Production-Scale Performance of Lipid Hyper-Accumulating Algae

May 21, 2013
Algae Platform Review

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WBS 9.1.2.5

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Goal Statement

Primary Goal for FY13

- Develop an integrated pipeline for predicting and validating outdoor performance of any industrial or novel algae strain of interest

Secondary Goal

- Evaluate the performance of a lipid hyper-accumulating strain in settings representative of industrial conditions

Quad Chart Overview

Timeline

- **Start Date: 10/1/12**
- **End Date: 9/30/17**
- **Percent Complete: 10%**

Barriers

- **Barriers addressed**
  - Ft-B. Sustainable Production
  - Ft-C. Feedstock Development
  - Ft-G. Feedstock Quality & Monitoring

Budget

- **Total Funding $225k**
- **FY12 $0k**
- **FY13 $225k**

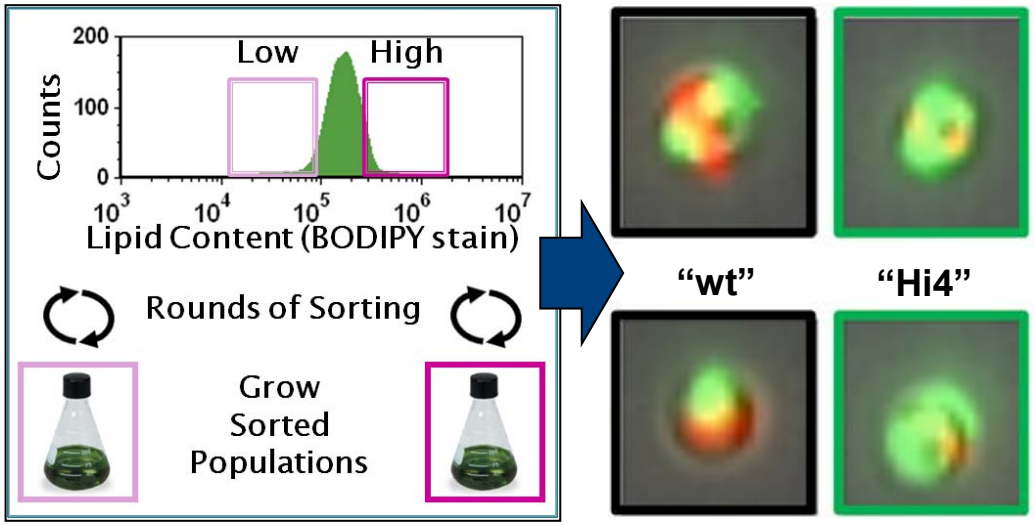
Partners

- **PNNL (Dr. M. Huesemann)**
- **Project management by monthly conference calls**

Project Overview: History

- Under NAABB, we developed a suite of techniques for algae characterization, which have been used to track the productivity and quality of algae during cultivation, harvesting, and extraction.

- We have isolated a population of *Picochlorum sp.* (formerly *Nannochloris sp.*) that shows a 2.8x increase in neutral lipid accumulation over the parent population.



- **This method for strain improvement is particularly compatible with immediate introduction to outdoor ponds, because the strains are *not* GMO and the selections can be conducted directly on industrially relevant strains.**

Project Overview: How Well Will These Strains Perform Outdoors?

The Problem

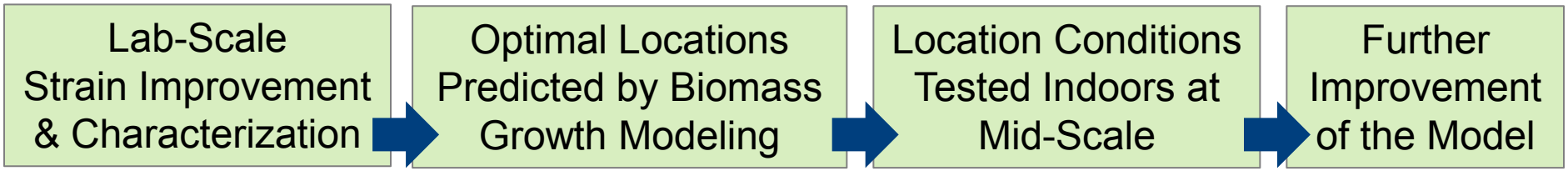
- **From flask to pond: Often algae strains do not perform as well or as predictably outdoors as they do in the lab**

How Do We Predict Which Strains Will Perform Best Outside?

