# DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

## Development of Algal Biomass Yield Improvements in an Integrated Process Phase 2

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David Hazlebeck
Global Algae Innovations



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# Goal Statement

## The goal is

to develop ***improved strains and cultivation methods*** to increase the algal biofuel intermediate yield by at least 70% and

to develop ***new drying and extraction technology*** to reduce the energy for downstream processing by at least 50%

to work in an ***integrated outdoor system that reduces the projected minimum selling price*** (MSP) of algae biomass by 20%

## Relevance to bioenergy industry

- Productivity is crucial to economic viability and sustainability of algal biofuel
- ABY1 solved harvesting & dewatering, so drying and extraction are now largest downstream energy use
- Fully-integrated system and cost metrics lead to commercially relevant new technologies

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# Quad Chart Overview

## Timeline

**10/2016 – 3/2019**
**90% Complete**

## Budget

| | Pre 2017 | 2017 | 2018 | 2019 + |
|---|---|---|---|---|
| DOE | 1040 | 2100 | 1300 | 560 |
| Cost Share | 260 | 525 | 325 | 140 |
| Partners<br>• UCSD<br>• TSD<br>• PNNL<br>• Qualitas Health<br>• NREL | | | <br>18%<br>5%<br>5%<br>3%<br>2% | |

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## Barriers

- Aft-B. Sustainable Algae Production
- Aft-C. Biomass Genetics and Development
- Aft-H. Overall Integration and Scale-Up
- Aft-I. Algal Feedstock On-Farm Preprocessing

**MYPP targets addressed**:

- 2020 algal yield of 3700 gal/ac-yr
- 2022 $n^{th}$ plant algal biofuel at $3/GGE

