DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Optimizing **S**election **Pre**ssures & Pest Management to Maximize Algal Biomass **Y**ield (OSPREY)

March 11, 2020
Advanced Algal Systems

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Project Overview – Impetus

- Algal industries are challenged by poor translatability of lab-scale R&D to larger systems
- We propose that the disconnect stems from:
  - a lab-centric approach (use of lab-cultivars, lab to field pipeline)
  - different selection pressures across environments

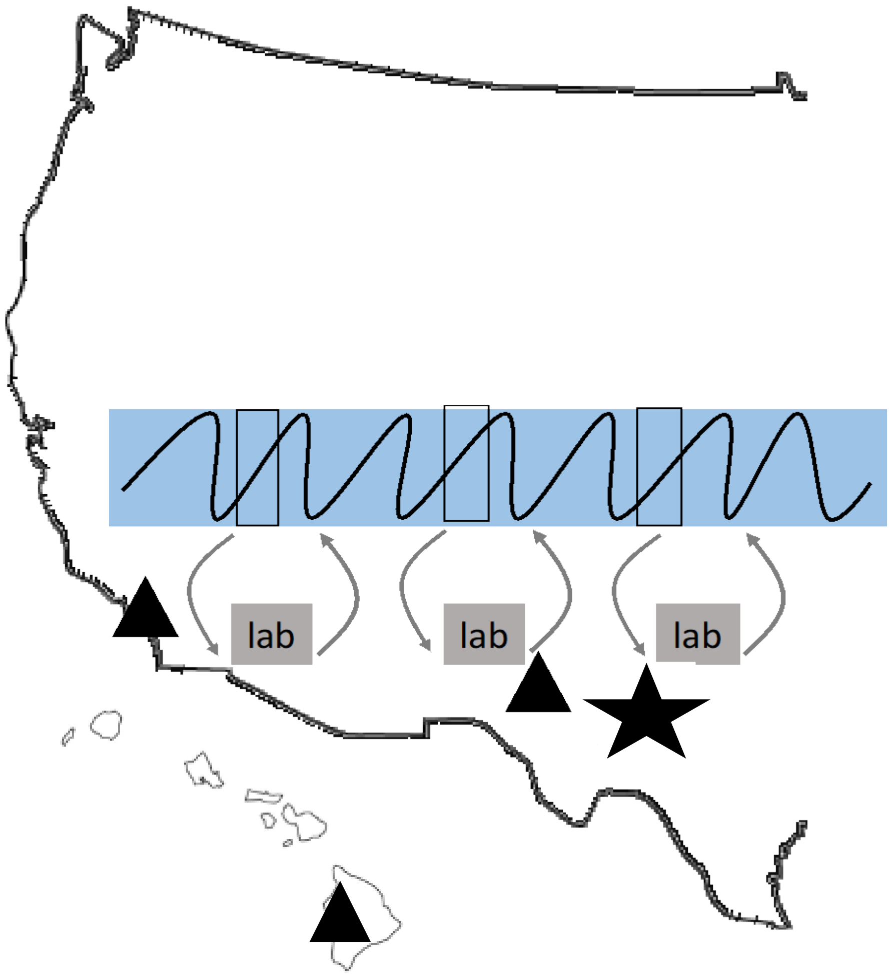
	Lab	**Field**
Positive pressures (increases desired traits)	NONE	Variable temperature, pests, commercial grade medium, natural sunlight
Negative Pressures (decrease desired traits)	Ideal temperature, lack of pests, permissive growth medium, poor light quality, low light	Frequent harvest, chemical treatment

Project Overview – Goals

We aim to: use a field-adapted industry strain and field-lab-field iterations across unique field sites to:

1. quantify and balance selection pressures across the lab and field
2. optimize processes including pest management

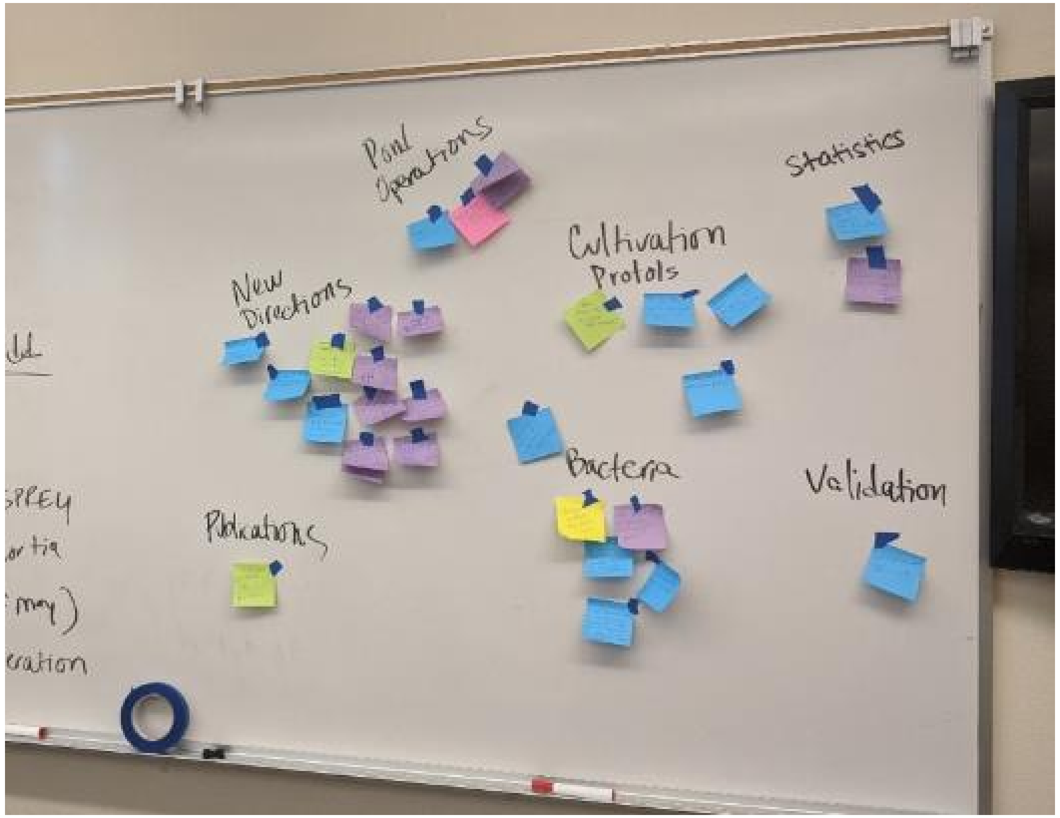
FIELD CULTIVATION	➢ quantify trait drift ➢ isolate field cultivars ➢ identify key pests
PROCESS IMPROVEMENTS	➢ optimize strain selection, maintenance, cultivation ➢ develop pest tracking tools ➢ improve strains via directed evolution & selection



We start in the field and will end in the field.

1. Management – Structure and Processes

- SOPO, Gantt Chart, Milestones, GNGs, KPPs serve as guidelines
- Frequent communication across team
  - initial PI kickoff
  - task workgroups
  - twice-monthly PI calls
  - twice-monthly team calls to share data, evaluate progress on tasks, discuss mitigation strategies
- Project monitoring (calls with Project Monitor every ~6 weeks, quarterly reports, interim verification)
- Risk control (risk mitigation matrix) and change control processes



1. Management – Roles and Responsibilities

- PI Alina Corcoran (NMC) and co-PI Shawn Starkenburg (LANL) responsible for project oversight, coordination, reporting

- Stakeholders/Industrial Partners
  - Jakob Nalley (Qualitas Health) – cultivation, testing kits and practices (Tasks 2, 4, 6)
  - Stephen Eacker (Phase Genomics) – metagenomics, interactome modeling (Tasks 4, 6)
  - Charley O'Kelley (Cyanotech Corp.) – cultivation, testing kits and practices (Tasks 2, 4, 6)

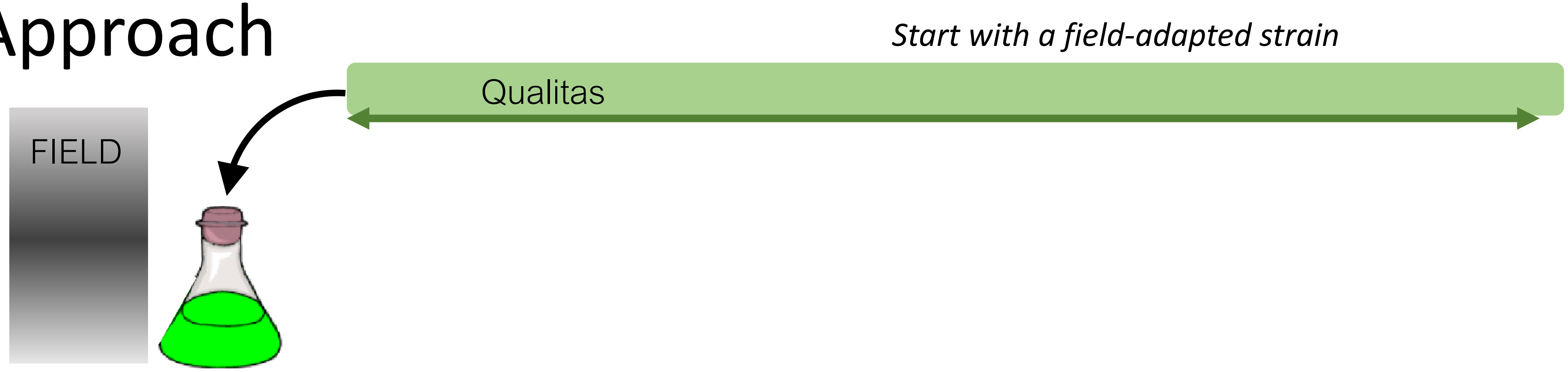
- National Laboratory Partners
  - Blake Hovde (LANL) – pest characterization, primers, field-deployable kits (Tasks 4, 6)

- Academic Partners
  - Omar Holguin (NMSU) – mutagenesis, carbon partitioning (Tasks 2, 5, 6)
  - Jonathan Shurin (UCSD) – cultivation, directed evolution (Tasks 2, 5)
  - Jason Quinn (CSU) – sustainability modeling (Task 6)