How Do We Cost-Effectively Validate Which Locations Are the Best?

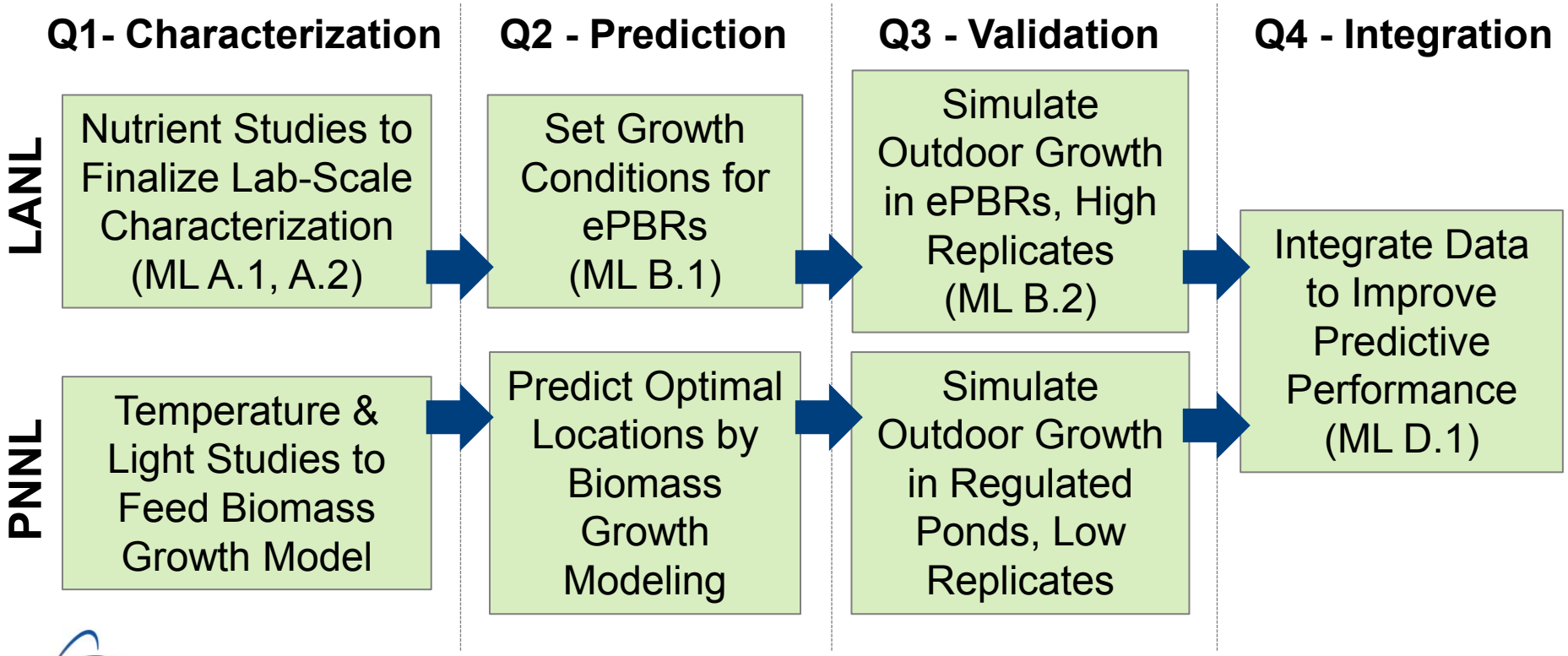
The Solution

- **Develop an integrated pipeline for predicting and validating outdoor performance of any industrial or novel algae strain of interest**



