SANDIA BIOSCIENCE

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Stability and Sustainability of Algal Mass Culture

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SFA Kickoff 2/10/2015

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U.S. DEPARTMENT OF ENERGY

NNSA National Nuclear Security Administration

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BIOENERGY TECHNOLOGIES OFFICE

Sandia National Laboratories

Projects to leverage for SFA

- Previous SNL LDRD projects
- Previous BETO funded projects
- Current Beto funded projects:
  - Nutrient Recycling Project
  - Algae Testbed Public Private Partnership (ATP3)
  - Algal Biomass Yield Projects
    - California Polytechnic University San Luis Obispo
    - Cellana LLC
- "Pond Crash" Testbed: SNL CA

Why is this important for biofuels

Standard DOE 4 Hectare Pond - $5/gge Scenario

Annual Productivity (calculated)	13g-AFDW/m2/day
Daily production per pond (40,000m2 per pond)	0.52 MT/day
Annual production per pond (330 days)	171.6 MT/pond/year
Algae Lipid Content	25%
Overall Algal Oil Yield	1000 gal_Algal_oil/acre/yr.
Biomass loss through predation	10-30%
Annualized Biomass lost per pond	17.2-51.5 MT/pond/year
Annualized Biomass lost per farm (101 ponds)	1737-5201 MT/farm/year
Algae Lipid Content	25%
Annualized lipid loss per farm per year	434-1300 MT/farm/year
Total value of lost algal lipid per farm per year	$608K -$1.82M/farm/year

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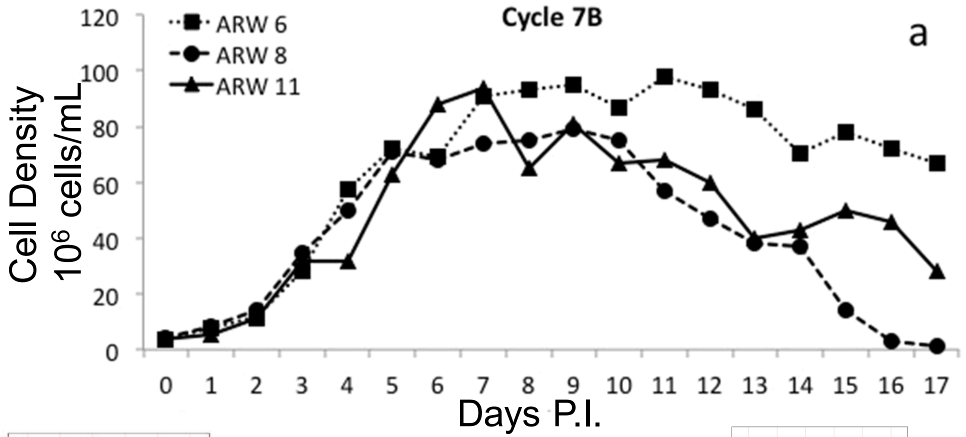
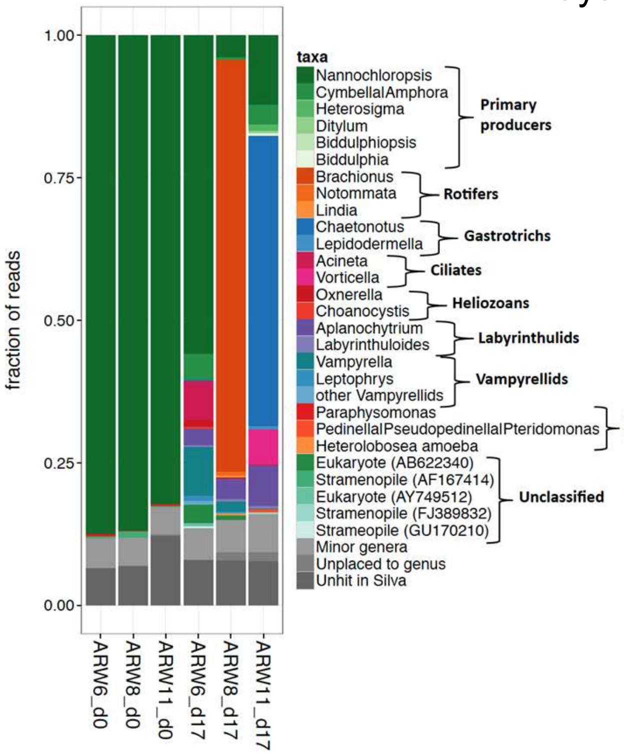
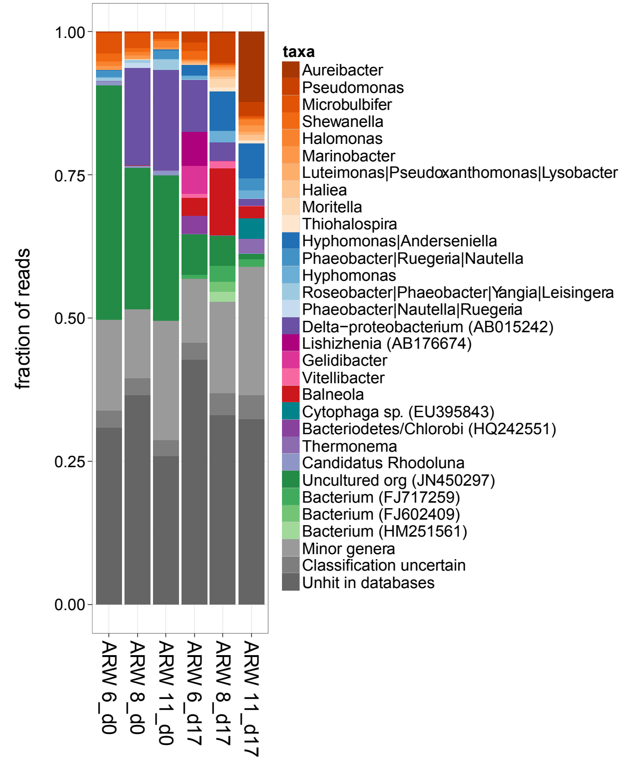
Forensic Analysis of Pond Crashes