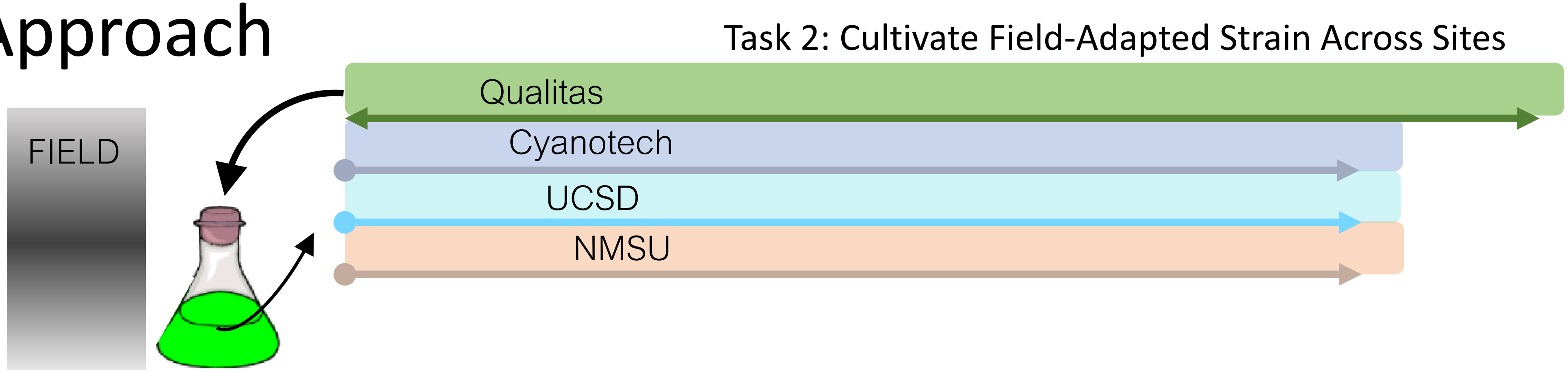
1. Management – Risks

Description	Risk	Impact	Risk Mitigation and Response Strategies
Technical Risks			
Baseline strain does not establish	Low	Medium	Co-culture multiple strains, letting the environment select for robust strain(s).
Desired traits are not genetically stable	Low	Medium	Maintain selection pressures at all times.
Evolution or mutagenesis does not change traits	Medium	Medium	Co-culture strains with broad genetic background. Conduct gene editing using omics studies to inform gene targets.
Enhanced traits derive from the microbiome	Low	Low	Re-introduce/seed microbiome with algae during scale up. Use HISCI system to identify isolate key members.
Resource Risks			
Equipment downtime	Medium	Medium	Given shared equipment across sites, temporarily reassign tasks.
Management Risks			
Personnel turnover	Low	Medium	Personnel are available who could assume the responsibilities of departed personnel. Cross-training.
Cut in funding	Low	High	Eliminate certain tasks and/or sub-tasks.

2. Approach

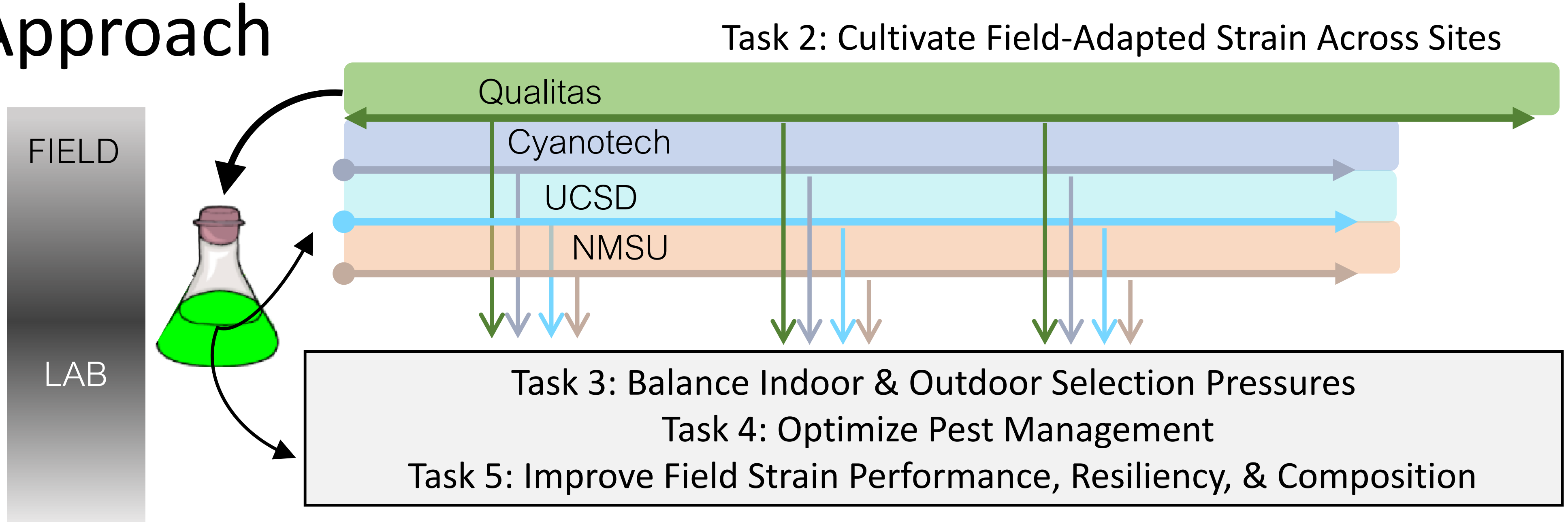


2. Approach

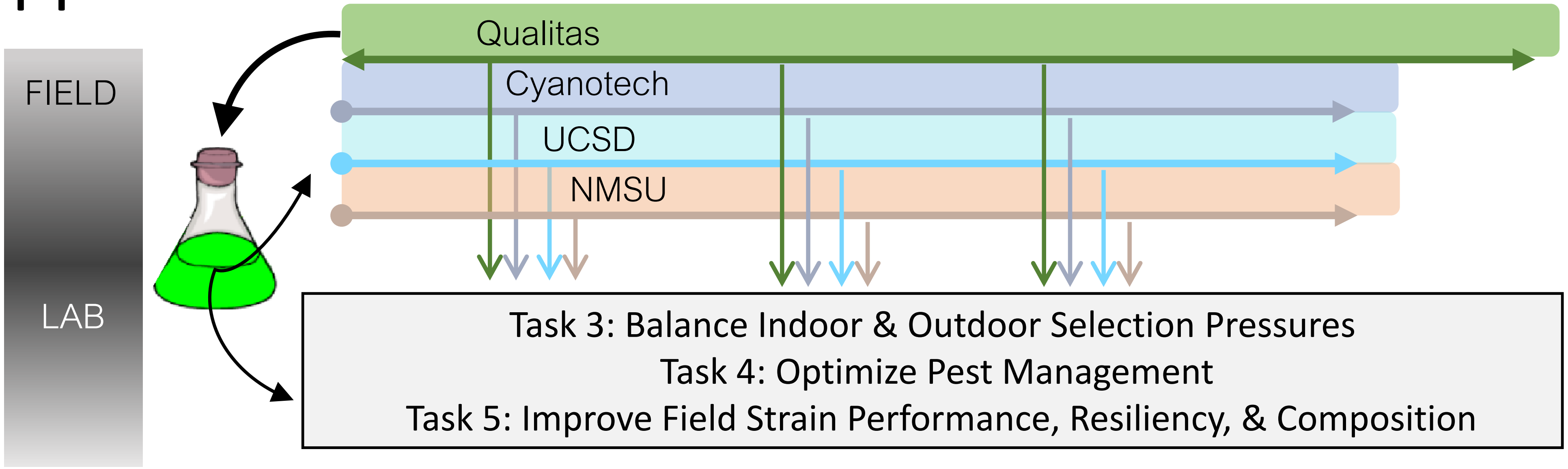


Task 2: Cultivate Field-Adapted Strain Across Sites
FIELD
Qualitas
Cyanotech
UCSD
NMSU

2. Approach

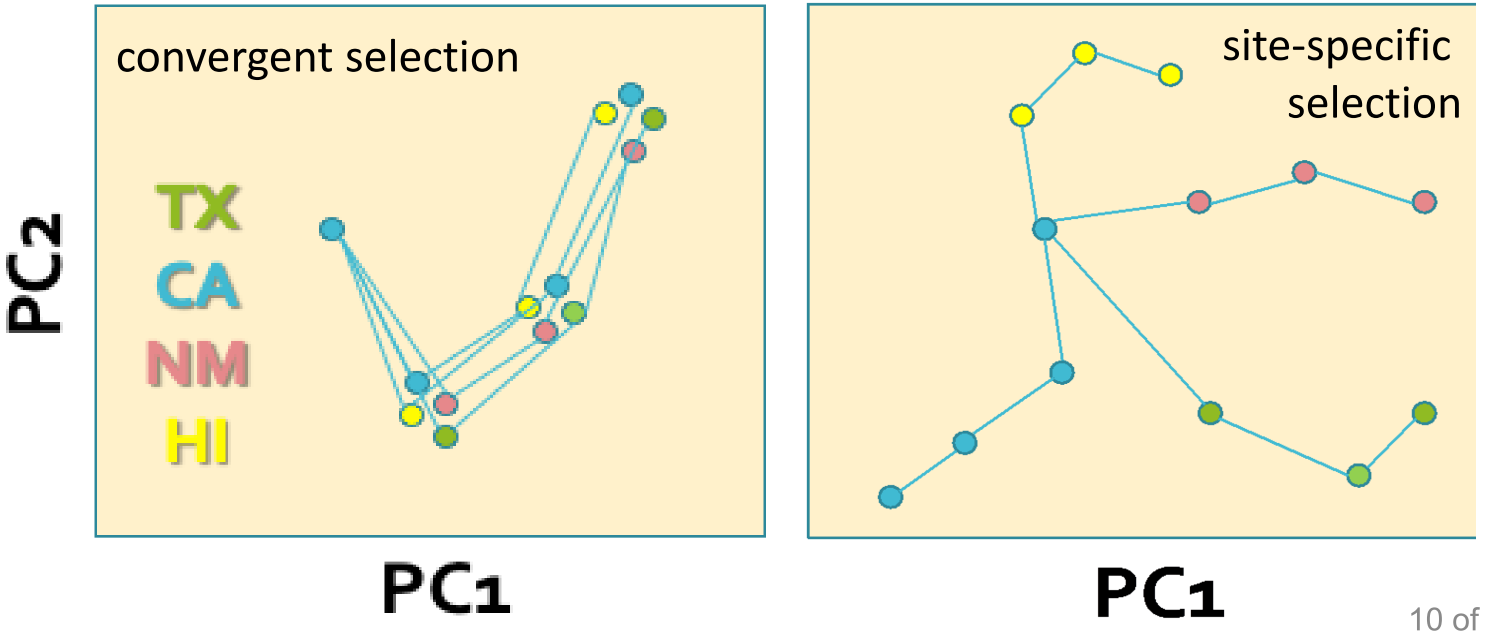

Task 2: Cultivate Field-Adapted Strain Across Sites
FIELD
LAB
Qualitas
Cyanotech
UCSD
NMSU
Task 3: Balance Indoor & Outdoor Selection Pressures
Task 4: Optimize Pest Management
Task 5: Improve Field Strain Performance, Resiliency, & Composition