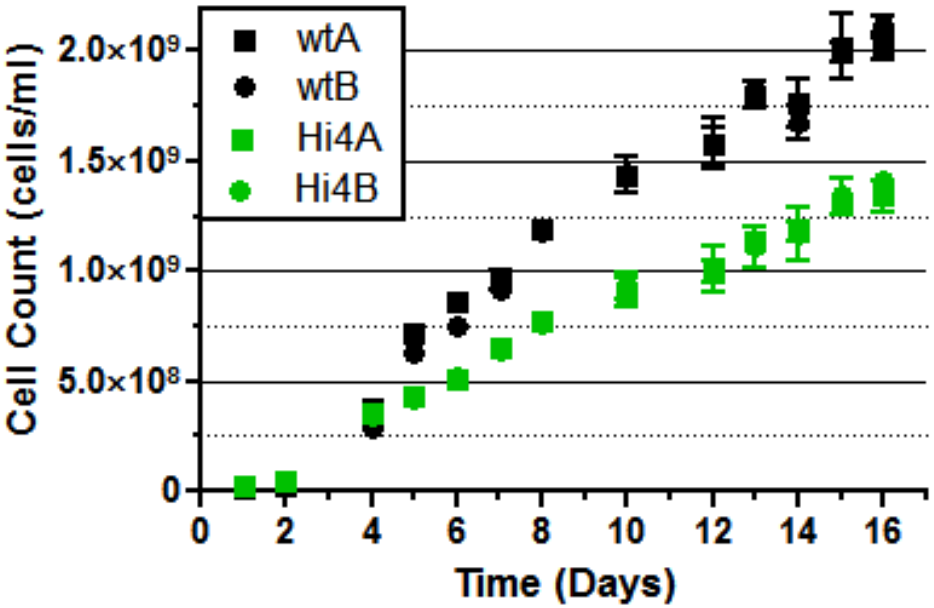
1 - Approach

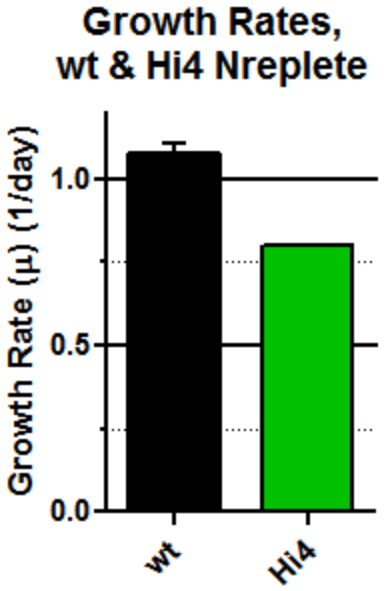
- **Use the parent (wt) and hyper-accumulating (Hi4) *Picochlorum sp.* strains as example strains for developing this pipeline.**



2 - Technical Accomplishments:

Lipid Hyper-Accumulator Cultivated Under Nutrient Replete Conditions (Q1 Milestone)