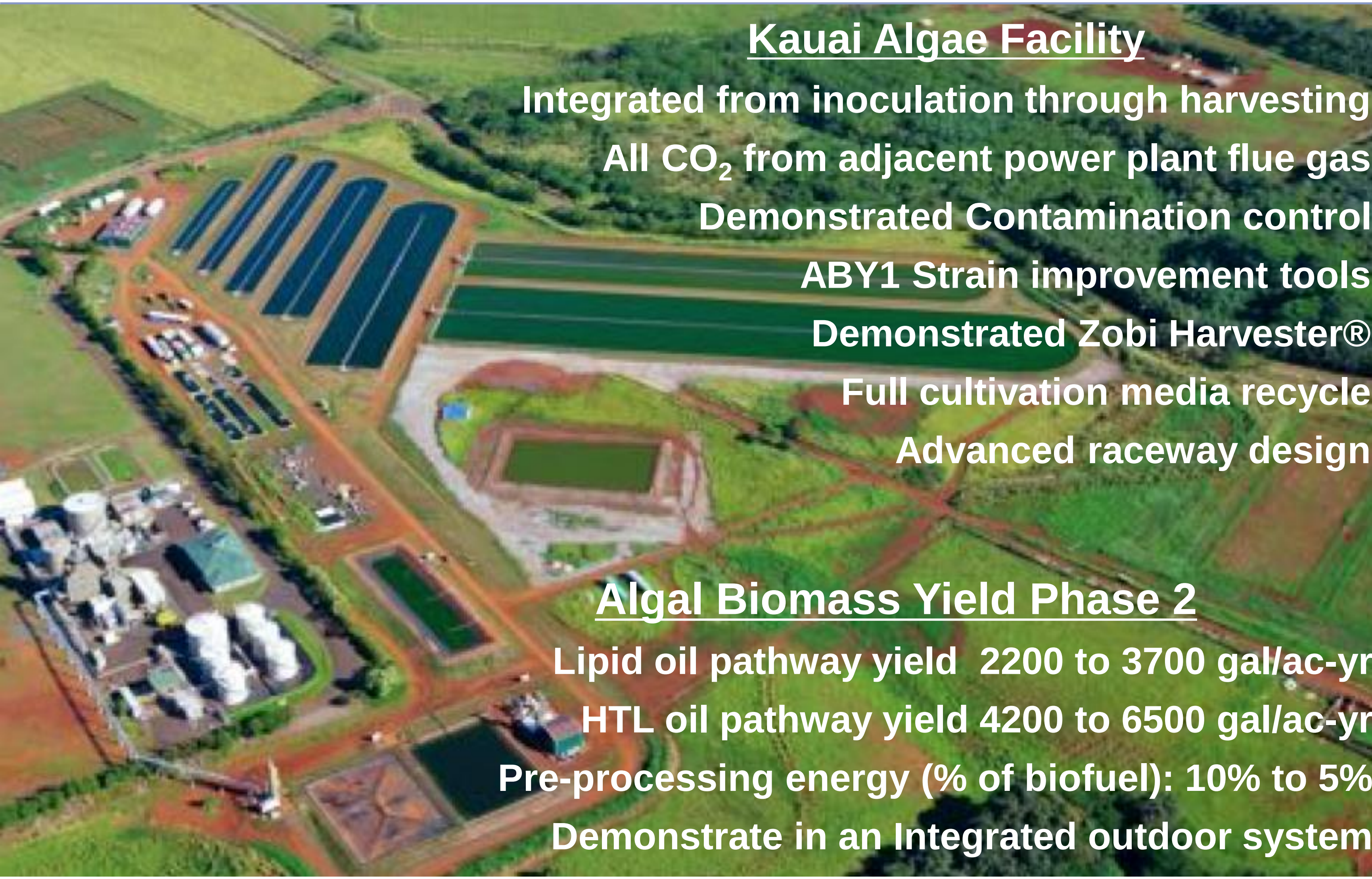
## Objective

Achieve higher productivity,
lower processing energy use,
and lower production cost

## End of Project Goal

70% increase in lipid productivity
50% reduction in processing energy
20% reduction in minimum selling price

# 1 - Project Overview History



**Kauai Algae Facility**

**Integrated from inoculation through harvesting**

**All $CO_2$ from adjacent power plant flue gas**

**Demonstrated Contamination control**

**ABY1 Strain improvement tools**

**Demonstrated Zobi Harvester®**

**Full cultivation media recycle**

**Advanced raceway design**

**Algal Biomass Yield Phase 2**

**Lipid oil pathway yield 2200 to 3700 gal/ac-yr**

**HTL oil pathway yield 4200 to 6500 gal/ac-yr**

**Pre-processing energy (% of biofuel): 10% to 5%**

**Demonstrate in an Integrated outdoor system**

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# 1 - Project Overview Goals

| Area | Baseline (ABY 1) | Goals | Results to date | % Change |
|---|---|---|---|---|
| Productivity: (gal oil/acre-year) | 2200 | 3700 | 3300 | 50% |
| Pre-processing: (% of the biofuel energy) | 9.6% | 4.8% | **2.5%** | -73% |
| Algae meal drying energy (% of algae biomass energy) | 35% | NA | **4.8%** | -86% |
| Integration: algae paste MSP ($/mt AFDW) | $597 | $499 | **$423** | -29% |
| Integration: farm energy (kwh/mt AFDW) | 270 | 205 | 245 | -9% |
| 5. Integration: MFSP ($/GGE) | $3.33[a] | $3.00 | **$2.51**[a] | -25% |

[a] Assumes $500/mt for the co-product algae meal

Note: HTL testing delayed, so only lipid results presented

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# Project Overview - Application of technology

- **Zobi harvester® is available as commercial product**
- **Broad use of technology in other DOE projects**
  - Currently prime or subcontractor with 8 different research teams
  - Partnered with 12 teams on recent DOE proposals
- **Technology part of integrated biorefinery scale-up project**
- **Established Global Algae Equipment as a vehicle to commercialize new equipment and instruments**

# 2 – Approach (Management)

**All technologies filtered through comprehensive cost model**

- Economically viable
- Integration impacts and opportunities

**Technology development map**

- Prioritize research
- Many options
- Quick advancement/early risk retirement
- Synergistic projects or opportunities

**Frequent telecoms to discuss results and opportunities**

- Rapid communication
- Synergistic projects and opportunities
- Cost and technology status/potential transparent to team