2. Approach

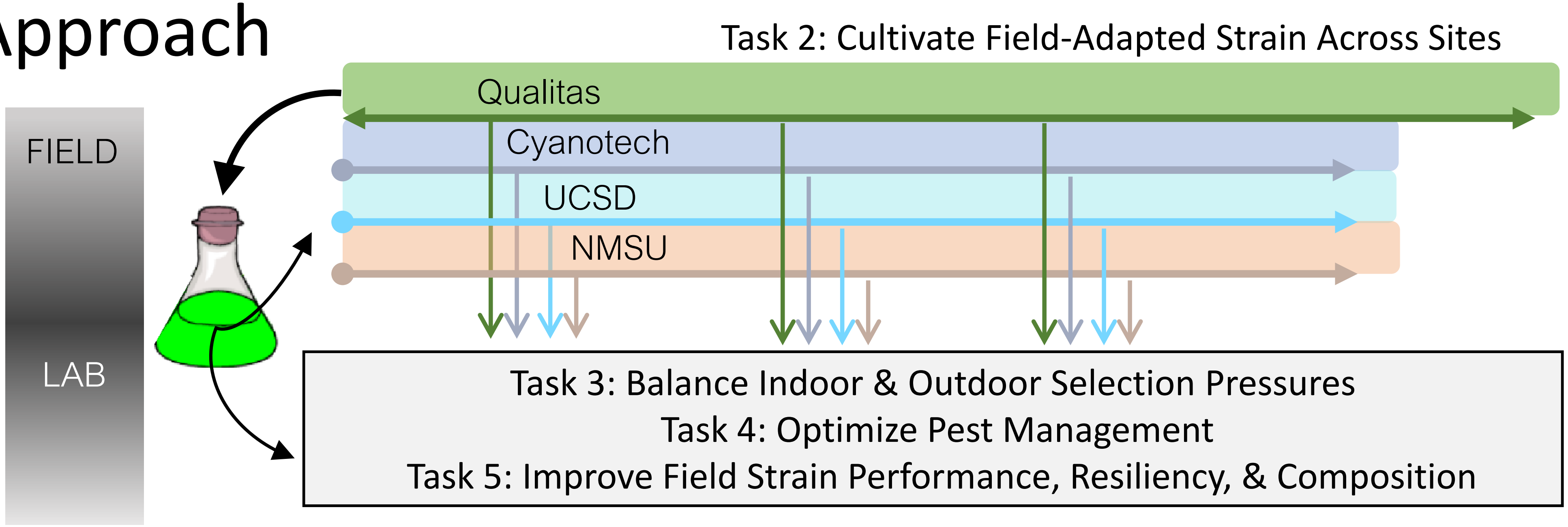


Task 3: Characterize the genotypes and phenotypes of the lab (3 cultivation modes, 3 labs) and field (4 sites) cultivars to determine trait loss, conservation, or enhancement.

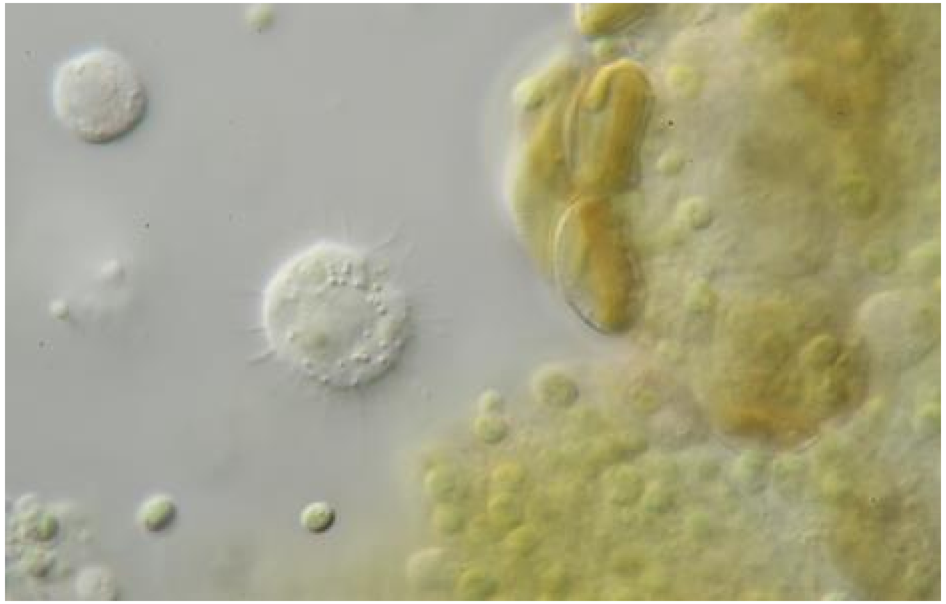
**technical challenge: target traits do not shift or shifts are only the result of microbiome changes.*



2. Approach

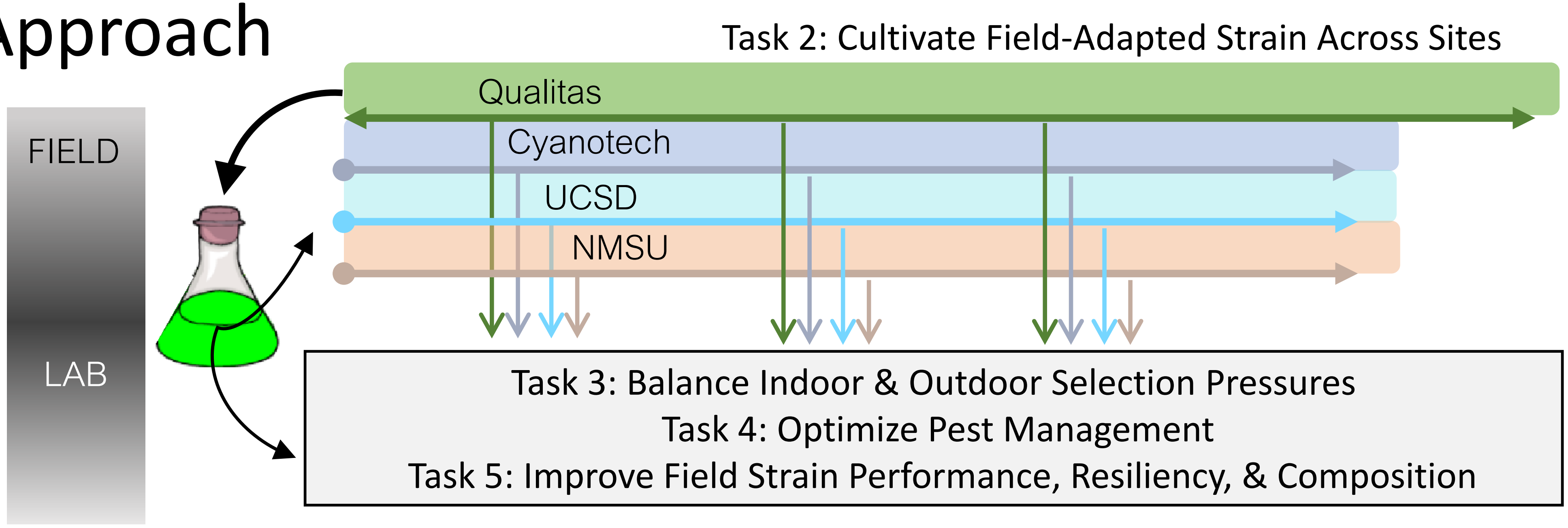


Task 4: Generate high quality metagenomes of outdoor cultivation systems, determine causative agents for shifts in pond productivity, and develop and deploy fieldable kits to monitor pests.



**technical challenge: pest pressure across the field sites is low and there are few crashes, limiting metagenomic data.*

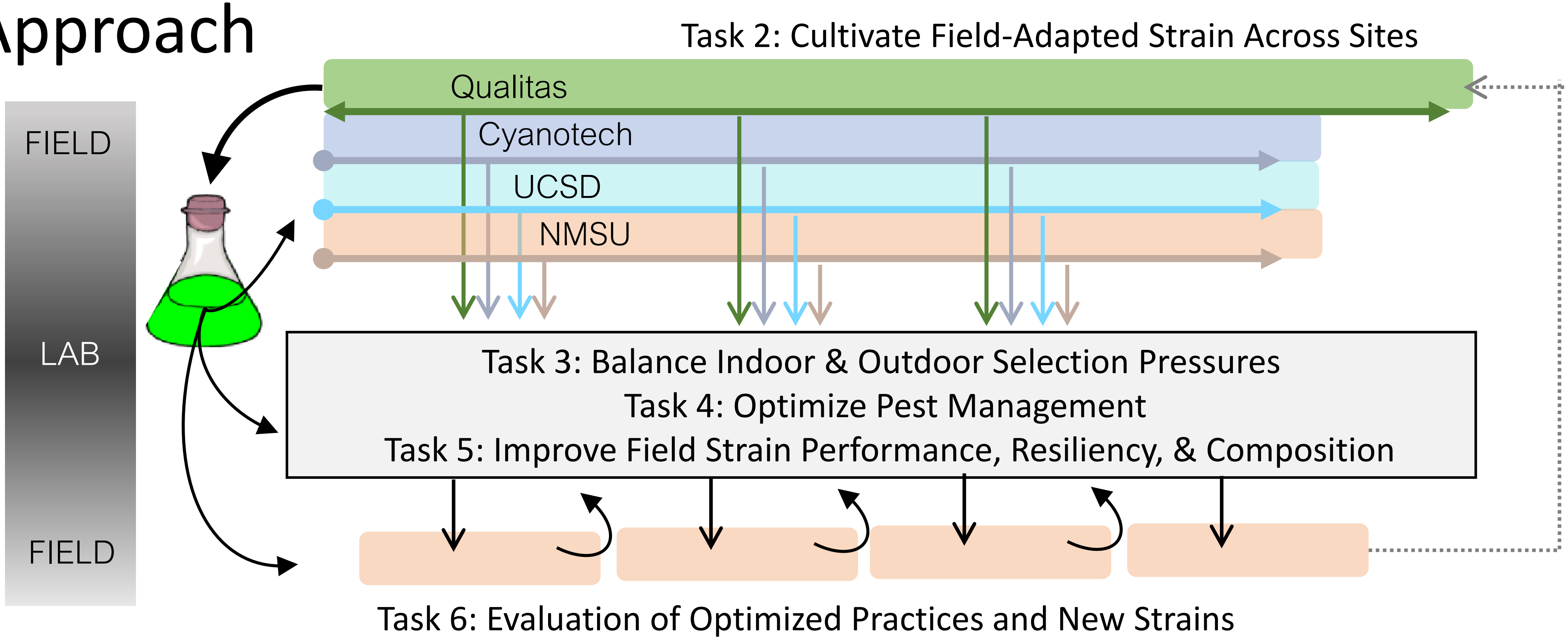
2. Approach

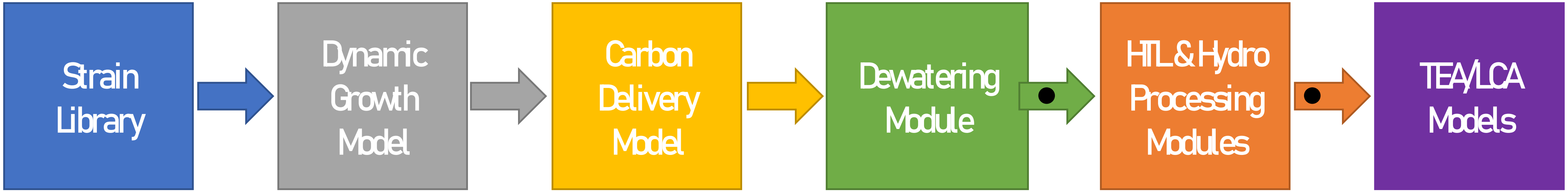


Task 5: Apply a combination of mutagenesis and directed evolution to a field-adapted strain improve its productivity, stability, and biomass composition.

