation. "Use of TAL Effector Nuclease (TALEN) Technologies for Targeted Gene Knockout and Gene Replacement". January 17, 2013
- Yuan, J., Kendall, A., (2012) "Life cycle assessment of second and third generation biofuels: a review of feedstocks, processes and environmental implications", *Growing the Bioeconomy Conference*, Banff, Alberta, Canada.
- M. Hildebrand: Development of Diatom Genetic Manipulation Tools; oral presentation at the Food and Fuel for the 21st Century, April 22-23, 2012, La Jolla, CA, USA.
- M. Hildebrand: Invited Keynote Speaker and Session Chair, The Second International Conference on Algal Biomass, Biofuels, and Bioproducts, "Evolutionary-based Differences in Microalgal Cellular Organization and Processes as Related to Biofuels Production" San Diego, CA, June 10-13, 2012.
- M. Hildebrand: Invited talk, "Improvement of Lipid Accumulation in Microalgae by Carbon Flux Metabolic Engineering." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Smith, S.R., Allen, A.E., Hildebrand, M.: Poster, "A global regulatory mechanism integrates carbon and energy metabolism in the diatom *Thalassiosira pseudonana.*" FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Taton A., E. Lis, D. M. Adin, G. Dong, S. Cookson, T. Swinney, E. King, S. A. Kay, S. S. Golden, and J. W. Golden (2012) - Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria. Proceedings of 14th International Symposium on Phototrophic Prokaryotes, Porto, Portugal, August 5 to 10. (TALK)
- Taton A., E. Lis, D. M. Adin, G. Dong, F. Unglaub, T. Swinney, E. King, R. Cook, N.E. Wright, S. A. Kay, S. S. Golden, and J. W. Golden (2012) - Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria. Development of Microalgae Industrial Biotechnology: from animal food to bioenergy, French BioBeach - November 12, San Diego, CA. (POSTER)

Presentations

Task C. Genetic Tools *(continued)*

- Golden: Speaker, Keystone conference on Biofuels, Singapore, "Developing Cyanobacteria for Production of Industrial Products and Fuels," March 2011
- J. W. Golden. Multiple seminars on "Heterocyst Development" and "Cyanobacterial Biotechnology" in China at the Hydrobiology Institute (Wuhan), Central Normal University (Wuhan), Qingdao Institute of Bioenergy and Bioprocess Technology (Qingdao), and the Shanghai Institute of Plant Physiology and Ecology (Shanghai), July 1-12, 2012. Invited presentations.
- J. W. Golden. Food and Fuel for the 21st Century, La Jolla, CA, May 11-13, 2012. "Cyanobacterial strains, genetic tools, crop protection, and nutrient supply". Oral presentation.
- Golden, S. (2013) - Developing Cyanobacteria as Platforms for Food and Fuel Production. FF21 Symposium, UCSD, La Jolla, CA. April 19-20 (Invited talk).
- Taton A., E. Lis, D. M. Adin, G. Dong, S. Cookson, T. Swinney, E. King, S. A. Kay, S. S. Golden, and J. W. Golden (2012) - Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria. Proceedings of 14th International Symposium on Phototrophic Prokaryotes, Porto, Portugal, August 5 to 10. (TALK)
- Taton A., E. Lis, D. M. Adin, G. Dong, F. Unglaub, T. Swinney, E. King, R. Cook, N.E. Wright, S. A. Kay, S. S. Golden, and J. W. Golden (2012) - Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria. Development of Microalgae Industrial Biotechnology: from animal food to bioenergy, French BioBeach - November 12, San Diego, CA. (POSTER)
- Rasala BA, Lee P, Ng J, Barerra D, Plucinak TM, Rosenberg J, Weeks D, Oyler G, Mayfield SP. "Genetic tools for microalgal research and biotechnology." Algal Biomass Summit, ABO meeting, Denver, Colorado, September 27, 2012. Poster presentation.
- Rasala BA and Mayfield, SP. "Development of Molecular Genetic Tools for Microalgae." SD-CAB Symposium 2012, Food and Fuel for the 21st Century. May 2012. Platform presentation.