**Go/No-go Metric on biofuel intermediate yield**

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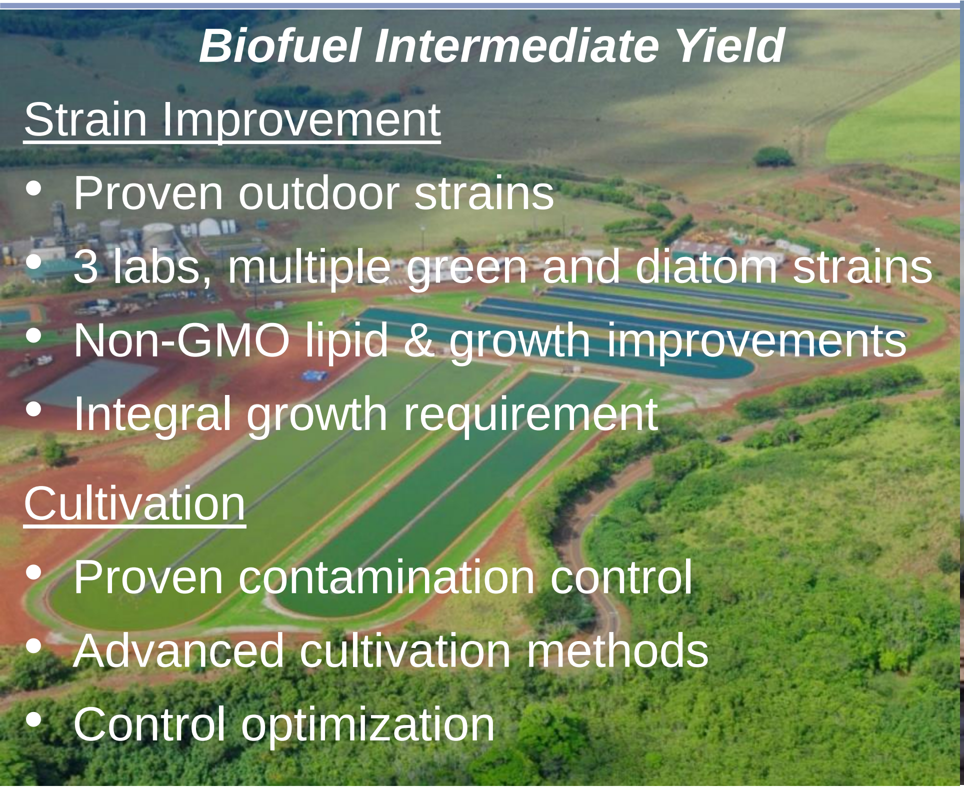
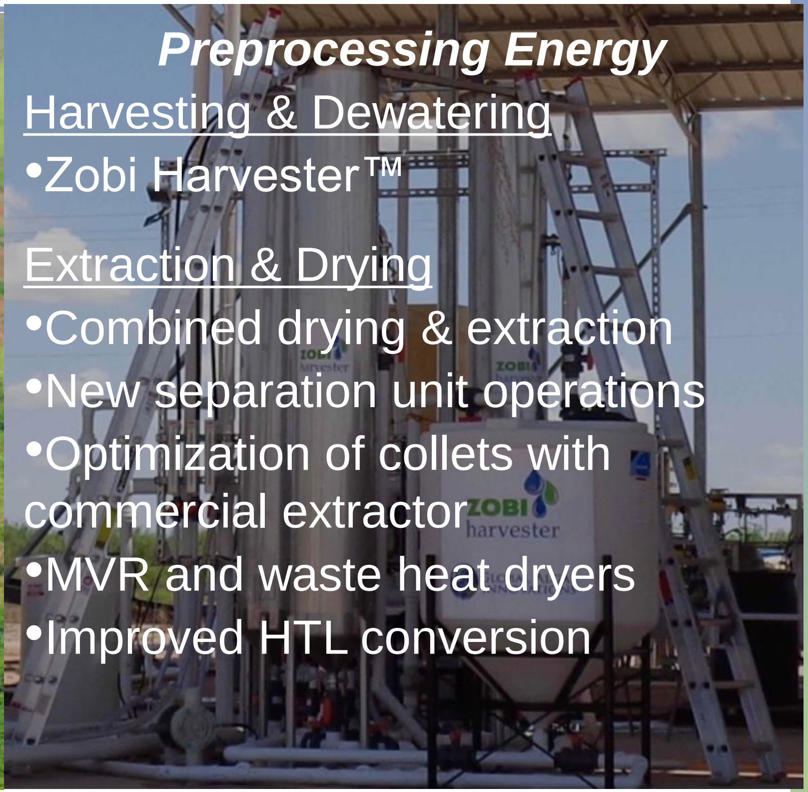
# 2 – Approach (Technical)