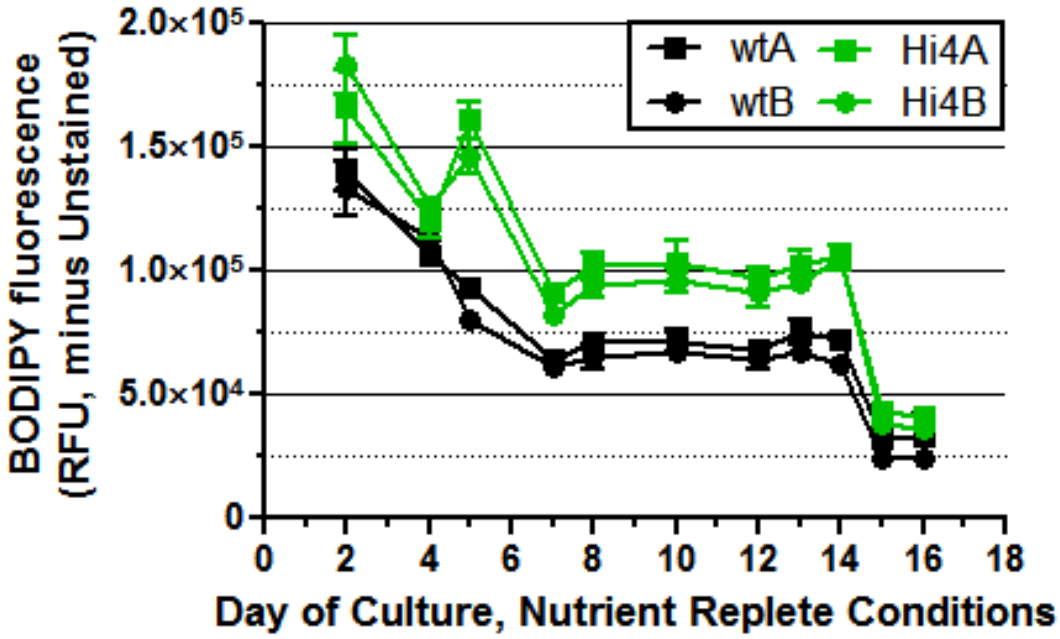
Biomass (g/L):
wt = 4.42
Hi4 = 4.36

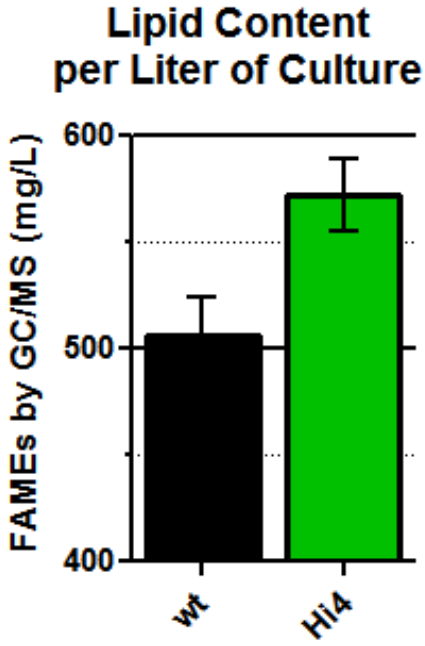
We hypothesize that the loss in cell counts in Hi4 is compensated for by an increase in cell size

- ***Maximum specific growth rate is slightly reduced in Hi4, but biomass accumulation is similar***
- ***Maximal productivities may depend on additional strain properties such as cell size***

2 - Technical Accomplishments:

Lipid Hyper-Accumulator Characterized Under Nutrient Replete Conditions (Q1 Milestone)

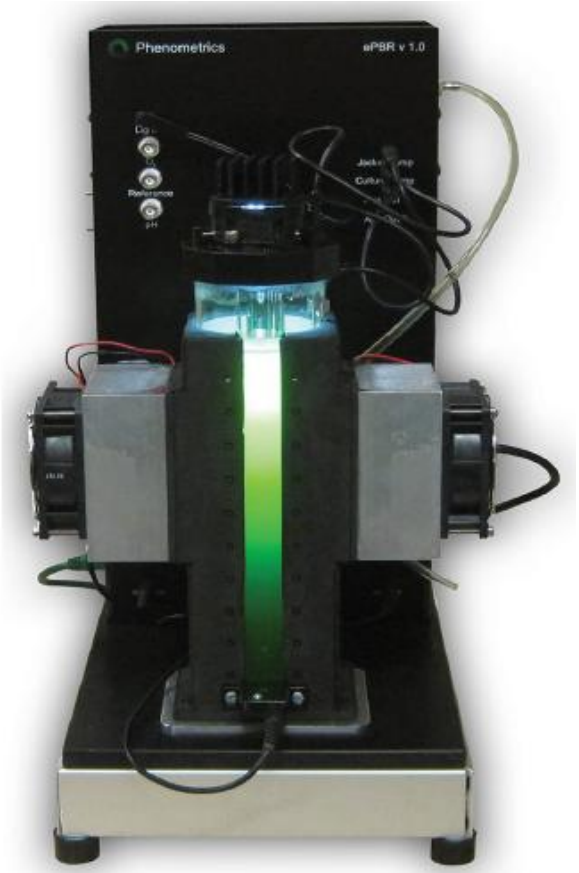




- ***Lipid content in Hi4 cultures is increased over wt, even under nutrient replete conditions***
- ***Increased productivities may be achieved with Hi4 by operating under continuous culture conditions***