**technical challenge:
evolution and selection do not change traits of interest, or change traits unfavorably.*

2. Approach





Task 6: Test process innovations and new strains in the field and utilize TEA, LCA, and dynamic growth modeling to evaluate the effects on the algal biofuel and bioproduct value chain.

2. Approach – Go/No-Go Points and Evaluation Metrics

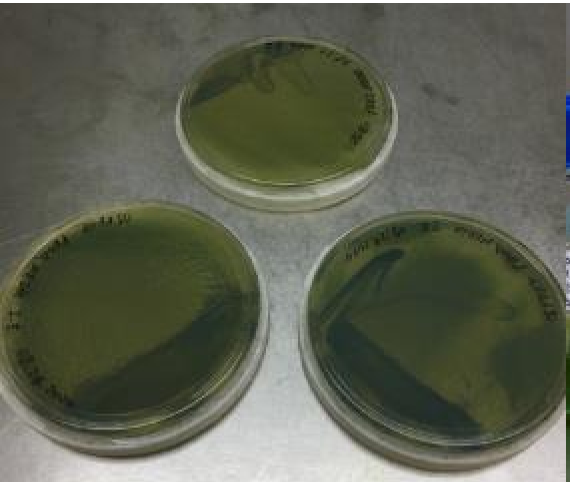
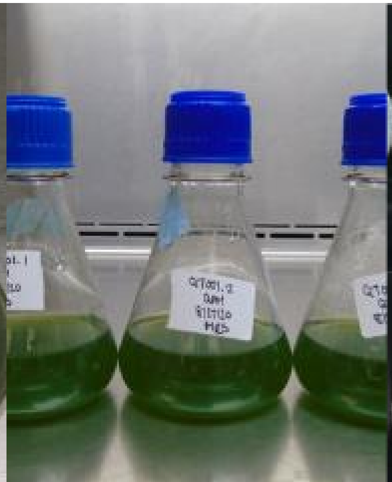
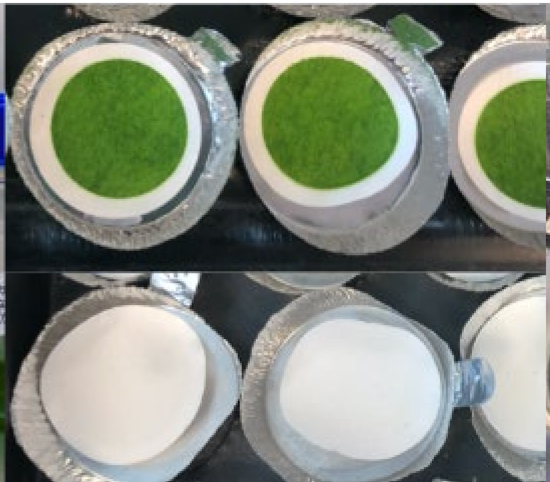
- **GNG 1: Technical & Cultivation Readiness Validated**
  - Met at end of BP1 (Validation, M1-M3)
  - Necessary to demonstrate that the team has the resources to do the project
- **GNG 2: Comparison of one mutant vs baseline strain and deployment of the beta version of the qPCR tool at the scale of 260L ponds in triplicate at one of the field sites.**
  - Will be met at end of BP2 (Characterization and Improvement, M4-21)
  - Important to show potential for our strain and process improvements to be advanced for further field testing
- **GNG 3: Three lab-to-field iterations that incorporate at least two of the following: trait stack, qPCR tool deployment, and process improvements based on trait drift in the lab and field to reach improvements in productivity, robustness and biomass composition compared to the baseline *Nannochloropsis* strain**
  - Will be met at end of BP3 (Development and Testing of New Practices and Strains, M22-39)
  - Important because it relies on all tasks and requires field-lab-field iterations
- Task-specific performance metrics: productivity, stability, biomass composition, lipid composition, genetic evolution, fuel yield, CUE, energy per unit C, CO_2 costs

3. Impact

- Two industrial partners engaged on the project team
  - Director of Applied Research at Cyanotech Corporation
  - Director of Agronomy at Qualitas Health, Inc.
- Outputs
  - Process improvements in strain improvement, maintenance, cultivation
  - Metagenomic database of pests across diverse sites
  - Fieldable pest-tracking kits
  - 50% improvement in harvest & robustness, 20% improvement in conversion yield

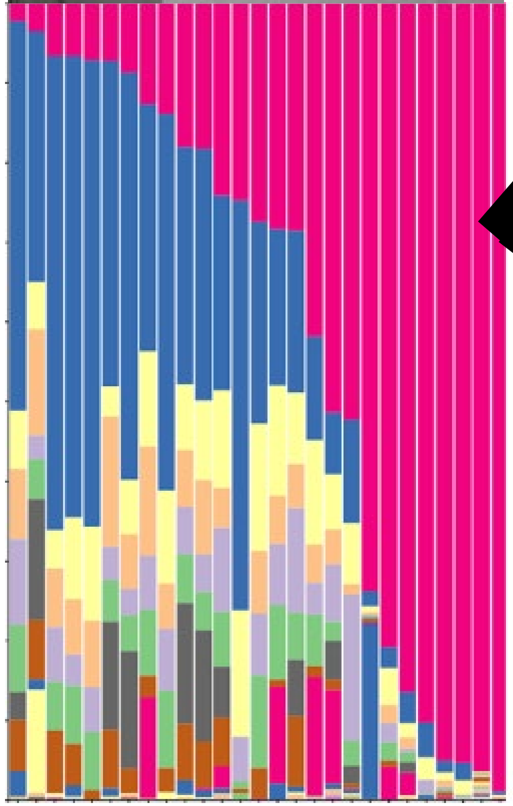
4. Progress & Outcomes

- Project currently in BP2, period focused on Strain Characterization and Improvement
- Major outcomes:
  1. Isolation of the field-adapted strain from Qualitas Heath ponds and establishment across the labs and additional field sites
  2. Initial trait characterization of lab and field cultivars
  3. Metagenome characterization across sites
  4. Application of selection and mutagenesis/selection to develop cold and hot tolerance

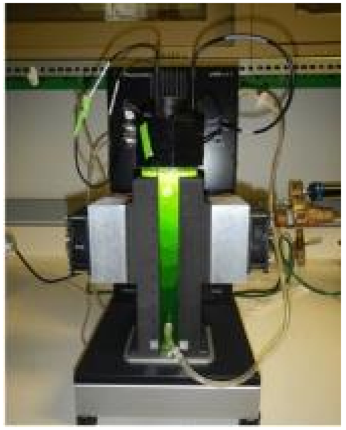





4. Progress & Outcomes: Strain Establishment (Task 2)

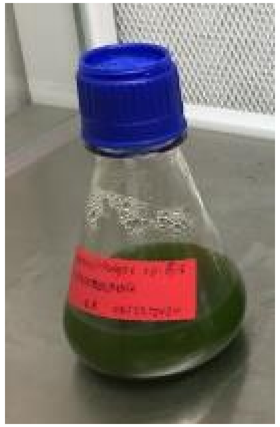
- Strain collected from Qualitas
- 24 isolates captured via flow cytometry
- Strain ID confirmed
- Isolate with fewest associated bacteria chosen for propagation
- Established in the lab and field across sites