***Biofuel Intermediate Yield***

Strain Improvement
- Proven outdoor strains
- 3 labs, multiple green and diatom strains
- Non-GMO lipid & growth improvements
- Integral growth requirement

Cultivation
- Proven contamination control
- Advanced cultivation methods
- Control optimization



***Preprocessing Energy***

Harvesting & Dewatering
- Zobi Harvester™

Extraction & Drying
- Combined drying & extraction
- New separation unit operations
- Optimization of collets with commercial extractor
- MVR and waste heat dryers
- Improved HTL conversion

***Top Challenges***

- Complexity of abiotic and biotic variation
- Translating lab to large-scale outdoor cultivation
- Inability to achieve early risk retirement for strain optimization
- Producing sufficient material for downstream processing work

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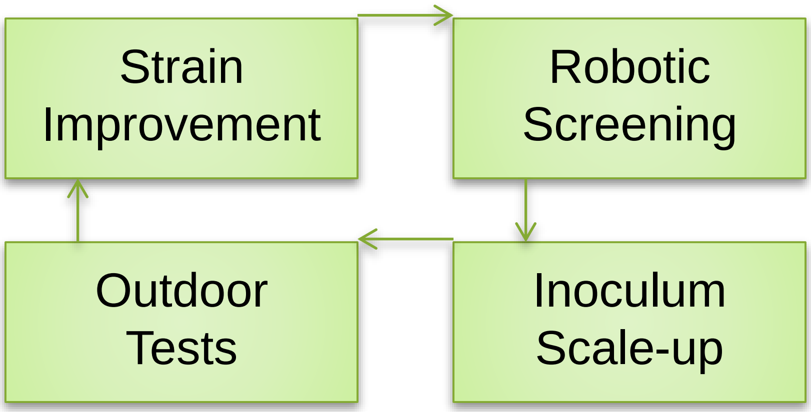
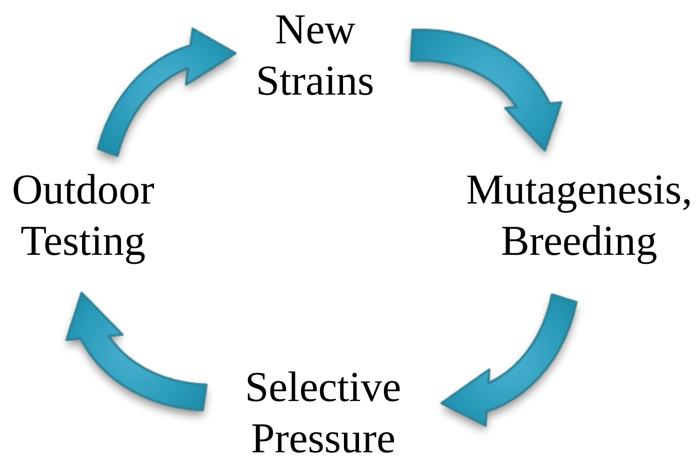
# Strain Improvement

## Rapid feedback

- New strains sets every 6 weeks




## Directed Evolution

- Specialty PBRs for selective pressure
- High oxygen, high light, shallow, high concentration, temperature control