2 - Technical Accomplishments:

Growth of Lipid Hyper-Accumulator & Parent Strain Established in Phenometrics ePBRs (Q2 Milestone)



www.phenometrics.com

- ***ePBRs designed to***
  - *Vary light intensity up to full sunlight*
  - *Mimic typical pond depths*
- ***Temperature regulated***
- ***Computer controlled***
- ***24 available at LANL***
- ***Hardware and script modifications were made to establish appropriate***
  - *CO_2 delivery*
  - *Gas diffusion*
  - *pH control*
  - *Rapid yet sterile sampling*
  - *Replenishment of evaporative loss*

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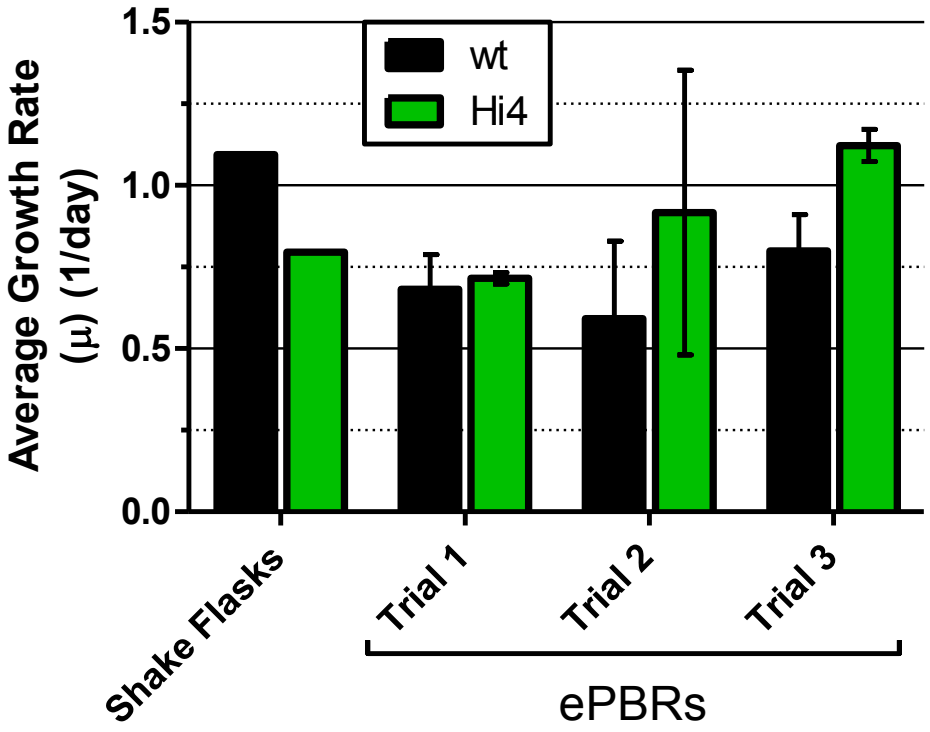


- ***Similar maximum specific growth rates were observed in the ePBRs, in comparison to previous shake flask experiments.***
- ***Light/Temp scripts received from PNNL for Q3 culturing***

3 - Relevance

"Feedstock Supply and Logistics R&D relates directly to, and strongly influences, all of the downstream elements of the Program's portfolio and their respective goals and objectives." – MYPP Nov. 2012

- **One MYPP performance goal is to "increase the projected productivity of large-scale algae cultivation", up to 30 g-m^2/day in 2012. We are contributing to this through:**
- **Strain Improvement (Feedstock Production)**
  - Contributes to any pathway requiring algae as a feedstock, as it focuses on improved production.