selected strain, pink shows *Nannochloropsis*



LANL

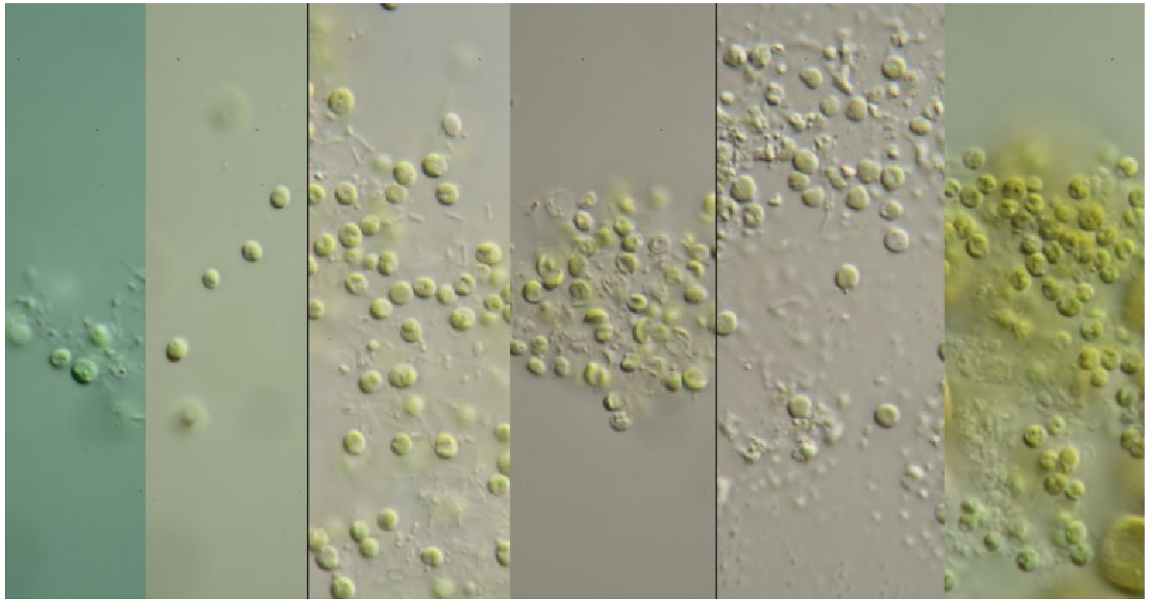


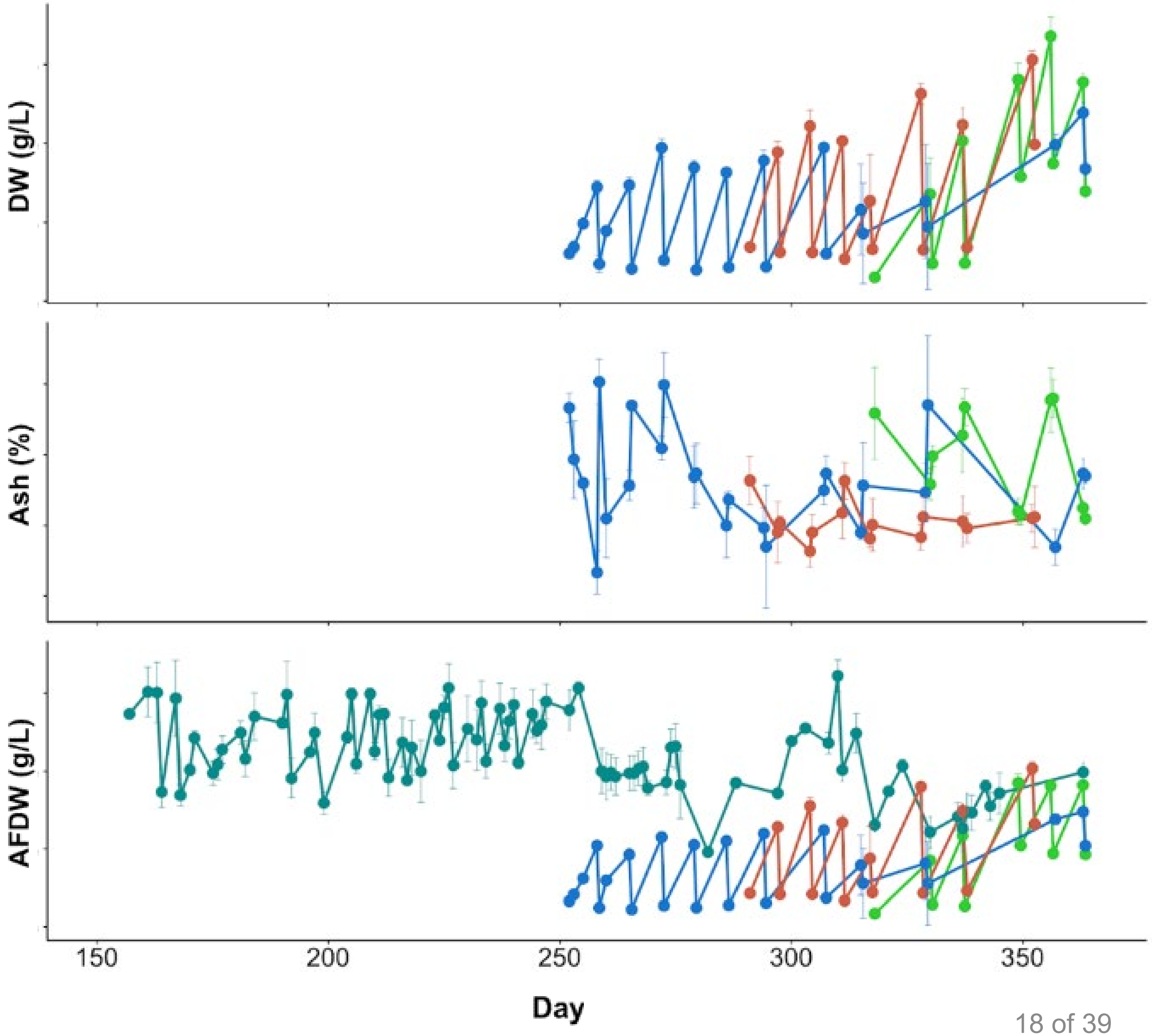
LANL, UCSD, NMSU

Cyanotech, UCSD, NMSU

4. Progress & Outcomes: Strain Establishment (Task 2)

- Three ponds established per site
- Processes
- Data collected analyzed
- Pest pond

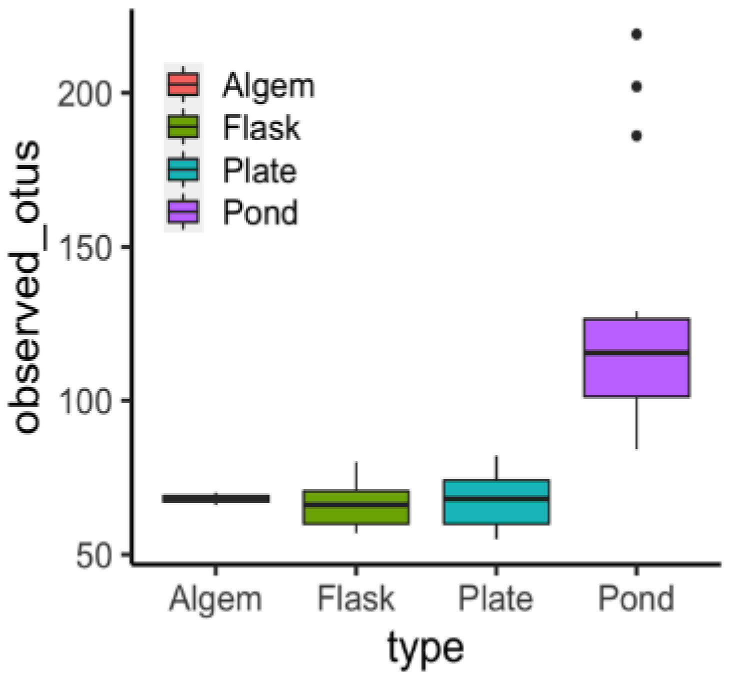




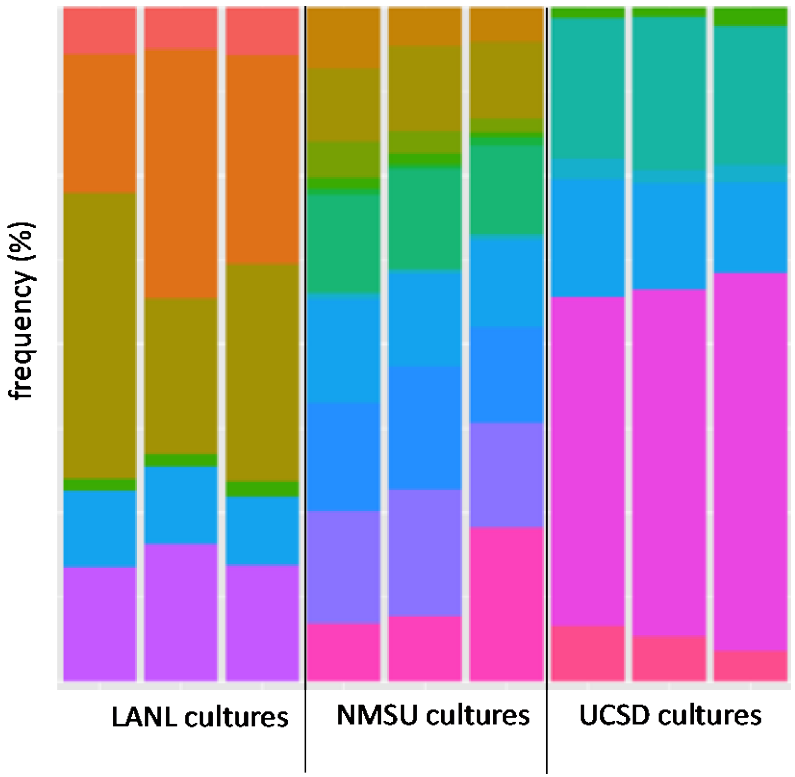
4. Progress & Outcomes: Trait Characterization (Task 3)

- Compared genotypic (18S, 16S, whole genome) and phenotypic (growth rate, biomass composition) traits of lab and field strains after 3 to 6 months in culture in a common lab environment ("common garden")
  - Batch culture (3 labs)
  - Plate storage (3 labs)
  - Semi-continuous culture in environmental photobioreactors (1 lab)
  - Field culture (4 sites) with the original microbiome and a reduced microbiome (chemically treated)

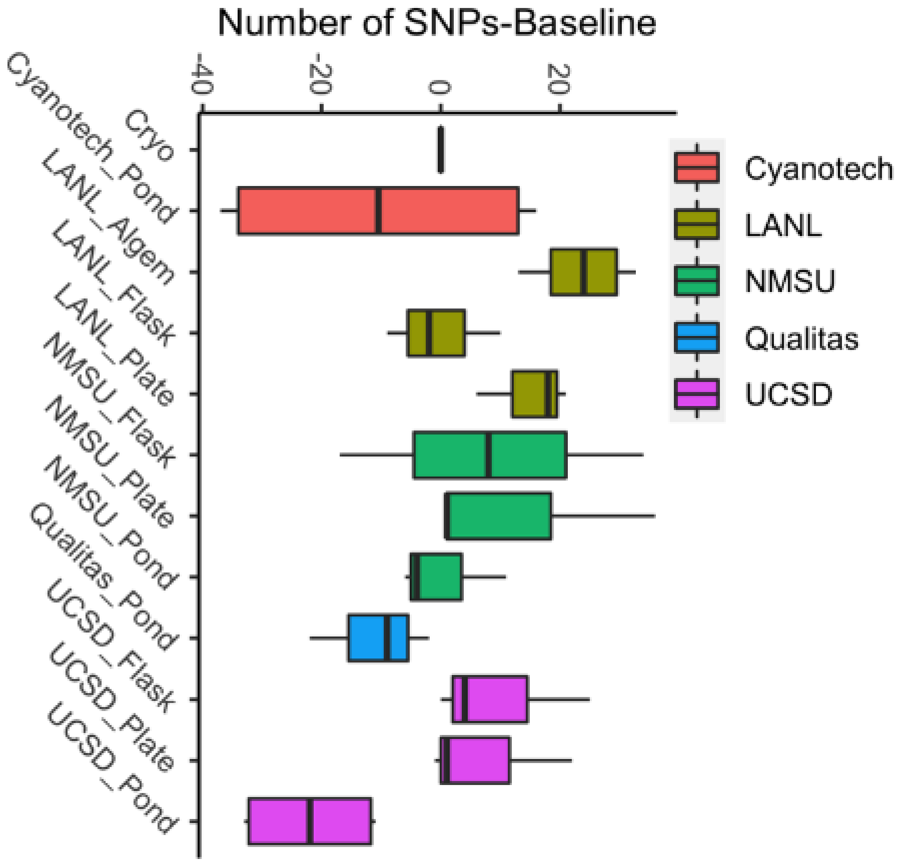
4. Progress & Outcomes: Trait Characterization (Task 3)



- Greatest diversity in pond samples
- Diversity similar across sites except Qualitas (using parent culture without initial isolation)