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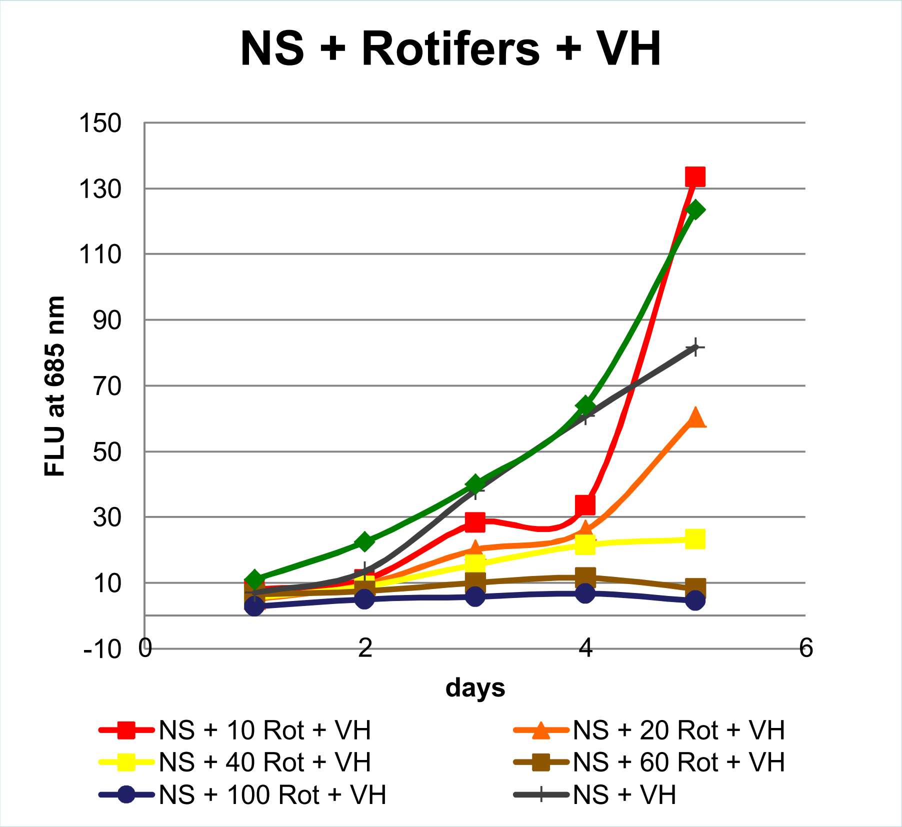
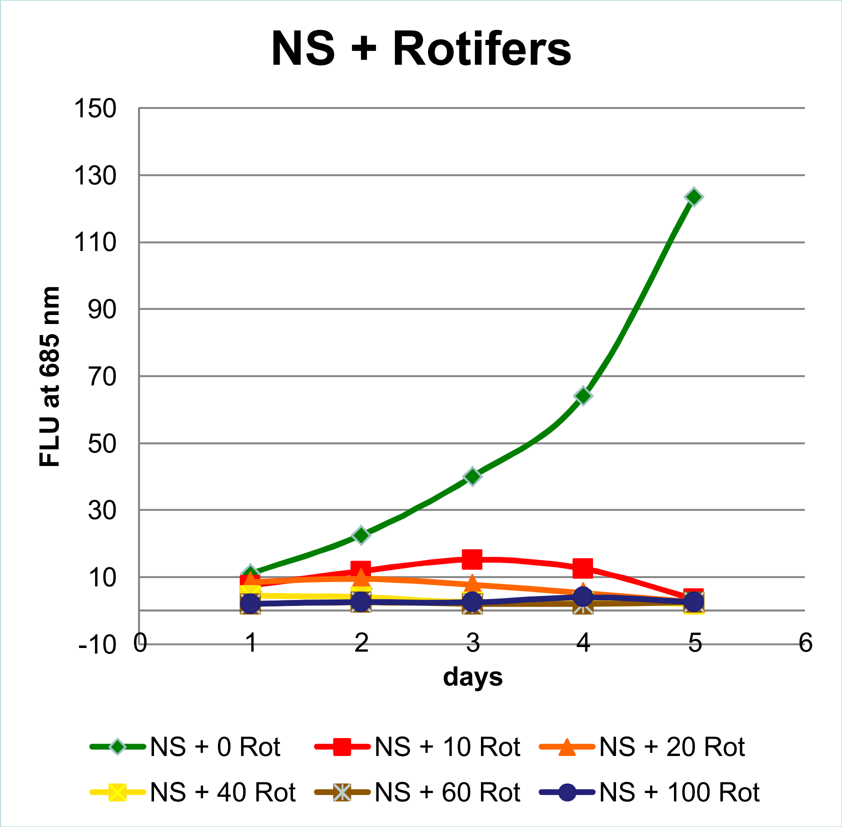
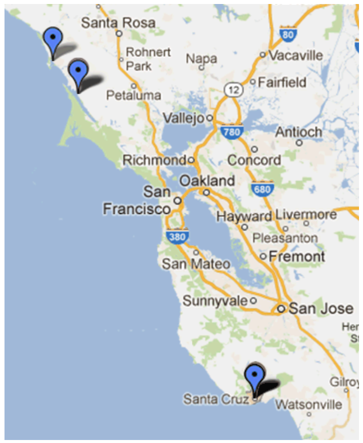
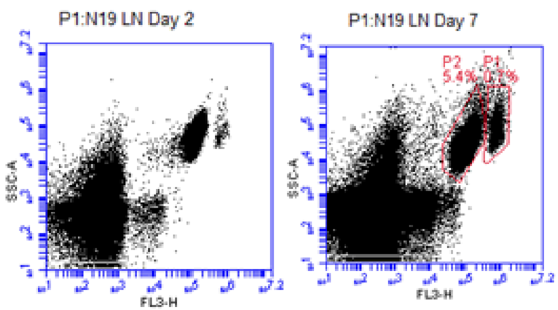
Probiotic strain Vh mitigates Rotifer grazing