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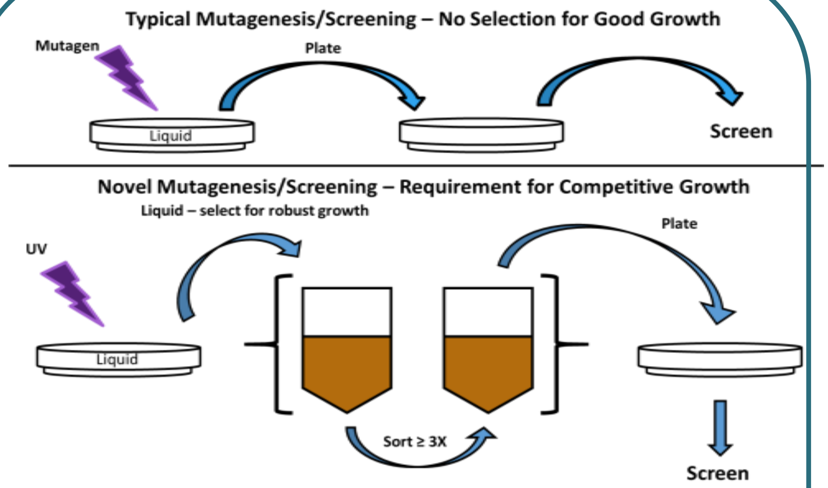
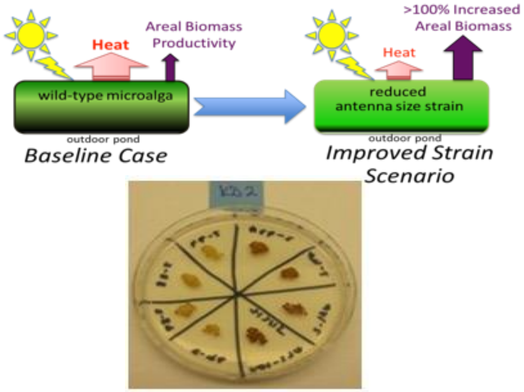
# Strain improvement



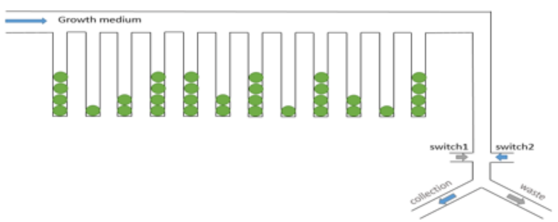

**Novel mutagenesis/high throughput fluorescent activated cell sorting**

- 3,600x's more efficient in viable mutants



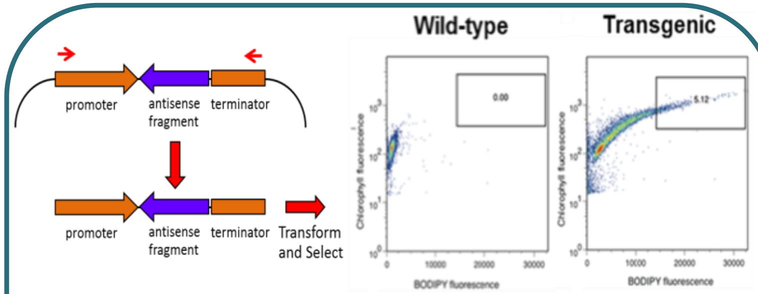

**Reduced photosystem antenna size**

- New strain lines started
- Reduced pigmentation lines on left




**Microfluidics for individual cell analyses**

- Sort for fastest growing cell lines




**Native sequence genetic manipulation**

- Avoids GMO classification
- Demonstrated antisense knockdown of CGI-58 lipase improved TAG content
- Can target light-harvesting pigments