Presentations

Task C. Genetic Tools *(continued)*

- Rasala BA and Mayfield SP. "Production of industrial enzymes in microalgae to enable cellulosic biofuels." American Chemical Society Spring 2012 National Meeting and Exposition. March 2012. Platform presentation.
- Specht EA, Mayfield SP: Genetic Manipulation of Chloroplast Gene Regulation. Poster presented at San Diego Center for Algal Biotechnology Annual Symposium, La Jolla CA, 13 May 2012.
- Specht, EA. Talk. Characterizing chloroplast gene regulatory elements to construct optimized synthetic regulatory regions. Talk, Quantitative Biology Winter Conference. Waikiki, Hawaii. February 18, 2013.
- Mayfield: Agilent (Santa Clara, CA; November 2011)
- Mayfield: Algal Biomass Organization (Minneapolis, MN; October 2011)
- Mayfield: Atlantic meets the Pacific Forum (La Jolla, CA; October 2011)
- Mayfield: Berlin Algae Conference (Berlin, Germany; December 2011)
- Mayfield: Environmental Entrepreneurs Breakfast (La Jolla, CA; October 2011)
- Mayfield: Microorganisms for Biofuel Production from Sunlight (Bielefeld, Germany; September 2011)
- Mayfield SP. Invited Talk. Washington University St. Louis, DOE biofuels Center, January 22, 2013.
- Mayfield SP. Invited Talk. Michigan State University, Department of Biochemistry, January 24, 2013.
- Mayfield SP. Invited talk. San Diego Science and Engineering Festival, March 20, 2013.
- Mayfield SP. Invited Talk. Life Technologies, Carlsbad CA, February 19, 2013.
- Mayfield SP. Invited Talk. California oversite committee meeting on Salton Sea, February 22, 2013.
- Mayfield SP. Talk. "Green Algae for Bio-products Production." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Rasala, B.A., Lee, P., Barrera, D., Haerizadeh, F., Peterson, T., Rosenberg, J., Oyler, G., Plucinak, T.M., Weeks, D., Mayfield, S. Poster. "Genetic tools for microalgal research and biotechnology." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.

Presentations

Task C. Genetic Tools *(continued)*

- Specht, E., Poster, "Genetic Manipulation of Chloroplast Gene Expression." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Gimpel, J., Poster, "Analysis of Heterologous Regulatory and Coding Regions in Algal Chloroplasts." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Georginanna, D.R., Davis, I.W., Elich, T., Mayfield, S.P., Poster, "Development of Functional Synthetic Promoters for *C. reinhardtii.*" FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Muff. T.J., Mayfield, S., Poster, "Transcriptional regulation in *Chlamydomonas reinhardtii*." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Tran, M., Camargo, L., Poster. "Disulfide bond formation as a rate limiting step in the accumulation of complex recombinant proteins." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Arnaud Taton, Ewa Lis, Dawn M. Adin, Guogang Dong, Scott Cookson, Federico Unglaub, Tyler Swinney, Edward King, Ron Cook, Nicole E. Wright, Steve A. Kay, Susan S. Golden & James W. Golden. Poster. "Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Emily Trentacoste, Jennifer R. Hull, Roshan Shrestha, Sarah R. Smith, Corine Glé, Aaron C. Hartmann, William H. Gerwick, Mark Hildebrand. Poster. "Metabolic Engineering of Lipid Catabolism Improves Lipid Yields from Microalgae." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Oyler: Noel EA, Kobayashi N, Barnes AL, Watson A, Rosenberg JN, Erikson GE, Van Etten J, Oyler GA. Integrated algae growth on anaerobic digester effluent: phylogeny and lipid compositions of Chlorella spp., ABO Algae Biomass Summit (Denver, CO), September 24-27, 2012.
- Oyler GA. *Chlamy* Chloroplast Expression VHH Antibodies and Protein Delivery Domains and VHH Antibodies for Oral Therapeutics, ABO Algae Biomass Summit (Denver, CO), September 27, 2012.

Presentations

Task C. Genetic Tools *(continued)*

- Weeks DP. Developing Surface Nanobodies Specific to *Chlamydomonas reinhardtii*, ABO Algae Biomass Summit (Denver, CO), September 27, 2012.
- Rosenberg: Rosenberg JN, Wan M, Kobayashi N, Andlay G, Balasubramanian A, Betenbaugh MJ, Oyler GA. A comparative analysis of Chlorella species' heterotrophic growth characteristics and lipid composition, ABO Algae Biomass Summit (Denver, CO), Biology Breakout Session: Analysis and Modification of Lipid Production, September 27, 2012.
- Rosenberg JN, Wauchope AD, Jiang WZ, Kang M, Kobayashi N, Tremblay JM, Shoemaker CB, Weeks DP, Hildebrand M, Betenbaugh MJ, Mayfield SP, Oyler GA. Development of a single-chain antibody toolkit to interrogate and manipulate the microalgal cell, ABO Algae Biomass Summit (Denver, CO), September 24-27, 2012.
- D. P. Weeks, Talk. "Use of TAL Effector (TALEN) technology for development of rice resistant to bacterial blight". FF21 Symposium, UCSD, La Jolla, CA. April 20, 2013.
- G. A. Oyler, Talk. "A Vision for Integrated Algae Systems using Chlorella." FF21 Symposium, UCSD, La Jolla, CA. April 19-20, 2013.
- Dismukes: ACS National Meeting (Denver, CO, August 2011)
- Dismukes: NSF Algal Biofuels Workshop, http://www.engr.colostate.edu/NSFalgaeworkshop/index.html (Arlington, VA; November, 2011);
- Kumaraswamy, G. Kenchappa. (2012). Metabolic engineering of Synechococcus sp. PCC 7002 for improved utilization of fixed carbon. Food and Fuel for 21st Century, May 11-13, La Jolla, CA.