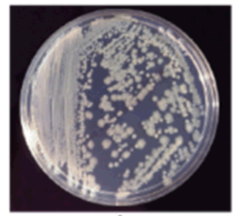


Isolation of probiotics to improve yield

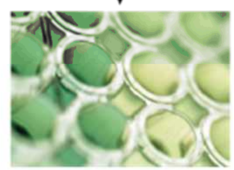




Environmental sampling



Isolation

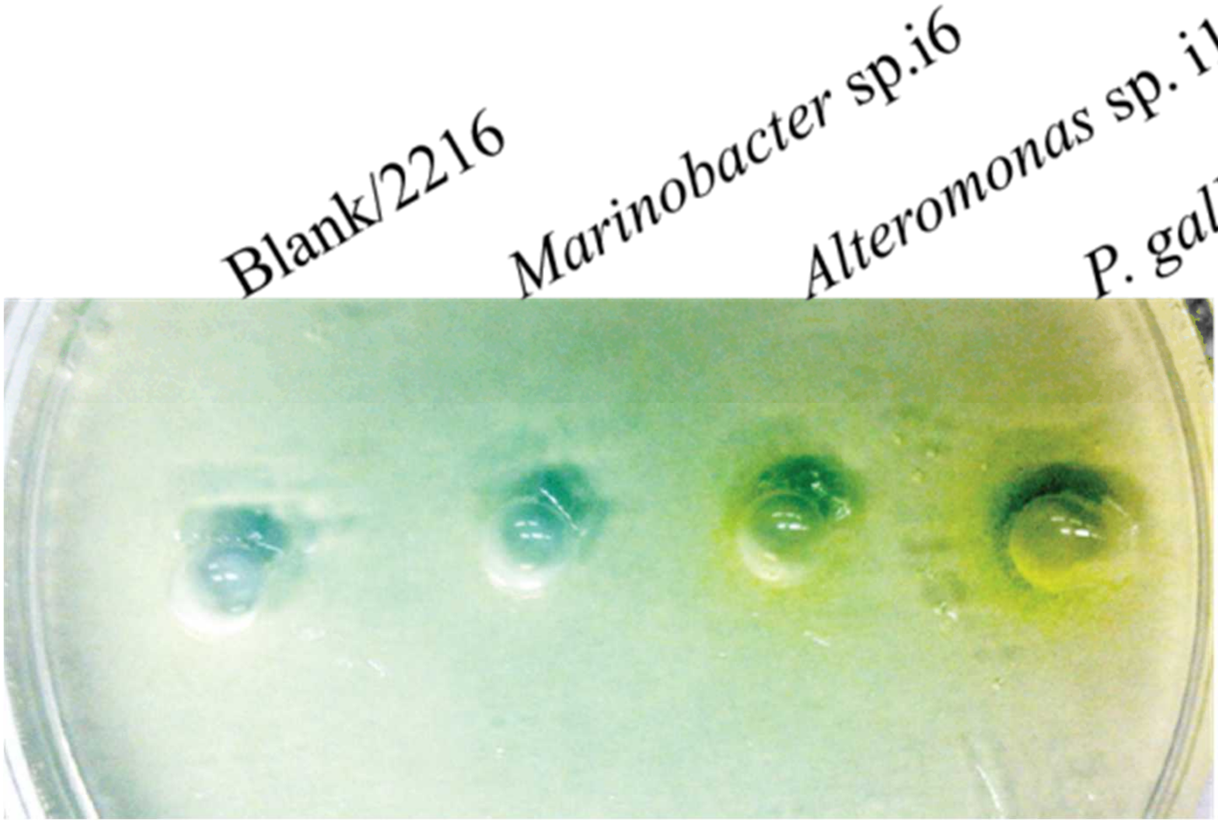


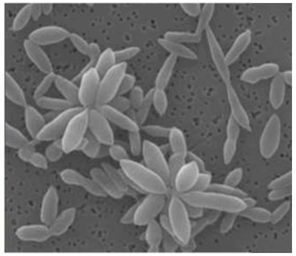
co-culture screen 200 µl

co-culture confirmation 8 ml

co-culture confirmation 60 ml

Probiotics: likely produce metabolites that enhances algal yield



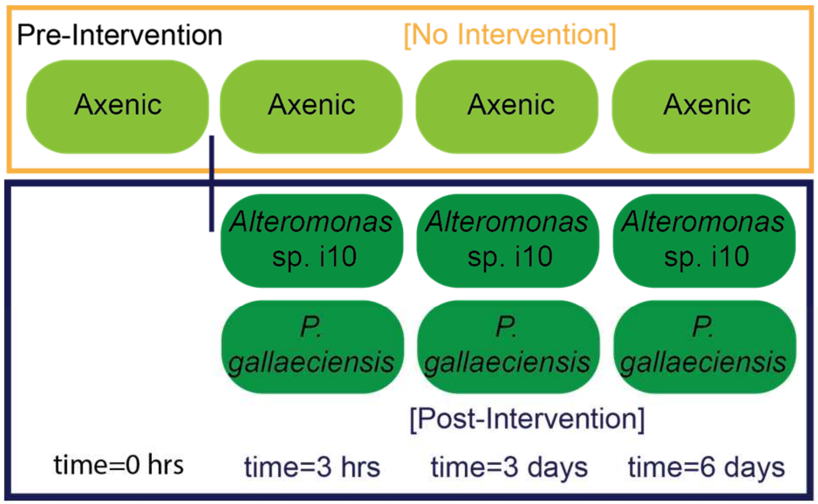


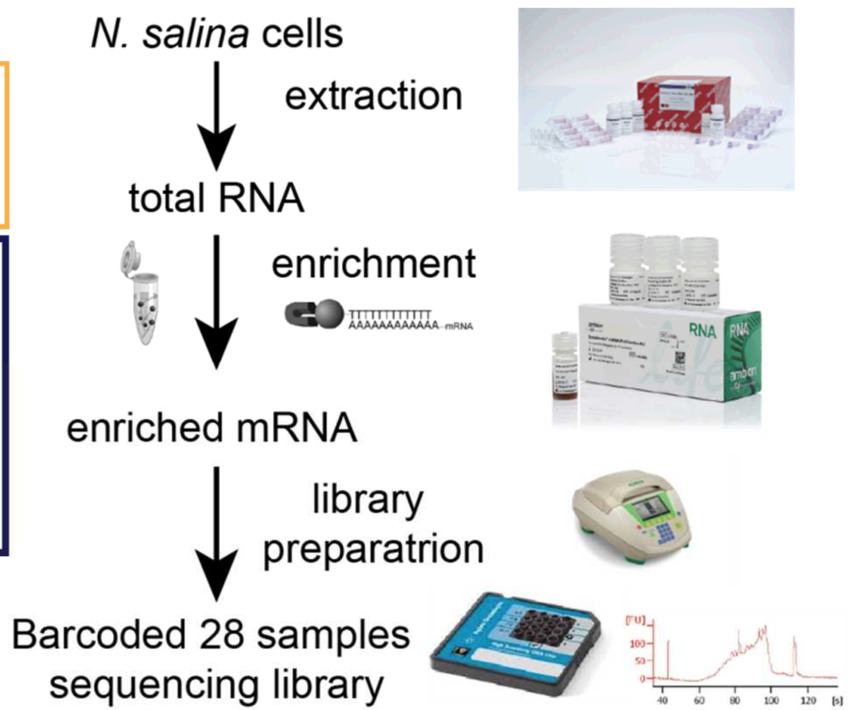
P. gallaenciensis

Probiotics + *N. salina* co-culture in outdoor mesocosms

8-week outdoor cultivation period (20L mesocosms)