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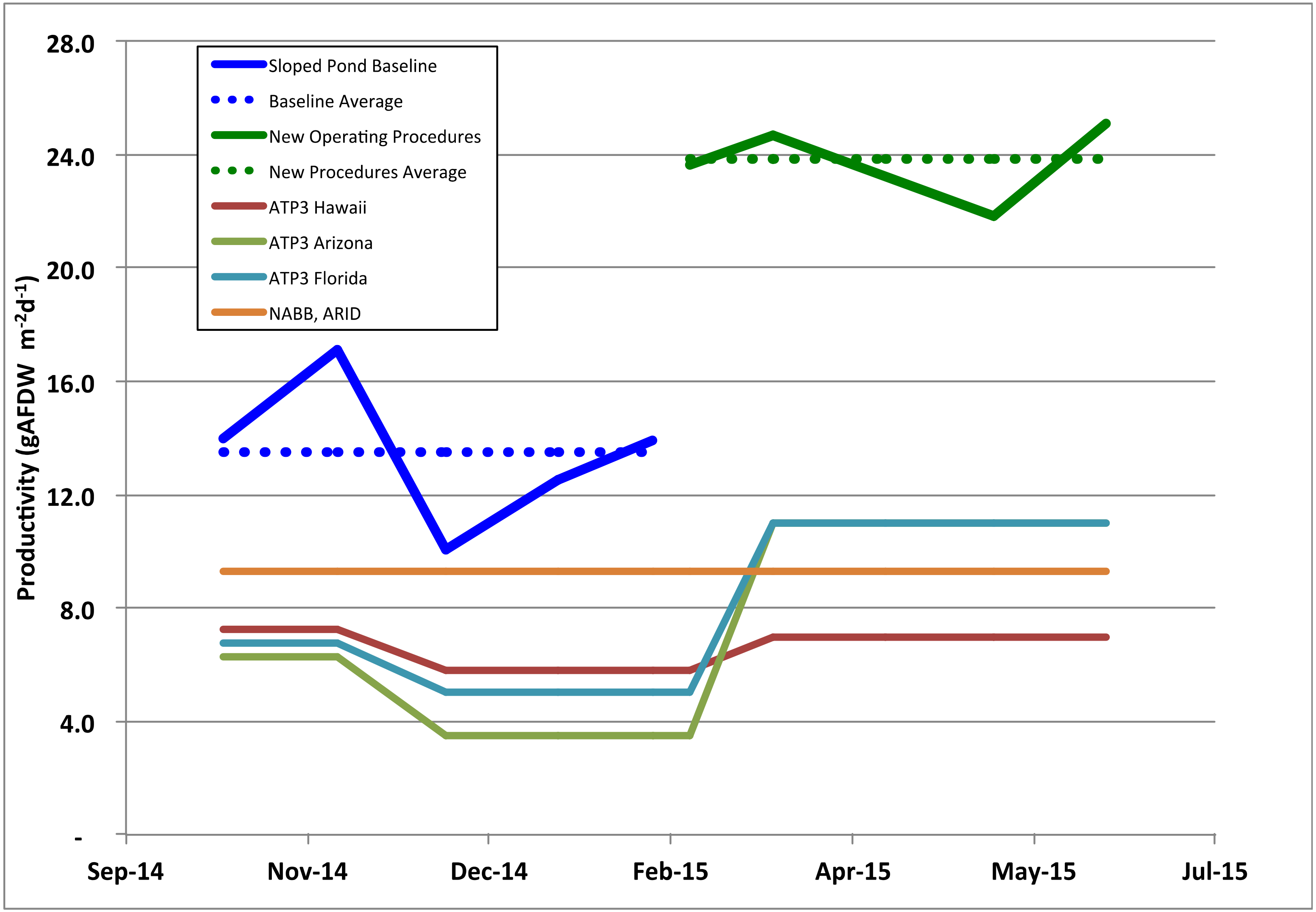
# Advanced cultivation methods

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# Cultivation Improvements

**Move to prior cultivation advances to smaller scale**

**Advanced cultivation**

- New set of advanced cultivation methods
- Control & media optimization for both growth & lipid formation
- Bacterial control strategies
- Cultivation system advances to amplify lipid trigger

**Tests utilize best strains available and comparison to control methods**

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# Pre-processing Energy

**Fully integrated with cultivation**

- Working with freshly harvested samples is essential
- Immediate identification of issues with new strains or cultivation
- Experience the diversity of culture conditions throughout year

**Harvesting**

- Finish longer term continuous operations
- Parametric studies to improve to enable further optimization