Algal Lipid Upgrading

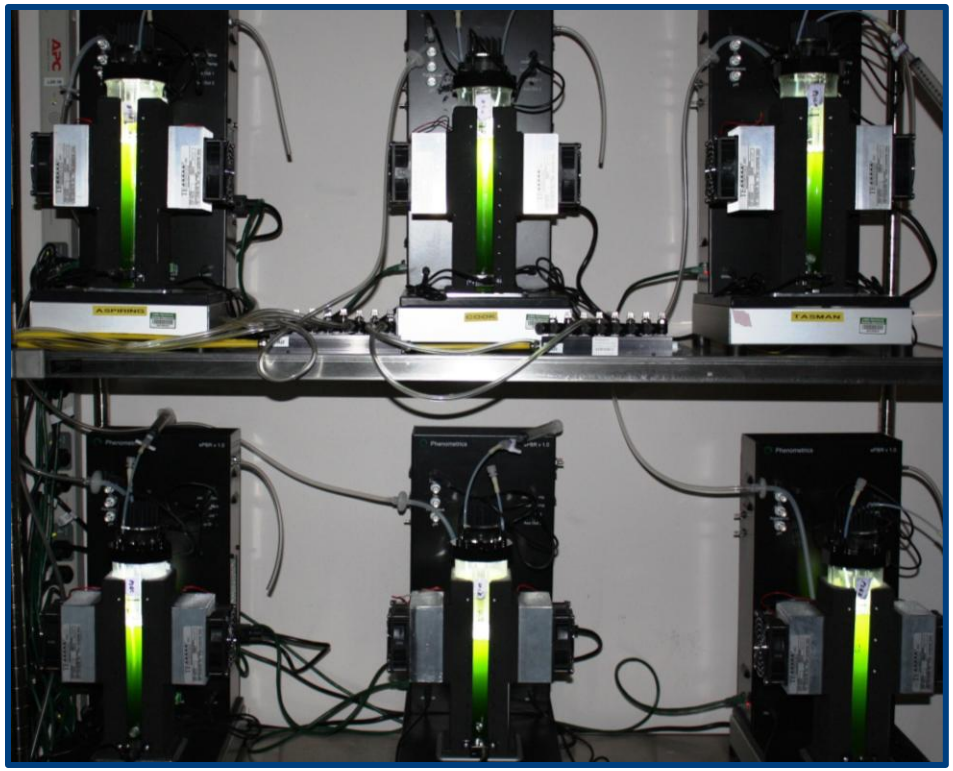
Whole Algae Hydrothermal Liquefaction

- **Locations for Maximal Performance (Resource Assessment)**
  - Improvement of the BAT with regard to specific strain data and varying environmental conditions.

4 - Critical Success Factors

- **Success Factors:**
  - Continued generation of improved/promising strains
  - Validation of use of ePBRs as lab-scale pond simulators
  - Validation of the biomass growth models
- **Technical Challenges:**
  - Generation of strains improved for additional properties: *e.g.* biomass production, increased growth rate, environmental tolerance
  - Reproducibility and climate simulation in ePBRs
  - Incorporation of cultivation parameters into the growth models
- **Success of this project will increase biomass productivities**
  - Lead to rapid & accurate identification of geographic locations optimally suited for maximal algae productivity
  - Further establish LANL's capability in generating improved algae strains with a variety of industrially relevant properties

5. Future Work (Q3, Q4)



- Simulate the climate of the predicted maximally productive month for wt and Hi4 in the ePBRs
- Harness the 24 ePBRs to conduct the experiment in replicates of 6
- Examine the effects of pH for future inputs to the BAT
- Compare results to larger scale but lower replicate data in environmentally controlled ponds (PNNL)

5. Future Work

- **Measure the energy required to harvest the wt and Hi4 strains (Q4).**
  - Hi4 is larger in size and therefore expected to require less energy to harvest (dewater)
  - We will quantify this difference to determine any additional cost savings that might be expected from using the improved strain