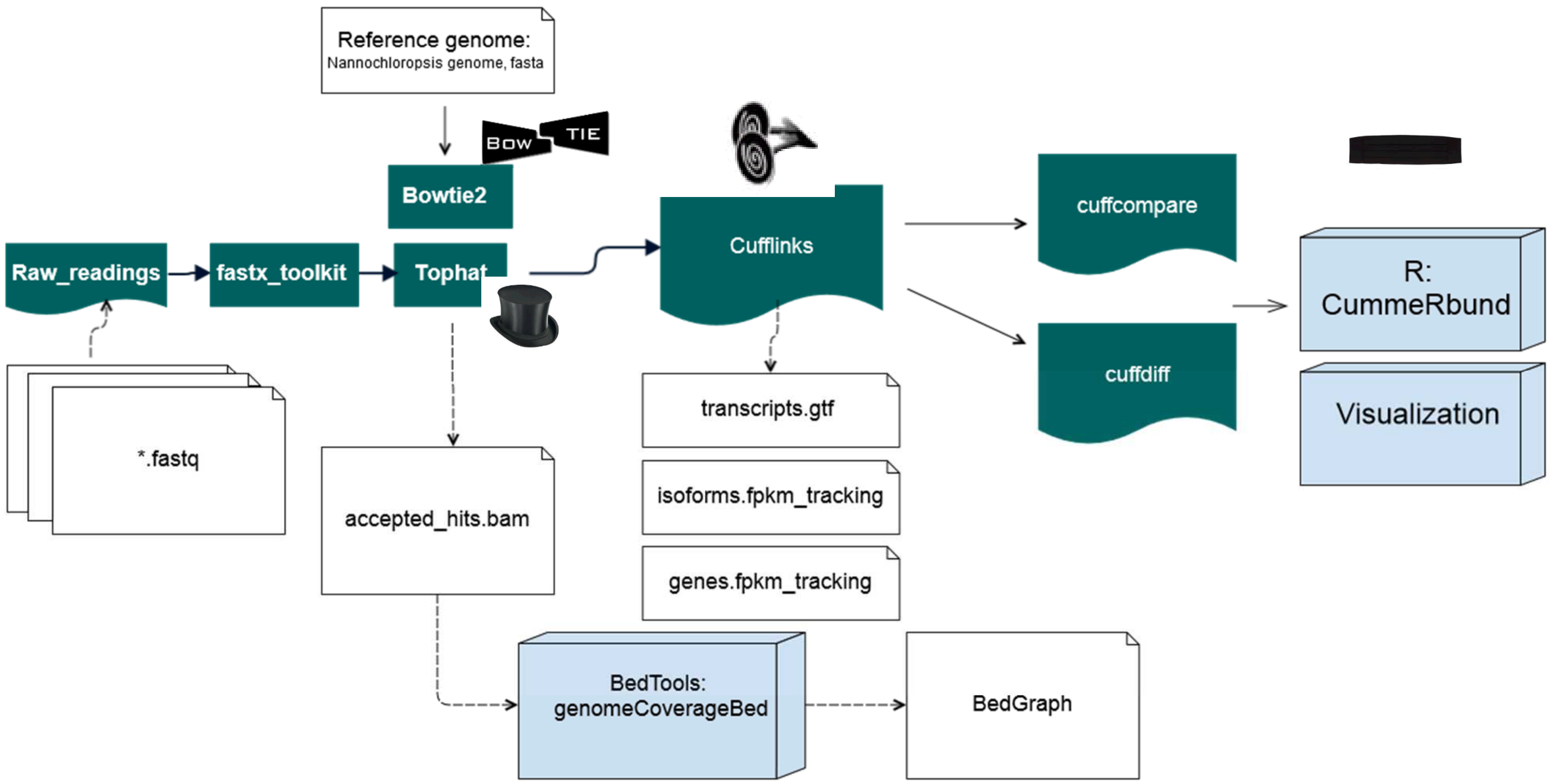
RNA-Seq experiment design





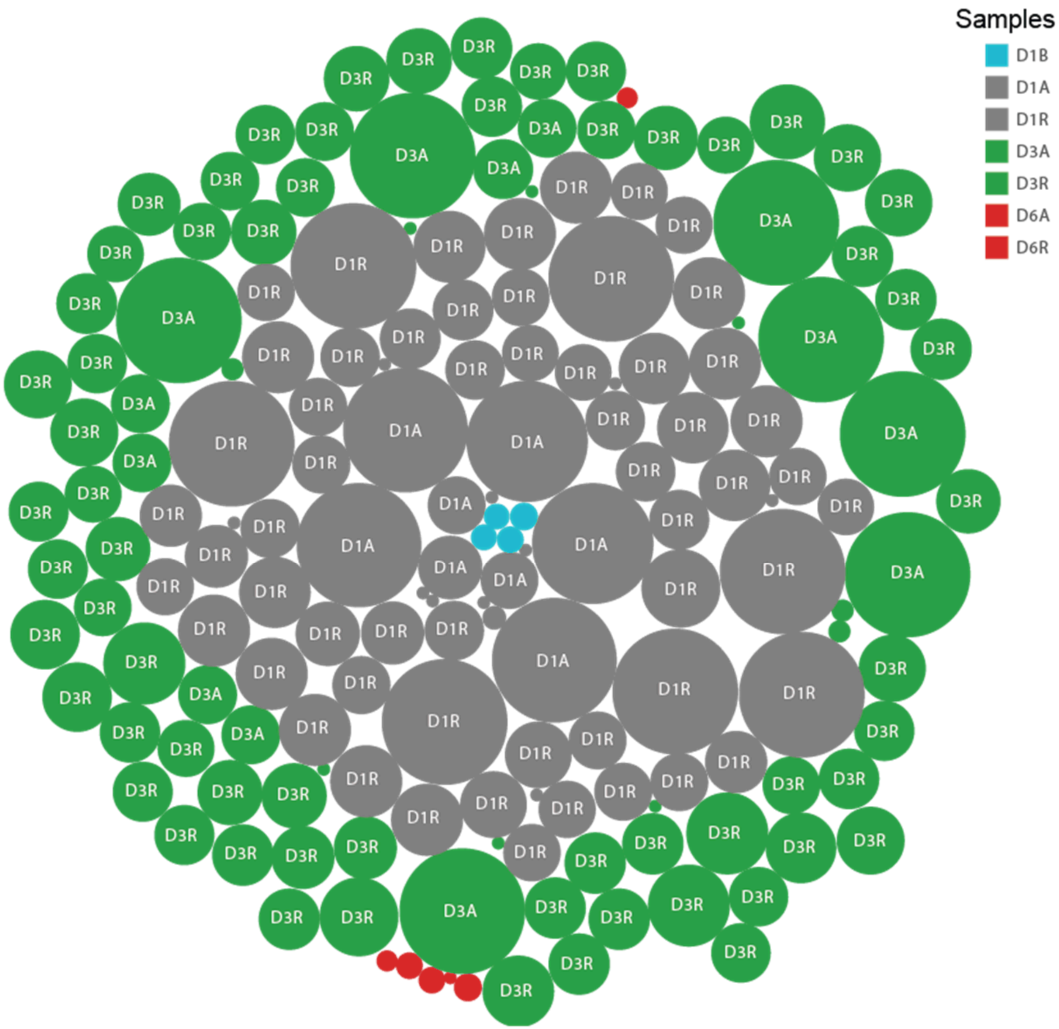
RNA Biol., 2013, 502-515

RNA-Seq analysis pipeline



Nature Protocol, 20
562-578

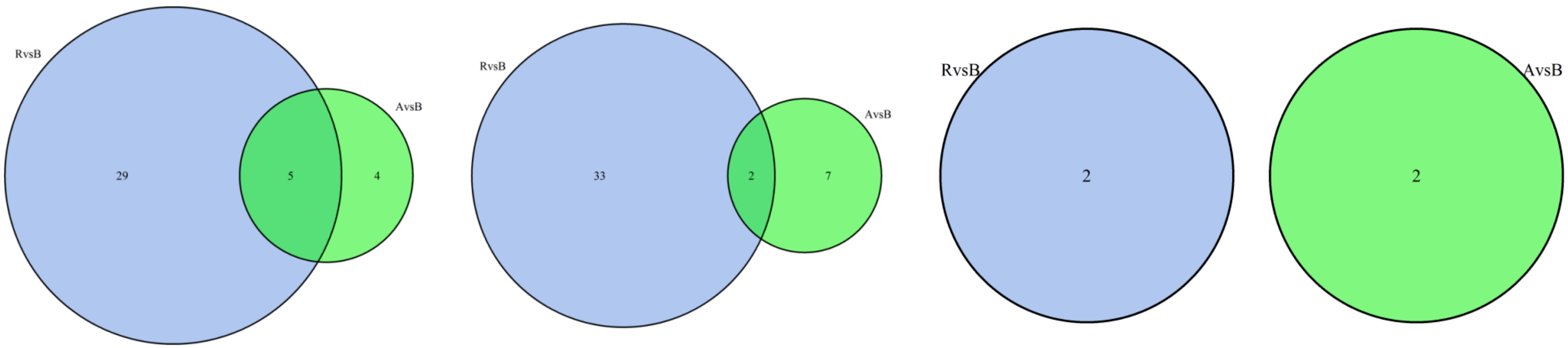
Tested bacteria quickly fire up the microalgal *N. salina* genes



Color shows details about samples. Size shows Fold Change. The marks are labeled by sample.

- **Benchmarking**: 3 hrs none treated vs. starting samples
- Intervention vs. none:
  - 3 hrs
  - 3 days
  - 6 days
- Bacterial isolates change *N. salina* transcriptome within several