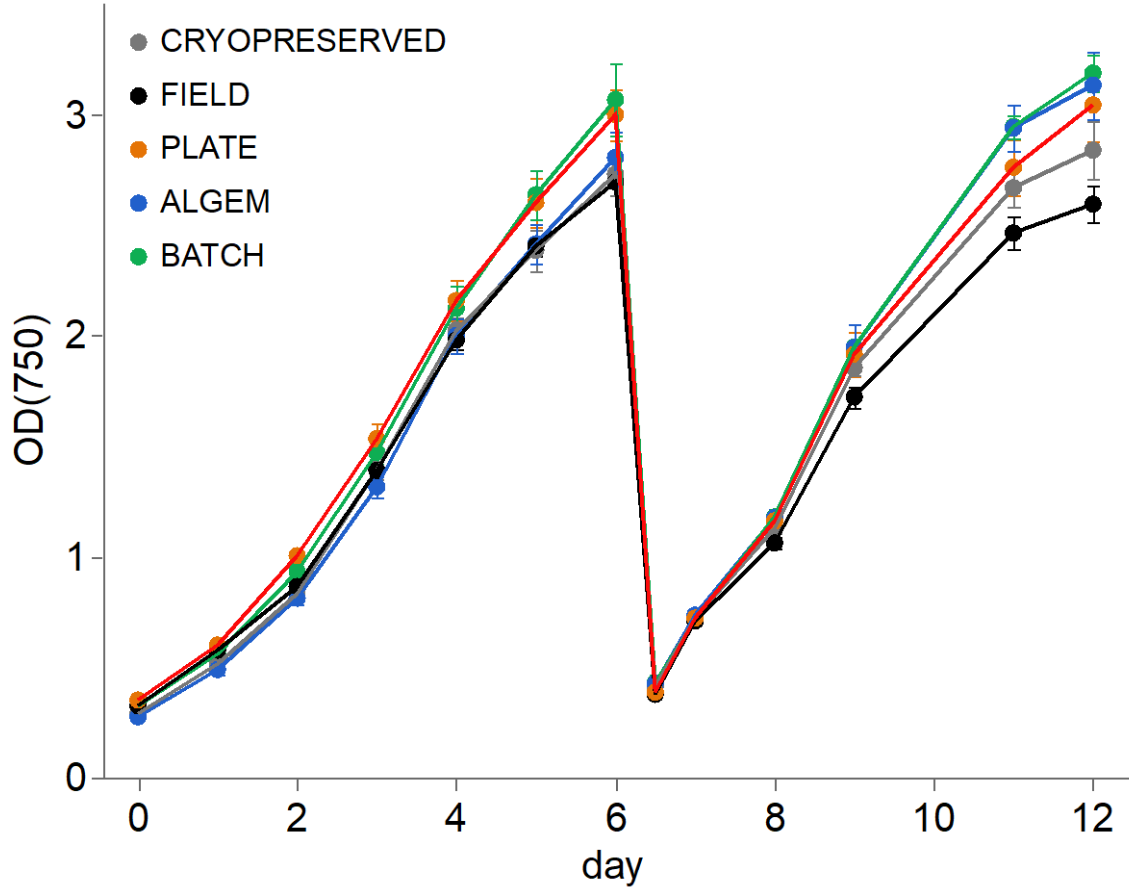


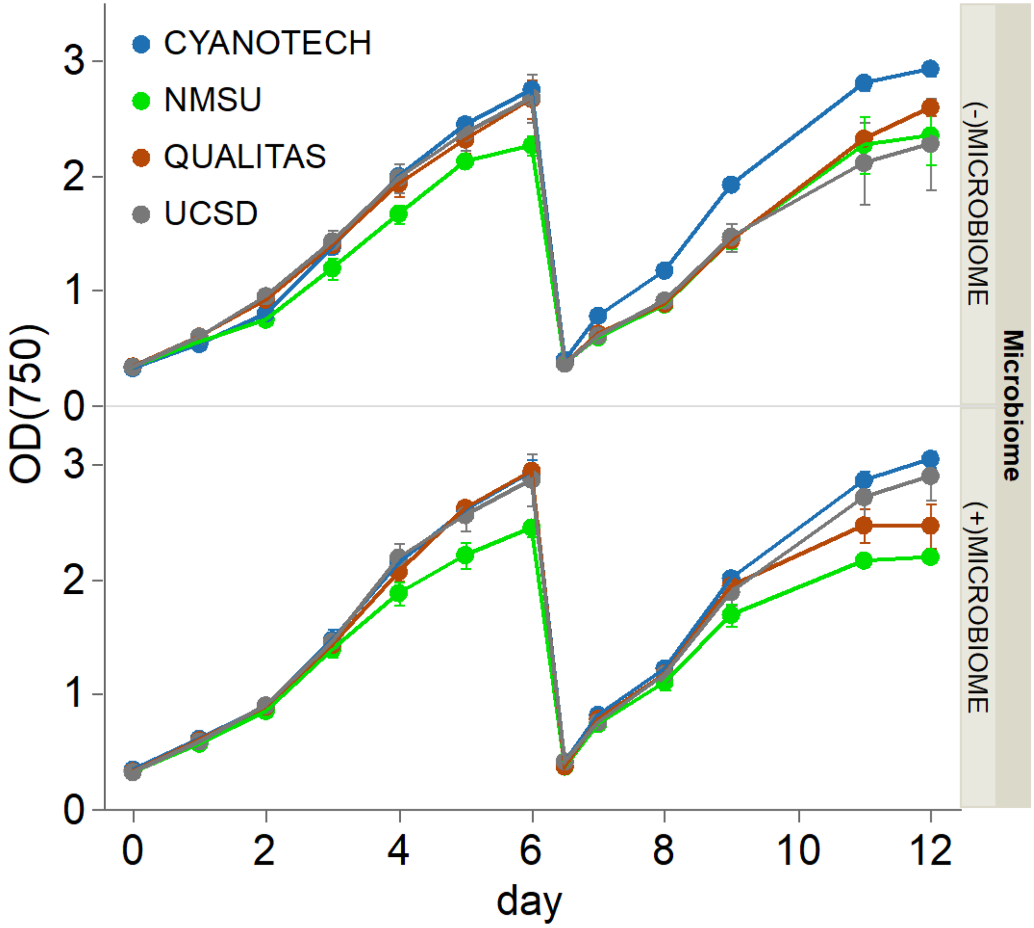
- Variation in bacterial community composition across labs, but not across replicates within a lab



- SNP level changes consistent with expectations
- Differences in variability across sites

4. Progress & Outcomes: Trait Characterization (Task 3)



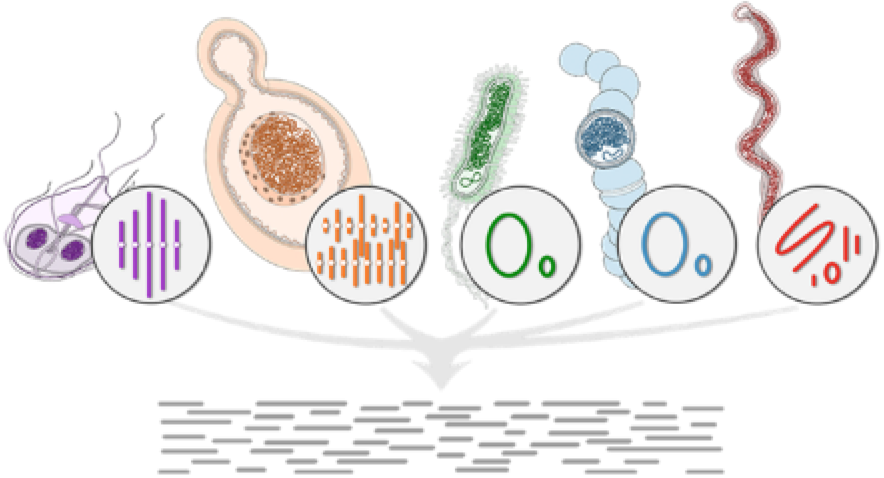


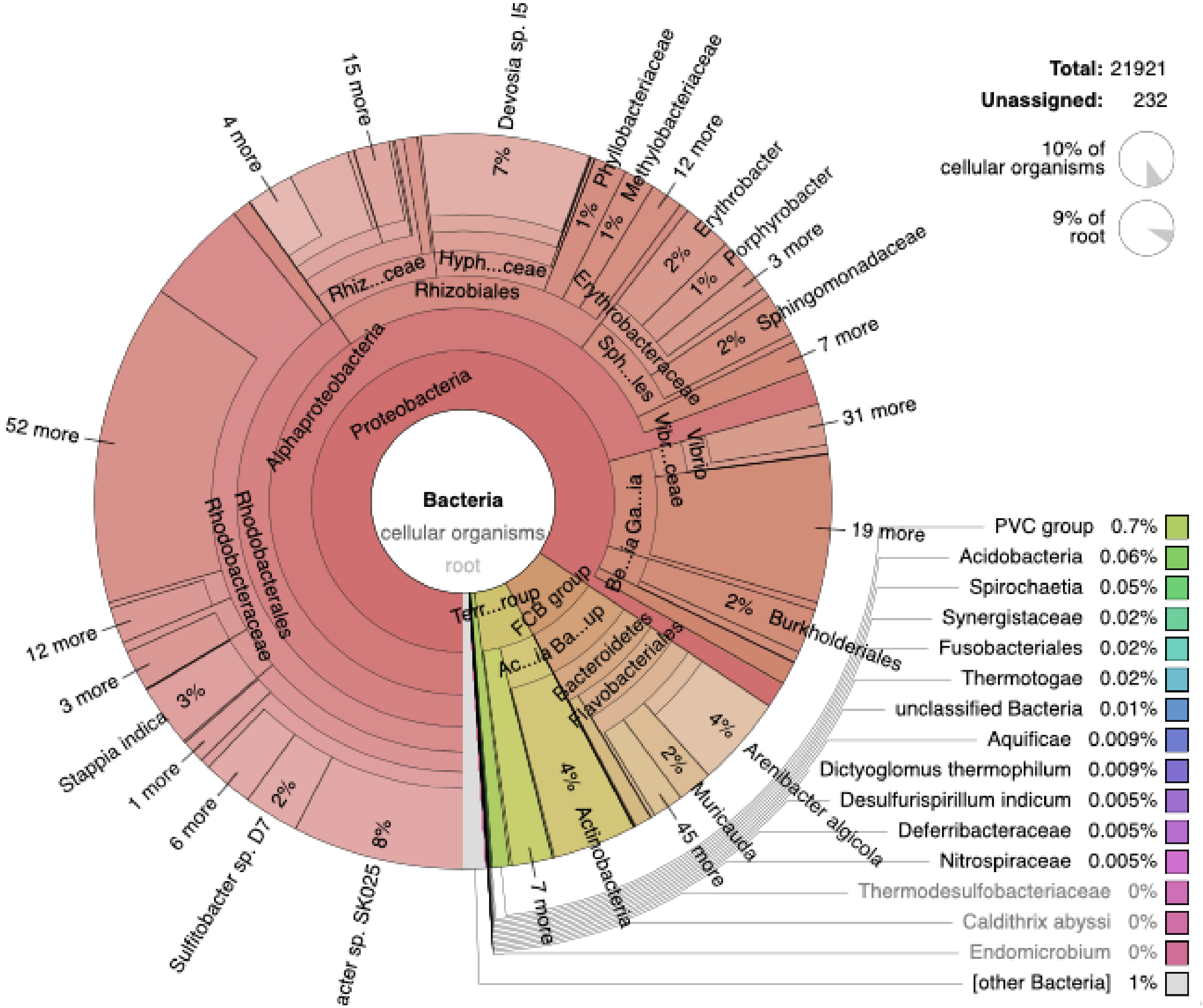
- Similar phenotypes in common garden
- Field cultures underperformed lab cultures & cryopreserved strain
- Some variability across cultivation modes

- In field cultures, 'Site x Microbiome' interactions, with different directionalities of microbiome effect

4. Progress & Outcomes – Pest Management (Task 4)

- Validated sample collection methods across sites, accounting for limitations at remote field sites (e.g., no Liquid N)
- Applied Proximeta approach to generate libraries for 18 metagenomes, to date