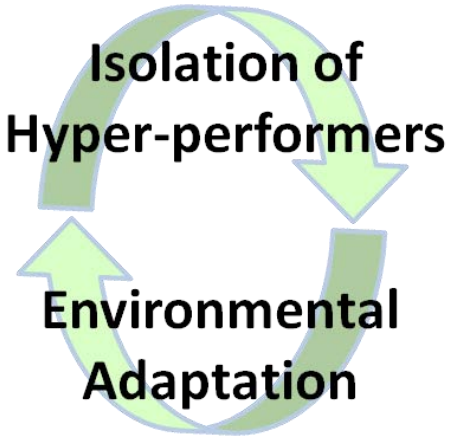
FY14 –

- **Focus on a freshwater strain, e.g. *Chlorella sorokiniana***
- **Generate an additional improved strain for the pipeline by cell sorting**
  - Explore new schemes for biomass improvement beyond lipid accumulation
- **Examine importance of cultivation conditions (*e.g.*, pH) to further improve the growth models**

5. Future Work

FY15 and beyond –

- **Generate additional improved strains for the pipeline by cell sorting**



- Hyper-performance:
  - Lipids
  - Biomass
- Adaptive Evolution
  - Temperature
  - Salinity
  - pH
- Relevant to wild type & mutagenized non-GMO strains, as well as GMO strains, as they come online

- **Characterize/model the improved strains**
- **Move strains to outdoor test beds**

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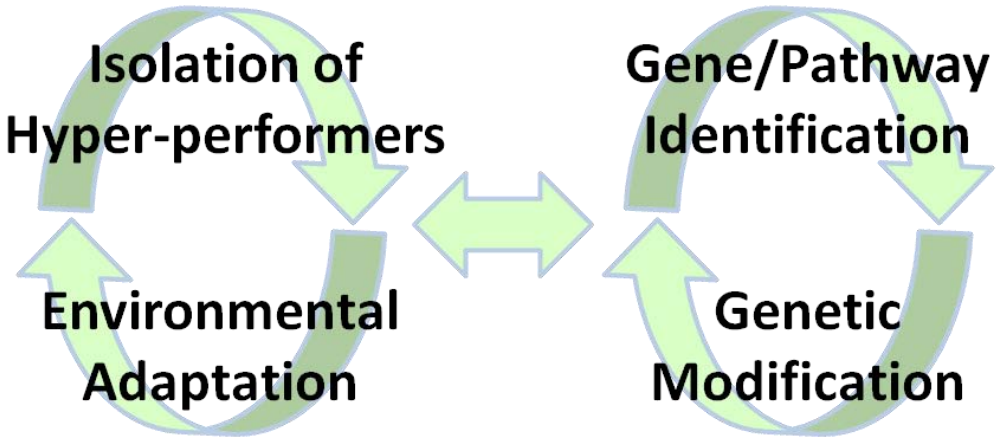

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Summary

- **Relevance: Improving algae strains and accurately predicting their performance in outdoor systems is critical to industry success.**
- **Ours is a low risk approach that takes improved strains from lab to mid-scale under climate-simulated conditions, with the goal of reaching outdoor test-beds next.**
- **We are meeting our milestones and are on track to establish this performance pipeline with the example *Picochlorum* strains.**
- **We are using the scripts from PNNL to simulate the climate of the continental U.S. location predicted to have optimal productivity.**
- **Cultivation conditions effecting the biomass growth model will be incorporated, as well as additional strains, in order to improve the model.**
- **There is potential for IP for the improved strains.**

Acknowlegements

- Scott Twary
- Amanda Barry
- Hiro Teshima
- Robin Yoshida

PNNL

- Michael Huesemann
- Mark Wigmosta

Additional Slides

(Not a template slide – for information purposes only)

- ***The following slides are to be included in your submission for Peer Evaluation purposes, but <u>will not be part of your Oral presentation</u>***
- ***You may refer to them during the Q&A period if they are helpful to you in explaining certain points.***

Responses to Previous Reviewers' Comments

- **N/A – this project is a new start in FY13**

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These additional slides will be included in the copy of your presentation that will be made available to the Reviewers.

Publications and Presentations

- **A prepared manuscript of the project's results is a deliverable for FY13.**

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