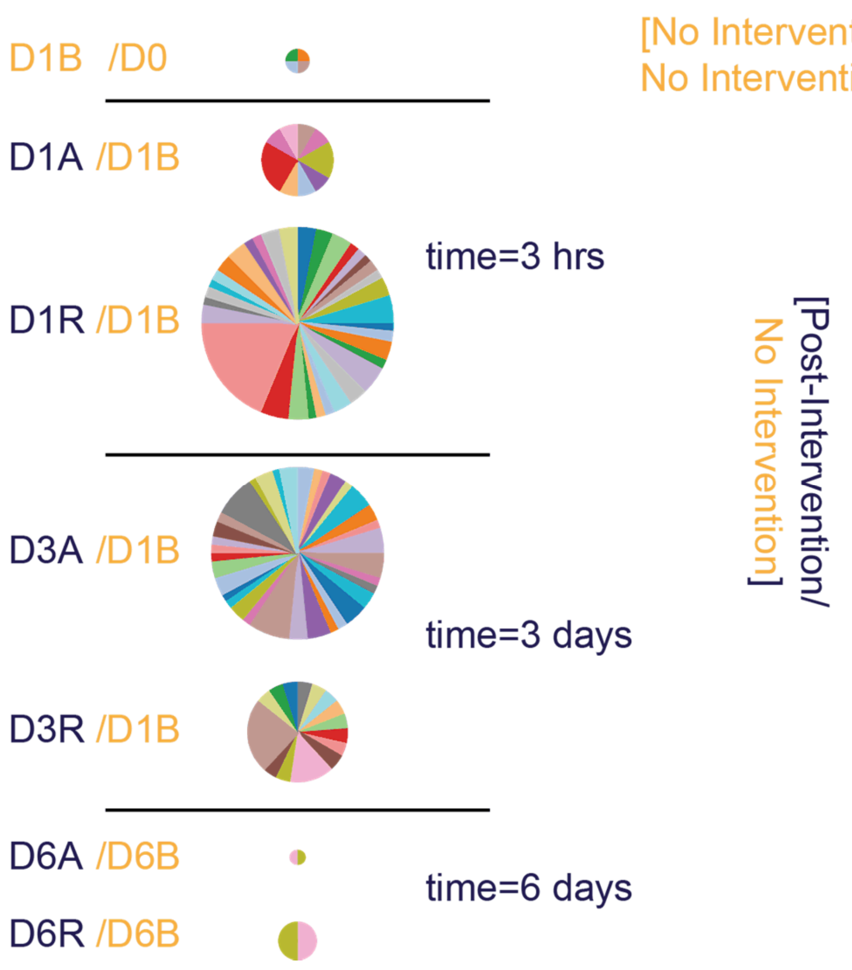
Shared/unique induced *N. salina* genes



3 hrs | 3 days | 6 days

- Total 81 genes to be differential expressions (DEs)
- Collectively, 38 and 42 DEs genes were respectively in 3-hrs and 3-days, but only 4 in 6-days samples
- Shared DE genes between two treatments suggest that changed biological processes are common in some parts when *N. salina*

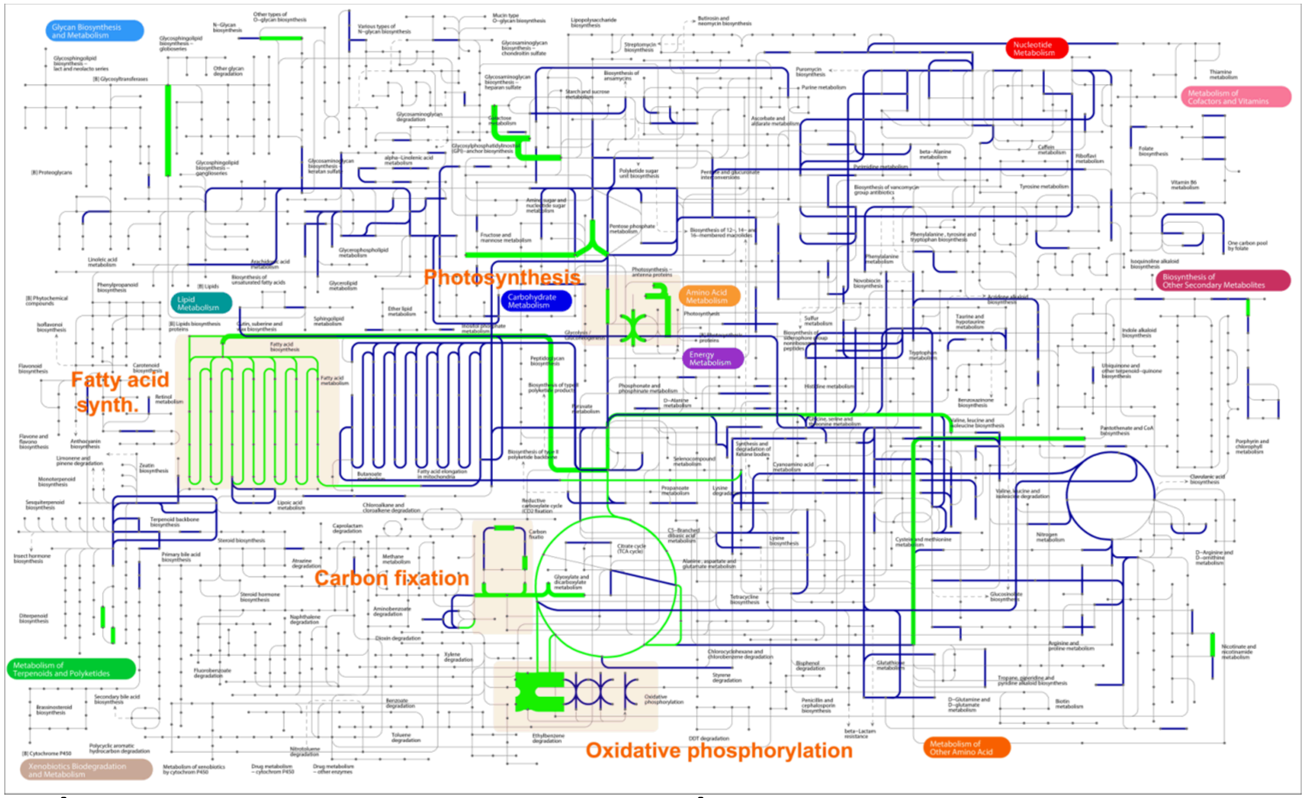
genes occurs in early *N. salina* exponential phase



- Gene Id (color) and transcriptional dissimilarities between samples (JS distance, size) broken down in different samples