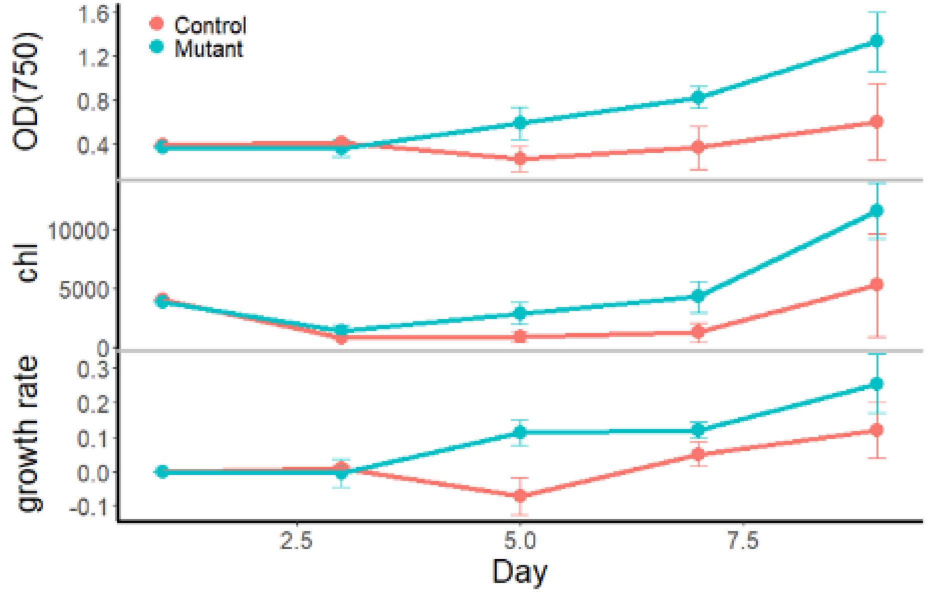


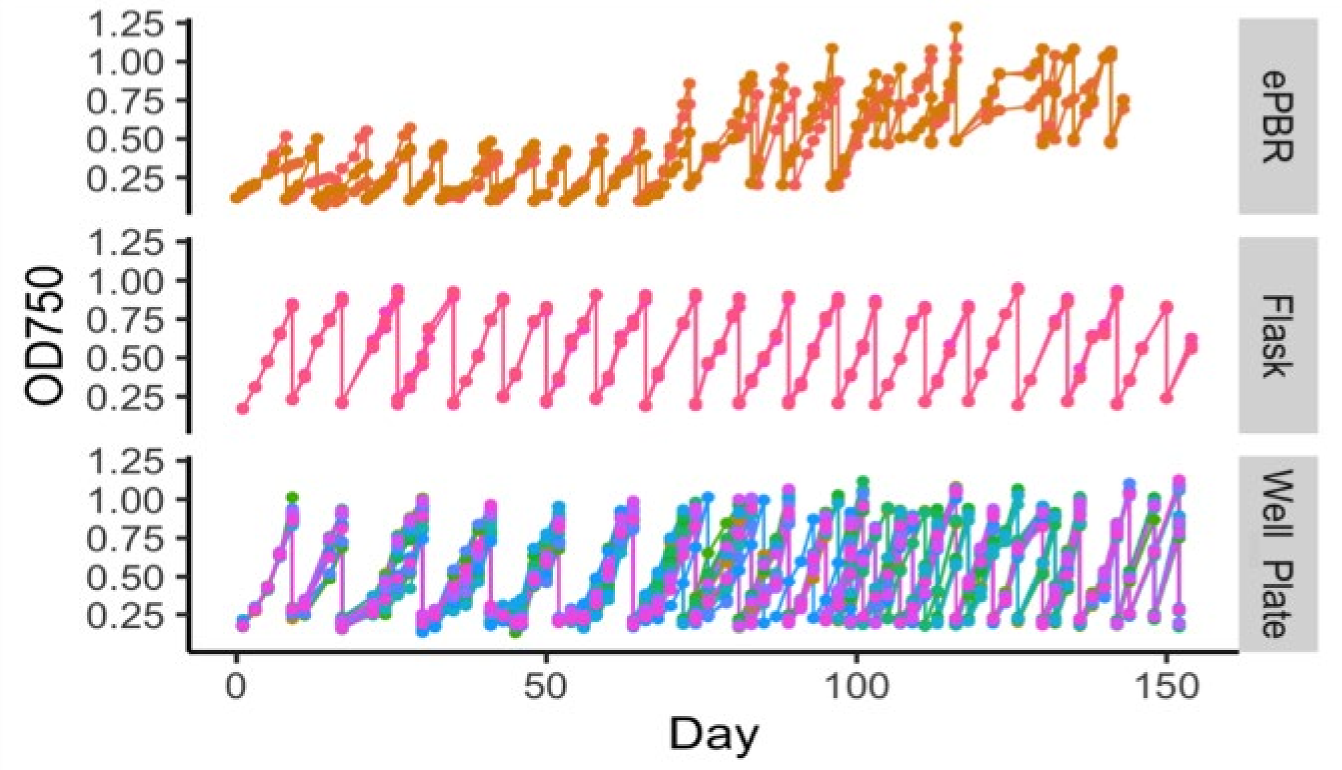


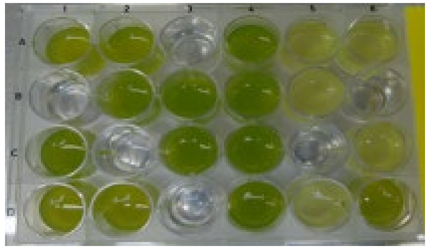
4. Progress & Outcomes – Strain Improvement (Task 5)

From a field adapted strain:

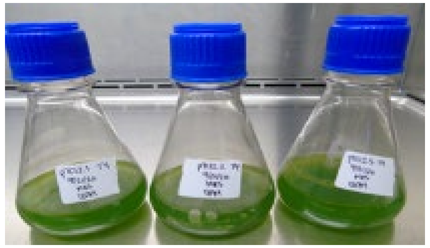
- Imposed cold selection (10-16°C) to a field-adapted strain for ~5 months (50 generations) in plates, flasks and environmental bioreactors
- Generated a mutant library (UV) and applied high temperatures to build a summer strain



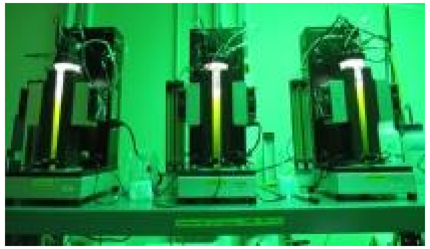




18 - 2mL wells



3 – 100 mL flasks



3 – 500 mL ePBRs

4. Progress & Outcomes – Milestones

Milestone		Status
Task 2	**Establishment and Maintenance of a Field-Adapted *Nannochloropsis* Strain Across New Field Sites**	
Milestone 2.1	Isolated and taxonomically verified field-adapted *Nannochloropsis* strain shipped to Las Cruces, NM; San Diego, CA; and Kailua Kona, HI for scale up	complete
Milestone 2.2	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	complete
Task 3	**Characterization of Trait Drift in the Field and Laboratory**	
Milestone	Whole genome shotgun, 18s, maximum specific growth rate, robustness and composition data will be compiled for at least 6 lab- and field-maintained isolates following 6 months in culture	on schedule
Task 4	**Optimization of Pest Management**	
Milestone 4.1	Assembly of >20 metagenomes from ponds located at a minimum of two field sites exhibiting low- and high productivity or health to identify putative pests or microbiome shifts	on schedule
Task 5	**Improvement of Field Strain Performance and Composition**	
Milestone 5.1	A library of *Nannochloropsis* strains is generated by mutagenesis and/or selection along gradients of CO2, temperature, and light environmental stressors	on schedule
Milestone 5.2	At least one *Nannochloropsis* strain is generated by mutagenesis and/or a 3- month selection from a cold temperature gradient demonstrating cold tolerance compared to the baseline strain	on schedule

Summary

- Project is unique because it starts in the field with a field-adapted strain, focuses on field—lab—field iterations, and builds process improvements by balancing selection pressures and optimizing pest management
- Project milestones met to date and upcoming milestones on schedule
- Strong project management (and project team!)

Timeline:

- Project Start: 1/1/2019
- Project end date: 12/30/2023

	FY20 Costed	Total Award
DOE Funding	$505,876.66	$4,999,47
Cost Share	$215,171	$1,290,354

Project Partners

- Los Alamos National Laboratory (Los Alamos, NM)
- Cyanotech Corporation (Kailua-Kona, HI)
- Phase Genomics, Inc. (Seattle, WA)
- Qualitas Health, Inc. (Imperial, TX)
- Colorado State University (Fort Collins, CO)
- New Mexico State University (Las Cruces, NM)
- University of California San Diego (San Diego, CA)

Project Goal: to generate process innovations rooted in established outdoor systems for strain selection, improvement, maintenance, cultivation, and pest detection and tracking –resulting in 50% improvement in harvest yield 50% improvement in robustness, and 20% improvement in conversion yield.

End of Project Milestone: Statistically significant improvements in productivity, robustness and biomass composition to reach targets of 50%, 50% and 20%, respectively, via changes in cultivation practices and the use of field cultivars and improved strains demonstrated during field cultivation campaigns at the scale of at least 200L.

FY19 BETO Multi-Topic FOA
AOI 1: Cultivation Intensification Processes for Algae (CIPA)

Additional Slides

Project-Specific KPPs

- **Task 2**. Establishment and Maintenance of a Field-Adapted *Nannochloropsis* Strain Across New Field Sites

KPP	Values	Units	Duration Scale
Productivity	B: 9.1 I: 9.1 F: 9.1	AFDW $g/m^2/day$	30 Days 100L ponds

B: Baseline, I: Intermediate, F: Final

Needed to establish & verify productivity at the new full time field sites (HI, CA, NM).

Project-Specific KPPs

- **Task 3**. Characterization of Trait Drift in the Field and Laboratory

KPP	Values	Units	Duration Scale
Productivity	B/I/F: 9.1	AFDW g/L/day	30 Days >100L ponds
Stability (pests, temperature, light)	B/I/F: TBD	% Loss/Gain of AFDW g/L/day	10 Days Lab/Flask
Composition	B/I/F: Protein:30-50% Lipid :10-15% Total Carbs: 30%	wt.%	B/I; 10 Days Lab/Flask F: 14 Days >100L
Genetic Evolution	B/I/F: DNA Sequence	SNPs/kbp	N/A Entire genome

Project-Specific KPPs

- **Task 4**. Optimization of Pest Management

KPP	Values	Units	Duration Scale
Stability (pests)	B: TBD I:25% increase F: 50% increase	% Recovery of AFDW g/m^2/day	B/I; 10 Days Lab/Flask F: 14 Days >100L

Project-Specific KPPs

- **Task 5**. Improvement of Field Strain Performance and Composition

KPP	Values	Units	Duration Scale
Productivity	B:9.1 I: 11 F: 13.7	AFDW g/L/day or $g/m^2/day$	B: 7 days / ePBR I: 7 days / 100L F: 14 days/ 100L
Lipid Composition	B:18* I: 21 F: 25	wt.%	B: 7 days / ePBR I: 7 days / 100L F: 14 days/ 100L

*estimated improvements based on nutrient replete conditions for *N. oceanica* as reported in Ji et al. Algal Research 7 (2015) 66–77

FOA-required KPPs

Biomass & Cultivation

KPP	Values	Units	Duration Scale
Productivity	B:9.25 I:11 F:13.7	AFDW g/m²/day	30 days >100L ponds
Biomass Composition Protein:30-50%	B: Lipid :10-15% Total Carbs: 30% I: Lipid :15% Total Carbs: 25% F: Lipid :20% Total Carbs: 20%	wt.%	30 days >100L ponds
Media Composition*	Nitrogen source: Urea Ammonium Nitrate-32 [N]:150 [P]: 40 pH: 8.0	mg/L	NA NA