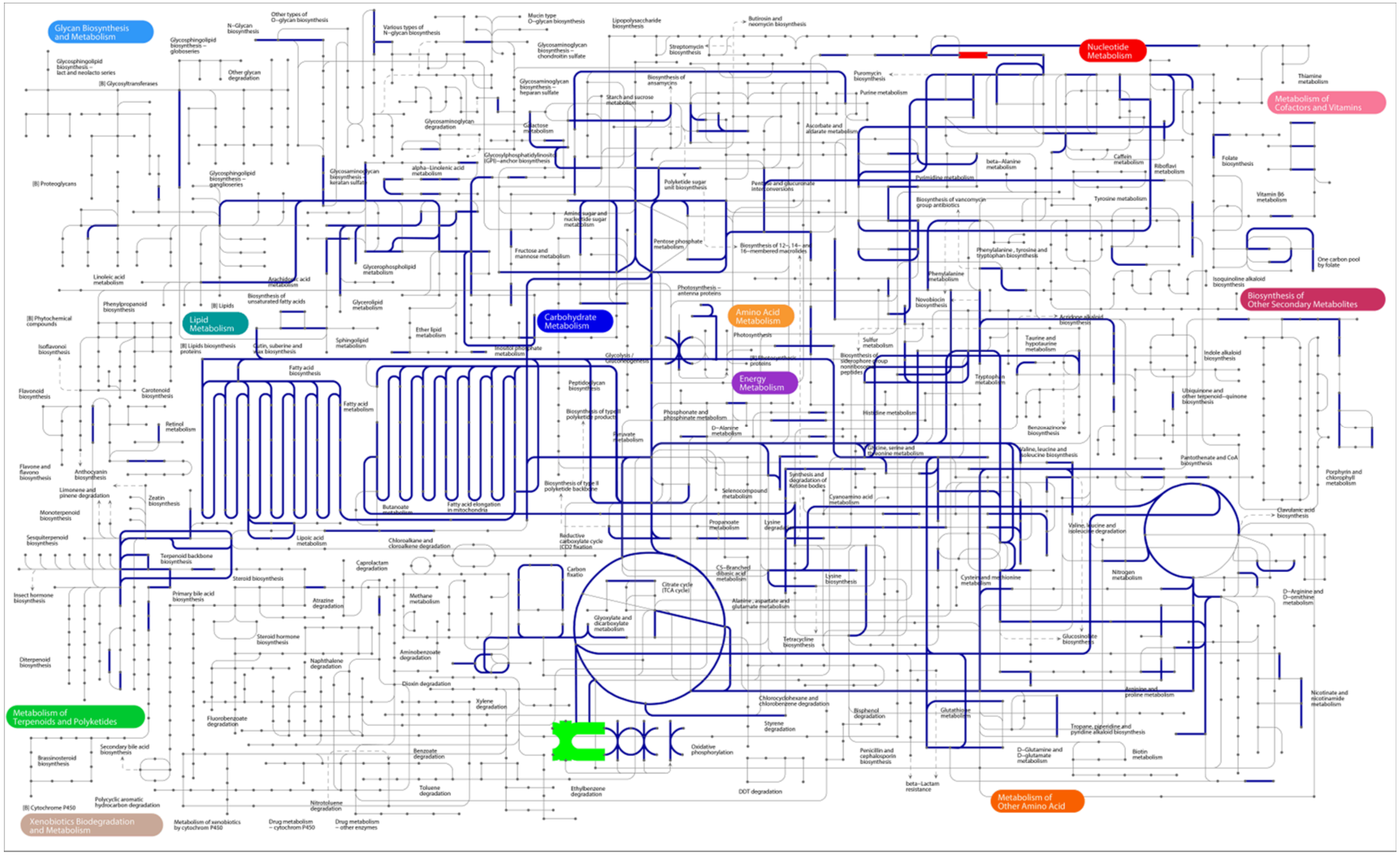
- Variance between intervention and none-intervention samples were pronounced in the

P. gallaeciensis induces *N. salina* metabolic genes overexpression



- Light gray not encoded in genome;
- Blue indicate genome encoded KEGG pathways that are no differentially expressed
- Up-regulated genes in green, **width** indicates expressio **fold change**
  - Photosynthesis, carbon fixation, and oxidative

Alteromonas sp.i10 likely does not directly affect *N.salina* metabolic pathway



- Only oxidative phosphorylation pathway changed

N. salina DE genes upon coculturing with *Alteromonas* i.10

gene_id	transcript_id	KO_terms	Function Description	log2_fold_change	p_value	q_value
XLOC_003238	TCONS_00003730	K01881	prolyl-tRNA synthetase [EC:6.1.1.15]	1.376503038	0.00005	0.00575684
XLOC_002629	TCONS_00003025	K01923	phosphoribosylaminoimidazole-succinocarboxamide synthase [EC:6.3.2.6]	-1.7268614	0.00055	0.0371352
XLOC_002629	TCONS_00003026	K01923	phosphoribosylaminoimidazole-succinocarboxamide synthase [EC:6.3.2.6]	-1.7268614	0.00055	0.0371352
XLOC_002629	TCONS_00003024	K01923	phosphoribosylaminoimidazole-succinocarboxamide synthase [EC:6.3.2.6]	-1.7268614	0.00055	0.0371352
XLOC_003540	TCONS_00004098	K02943	large subunit ribosomal protein LP2	1.123334999	0.00025	0.0211977
XLOC_003540	TCONS_00004101	K02943	large subunit ribosomal protein LP2	1.123334999	0.00025	0.0211977
XLOC_001276	TCONS_00001464	K03883	NADH-ubiquinone oxidoreductase chain 5 [EC:1.6.5.3]	3.221278679	0.00005	0.00575684
XLOC_001276	TCONS_00001463	K03935	NADH dehydrogenase (ubiquinone) Fe-S protein 2 [EC:1.6.5.3 1.6.99.3]	3.221278679	0.00005	0.00575684
XLOC_000686	TCONS_00000780	K05916	nitric oxide dioxygenase [EC:1.14.12.17]	1.075718668	0.00005	0.00575684
XLOC_003540	TCONS_00004100	K10206	LL-diaminopimelate aminotransferase [EC:2.6.1.83]	1.123334999	0.00025	0.0211977
XLOC_003540	TCONS_00004097	K10206	LL-diaminopimelate aminotransferase [EC:2.6.1.83]	1.123334999	0.00025	0.0211977
XLOC_003540	TCONS_00004099	K10206	LL-diaminopimelate aminotransferase [EC:2.6.1.83]	1.123334999	0.00025	0.0211977
XLOC_001943	TCONS_00002220	K14950	cation-transporting ATPase 13A1 [EC:3.6.3.-]	-11.54098526	0.0008	0.0488849
XLOC_003977	TCONS_00004613			-11.39417863	0.0008	0.0488849
XLOC_000792	TCONS_00000901			-12.4327037	0.00045	0.0319617
XLOC_001329	TCONS_00001525			-14.09135274	0.0005	0.0341812
XLOC_002169	TCONS_00002493			-10.97111241	0.0004	0.0295622
XLOC_003377	TCONS_00003909			-11.6463877	0.0005	0.0341812
XLOC_003637	TCONS_00004214			-10.92059849	0.00005	0.00575684
XLOC_004874	TCONS_00005650			-14.22597559	0.00005	0.00575684
XLOC_005229	TCONS_00006052			-13.34885261	0.0003	0.0252415
XLOC_003610	TCONS_00004183			-1.518828197	0.00005	0.00575684
XLOC_002687	TCONS_00003092			-1.034370868	0.0006	0.0400171
XLOC_003068	TCONS_00003524			1.067092565	0.00005	0.00575684
XLOC_002126	TCONS_00002447			1.183858401	0.00005	0.00575684
XLOC_003283	TCONS_00003784			3.300011139	0.00005	0.00575684
XLOC_003283	TCONS_00003786			3.300011139	0.00005	0.00575684
XLOC_003283	TCONS_00003785			3.300011139	0.00005	0.00575684
XLOC_000962	TCONS_00001092			11.77974808	0.0008	0.0488849
XLOC_001503	TCONS_00001729			12.61478575	0.00045	0.0319617
XLOC_001818	TCONS_00002082			13.06323327	0.0001	0.0104171
XLOC_002069	TCONS_00002375			12.32478811	0.0007	0.0453053
XLOC_002345	TCONS_00002714			13.43089679	0.00015	0.014144
XLOC_002644	TCONS_00003044			12.27369654	0.00015	0.014144
XLOC_004244	TCONS_00004928			12.63699159	0.00005	0.00575684