*Other nutrients/trace elements:0.5 mg/L Fe, remaining from source water

FOA-required KPPs

TEA & LCA

KPP	Values	Units	Duration Scale
Fuel Yield	B: 62 I: 72 F: 86	gasoline-gallon equivalency (GGE)	NA
CUE	B/I/F: 5.933	g CO_2/g AFDW Algae Biomass	4 weeks >100L Ponds
Energy per unit C	B/I/F: Natural gas flue gas 927.3 Coal flue gas 274.7 Pure CO_2 37.1	kJ/g of CO_2 into cultivation system	Scale: nth plant
CO_2 Cost	B/I/F: Natural gas flue gas $0.13509 Coal flue gas $0.04623 Pure CO2 $0.00204	$/g of CO2 into cultivation system	Scale: nth plant

Task-specific GANTT charts follow

Type		Activity	Strt	Dur
Task	2.0	Establishment and Maintenance of a Field-Adapted *Nannochloropsis* Strain Across New Field Sites	4	36
Subtask	2.1	Isolate the Nannochloropsis strain and establish it at each of the three field sites	4	5
Milestone	2.1.	Isolated and taxonomically verified field-adapted Nannochloropsis strain shipped to Las Cruces, NM; San Diego, CA; and Kailua Kona, HI for scale up	6	1
Subtask	2.2.	Maintain and sample outdoor cultivation systems in Imperial, TX; Las Cruces, NM; San Diego, CA; and Kailua Kona, HI	7	33
Subtask	2.3.	Compile and provide biomass, productivity, and environmental data from continuously cultured outdoor strains at each site	7	33
Milestone	2.2	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	9	1
Milestone	2.3	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	18	1

BP 2 (18 mo)																		BP 3 (18 mo)																	
Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23
4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39

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Task	2.0	Establishment and Maintenance of a Field-Adapted *Nannochloropsis* Strain Across New Field Sites	4	36
Subtask	2.1	Isolate the Nannochloropsis strain and establish it at each of the three field sites	4	5
Milestone	2.1.	Isolated and taxonomically verified field-adapted Nannochloropsis strain shipped to Las Cruces, NM; San Diego, CA; and Kailua Kona, HI for scale up	6	1
Subtask	2.2.	Maintain and sample outdoor cultivation systems in Imperial, TX; Las Cruces, NM; San Diego, CA; and Kailua Kona, HI	7	33
Subtask	2.3.	Compile and provide biomass, productivity, and environmental data from continuously cultured outdoor strains at each site	7	33
Milestone	2.2	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	9	1
Milestone	2.3	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	18	1
Task	3.0	Characterization of Trait Drift in the Field and Laboratory	7	33
Subtask	3.1	Maintain the baseline field-adapted strain following three different laboratory cultivation practices across each of three laboratories	7	33
Subtask	3.2	Characterize the genotype and phenotype of lab- and field-maintained strains	12	28
Milestone	3.1.	Whole genome shotgun, 18s, maximum specific growth rate, robustness and composition data will be compiled for at least 6 lab- and field-maintained isolates following 6 months in culture	12	1
Milestone	3.2.	Whole genome shotgun, 18s, maximum specific growth rate, robustness and composition data will be compiled for at least 6 lab- and field-maintained isolates following 6 months in culture	18	1
Subtask	3.3.	Modify laboratory maintenance and cultivation protocols based on quantification of trait drift	22	18

BP 2 (18 mo)																		BP 3 (18 mo)																	
Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23
4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39

Type		Activity	Strt	Dur
Task	2.0	Establishment and Maintenance of a Field-Adapted *Nannochloropsis* Strain Across New Field Sites	4	36
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Subtask	3.3.	Modify laboratory maintenance and cultivation protocols based on quantification of trait drift	22	18
Task	4.0	Optimization of Pest Management	4	36
Subtask	4.1.	Characterize the metagenome of field-adapted cultivars	4	36
Milestone	4.1	Assembly of >20 metagenomes from ponds located at a minimum of two field sites exhibiting low- and high productivity or health to identify putative pests or microbiome shifts	24	1
Subtask	4.2.	Develop pest tracking tools for common, disruptive algal pests	6	34
Milestone	4.2	Comparative metagenomic analysis of high and low productivity ponds from the industrial site completed	30	1
Milestone	4.3	Three candidate promoter suites selected from metagenome sequences for pest tracking PCR primers complete	36	1
Subtask	4.3	Develop field-test protocols and kits	22	18

BP 2 (18 mo)																	
Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21
4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21

BP 3 (18 mo)																	
Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23
22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39

BP2 (18 mo): Apr-20 (4), May-20 (5), Jun-20 (6), Jul-20 (7), Aug-20 (8), Sep-20 (9), Oct-20 (10), Nov-20 (11), Dec-20 (12), Jan-21 (13), Feb-21 (14), Mar-21 (15), Apr-21 (16), May-21 (17), Jun-21 (18), Jul-21 (19), Aug-21 (20), Sep-21 (21)

BP 3 (18 mo): Oct-21 (22), Nov-21 (23), Dec-21 (24), Jan-22 (25), Feb-22 (26), Mar-22 (27), Apr-22 (28), May-22 (29), Jun-22 (30), Jul-22 (31), Aug-22 (32), Sep-22 (33), Oct-22 (34), Nov-22 (35), Dec-22 (36), Jan-23 (37), Feb-23 (38), Mar-23 (39)

Type		Activity	Strt	Dur
Task	2.0	Establishment and Maintenance of a Field-Adapted *Nannochloropsis* Strain Across New Field Sites	4	36
Subtask	2.1	Isolate the Nannochloropsis strain and establish it at each of the three field sites	4	5
Milestone	2.1.	Isolated and taxonomically verified field-adapted Nannochloropsis strain shipped to Las Cruces, NM; San Diego, CA; and Kailua Kona, HI for scale up	6	1
Subtask	2.2.	Maintain and sample outdoor cultivation systems in Imperial, TX; Las Cruces, NM; San Diego, CA; and Kailua Kona, HI	7	33
Subtask	2.3.	Compile and provide biomass, productivity, and environmental data from continuously cultured outdoor strains at each site	7	33
Milestone	2.2	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	9	1
Milestone	2.3	Metrics of productivity, pond health, and environmental datasets compiled, delivered, and analyzed for all field sites covering the project period to date	18	1
Task	3.0	Characterization of Trait Drift in the Field and Laboratory	7	33
Subtask	3.1	Maintain the baseline field-adapted strain following three different laboratory cultivation practices across each of three laboratories	7	33
Subtask	3.2	Characterize the genotype and phenotype of lab- and field-maintained strains	12	28
Milestone	3.1.	Whole genome shotgun, 18s, maximum specific growth rate, robustness and composition data will be compiled for at least 6 lab- and field-maintained isolates following 6 months in culture	12	1
Milestone	3.2.	Whole genome shotgun, 18s, maximum specific growth rate, robustness and composition data will be compiled for at least 6 lab- and field-maintained isolates following 6 months in culture	18	1
Subtask	3.3.	Modify laboratory maintenance and cultivation protocols based on quantification of trait drift	22	18
Task	4.0	Optimization of Pest Management	4	36
Subtask	4.1.	Characterize the metagenome of field-adapted cultivars	4	36
Milestone	4.1	Assembly of >20 metagenomes from ponds located at a minimum of two field sites exhibiting low- and high productivity or health to identify putative pests or microbiome shifts	24	1
Subtask	4.2.	Develop pest tracking tools for common, disruptive algal pests	6	34
Milestone	4.2	Comparative metagenomic analysis of high and low productivity ponds from the industrial site completed	30	1
Milestone	4.3	Three candidate promoter suites selected from metagenome sequences for pest tracking PCR primers complete	36	1
Subtask	4.3	Develop field-test protocols and kits	22	18
Task	5.0	Improvement of Field Strain Performance and Composition	6	34
Subtask	5.1.	Generate and characterize mutant strains	8	14
Milestone	5.1	A library of *Nannochloropsis* strains is generated by mutagenesis and/or selection along gradients of CO2, temperature, and light environmental stressors	15	1
Milestone	5.2	At least one *Nannochloropsis* strain is generated by mutagenesis and/or a 3- month selection from a cold temperature gradient demonstrating cold tolerance compared to the baseline strain	15	1
Subtask	5.2	Optimize biomass composition and carbon allocation	8	18
Subtask	5.3	Conduct directed evolution experiments to quantify phenotypic change under different selective forces	18	12
Milestone	5.3	At least one mutant is identified in laboratory screenings that demonstrates a 10% improvement in productivity, robustness or biomass composition over the baseline strain	27	1
Subtask	5.4	Conduct at least 2 iterative rounds of UV mutagenesis with increasing selection pressures, focusing on pressures that have previously generated improvements or potential for improvements	27	13

Type		Activity	Strt	Dur
Task	6.0	Evaluation of Optimized Practices and New Strains	4	45
Subtask	6.1	Develop an engineering processes model for analysis of improvements	4	8
Subtask	6.2	Conduct concurrent TEA and LCA	12	10
Milestone	6.1	Analysis of economic impact on the biorefinery of carbon partitioning/modulation (loss of carbs and an increase in different lipid content/pools)	18	1
Go/No-Go	2	Comparison of one mutant vs baseline strain and deployment of the beta version of the qPCR tool at the scale of 260L ponds in triplicate at one of the field sites.	21	1
Subtask	6.3	Test improved cultivation practices and strains in the field	22	25
Go/No-Go	3	Three lab-to-field iterations that incorporate at least two of the following: trait stack, qPCR tool deployment, and process improvements based on trait drift in the lab and field to reach improvements in productivity, robustness and biomass composition compared to the baseline *Nannochloropsis* strain	39	1
Subtask	6.4	Conduct dynamic growth modeling	23	17
Milestone	6.3	Environmental impact on global warming potential with a comparison to the renewable fuel standard quantified	33	1
Subtask	6.5	Integrate results from all tasks to make recommendations for cultivation and pest management strategies	40	9
Subtask	6.6	Conduct temporally resolved TEA and LCA	40	9
Milestone	6.4	Field deployable qPCR assay field tested 3 times at 2 remote field sites	42	1
Milestone	6.5	Recommendations for strain maintenance, cultivation, improvement, and pest management drafted, considering implementation	45	1
Milestone	6.6	Quantification of economic ($ GGE^{-1}) and environmental impact (g-$CO_{2,eq}$ MJ^{-1}) of advancements compared to DOE targets	48	1
End of Project Goal		**Statistically significant improvements in productivity, robustness and biomass composition to reach targets of 50%, 50% and 20%, respectively, via changes in cultivation practices and the use of field cultivars and improved strains demonstrated during field cultivation campaigns at the scale of at least 200L.**	48	1

BP2 (18 mo)																	
Apr-20	May-20	Jun-20	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21
4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21

BP3 (18 mo)																	
Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Jul-22	Aug-22	Sep-22	Oct-22	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23
22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39

BP4 (9 mo)								
Apr-23	May-23	Jun-23	Jul-23	Aug-23	Sep-23	Oct-23	Nov-23	Dec-23
40	41	42	43	44	45	46	47	48