- Majority of genes do not have KO terms
- Nitric oxide dioxygenase catalyzes NO, an important sec
- Much is still unknown about the *Alteromonas* i.10 biolog that causes *N. salina* growth promotion

Nutrient recycling project is a partnership between national lab, university and industry

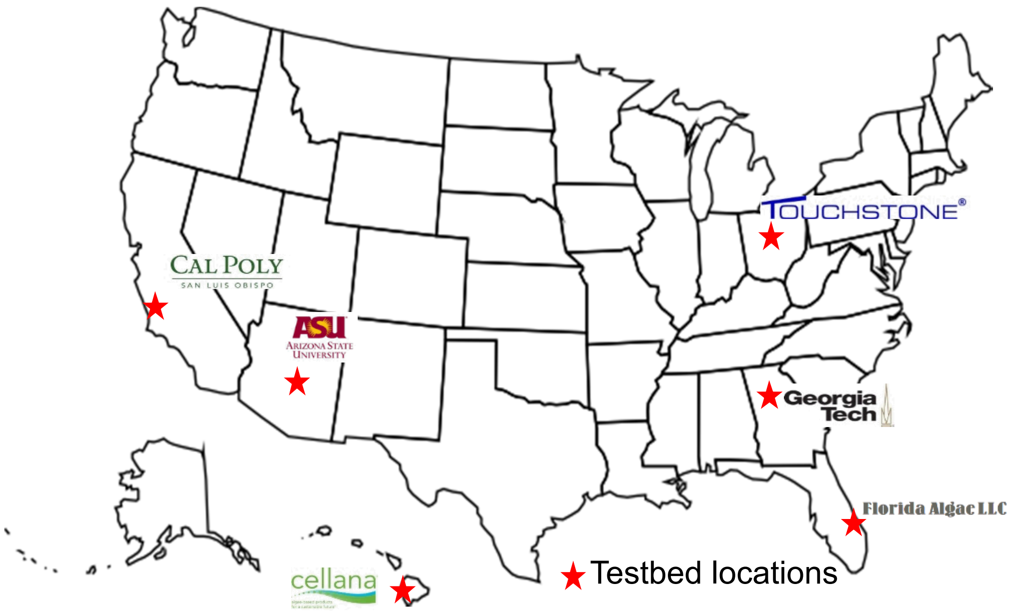
- Laboratory to pilot/field scale
- Sandia National Labs
  - Project Lead
  - Biochemistry/Precipitation Science
- Texas AgriLife Research (TAMU):
  - 12 X 550 L replicate ponds
  - 10,000 L ponds
  - *Nannochloropsis salina*
  - *Phaeodactylum tricornutum*
  - *PBRs*
  - *90 day growth trial*
  - Marine species
    - *Nannochloropsis salina*
    - *Phaeodactylum* tricornutum
      - (NAABB strains)
- OpenAlgae
  - TAG extraction
  - DAG extraction
    - Converted phospholipids

Sandia Algal Testbed

- 3 X 1000L raceway ponds (@ 20cm depth)
- 2700 μE LED lighting (~600 LEDs/p
- Temperature controlled
- CO_2 sparging system
- Automated logging of pH, Temp,
- Fluorometer, Coulter counter, flow cytometer
- SNL spectroradiometic monitoring

ATP^3 Consortium

- 6 X 1000 L replicate ponds in 5 geographically distinct locations
- *Nannochloropsis oceanica*
- *Chlorella sp* DOE 1412



Algal Biomass Yield: Cal Poly SLO

- Freshwater natural algal assemblages grown on primary effluent
- Original Oswald pond system
- ~1000L raceways (μBio Eng)

Algal Biomass Yield: Cellana LLC

- 1000L Ponds
- Large tubular PBRs
- 0.1 acre algal raceways
- Batch cultivation
  - Native HI strain
- Interested in probiotic strains
  - Potential for collaboration

Acknowledgments

DOE EERE BioEnergy Technology Office

Sandia National Labs

- **Eizadora Yu**
- Ryan Davis
- John Hewson
- Pamela Lane
- Nicholas Wyatt
- **Haifeng Geng**
- **Mary Tran-Gyamfi**

AgriLIFE RESEARCH
Texas A&M System

ATP3
Algae Testbed
Public-Private Partnership

OpenAlgae

cellana
algae-based products for a sustainable future

CAL POLY
SAN LUIS OBISPO

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Increasing sustainability by closing the nutrient recycle