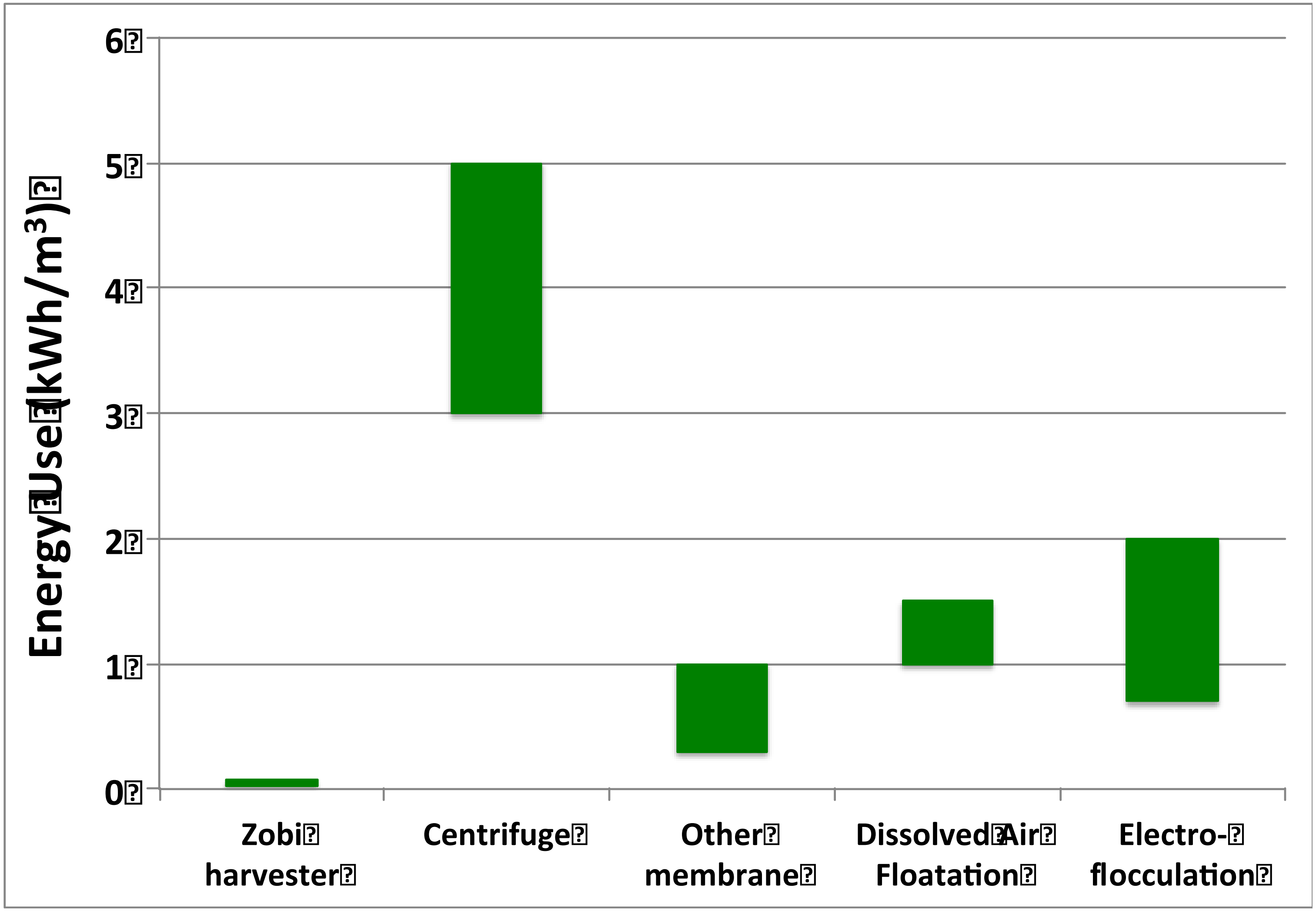
**Lipid Extraction - Focus on early risk retirement**

- Prioritize and test alternatives for each unit operation to attain a new low energy, low cost approach
- Develop the approach into robust process

**Hydrothermal liquefaction**

- Optimize cultivation/strain conditions for higher HTL yield without reducing the biomass productivity

# Zobi Harvester™ - very low energy use

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# 3- Technical Accomplishments, Progress and Results

# Strain Development
# Scripps Institution of Oceanography

<u>Two genetic manipulation strategies for strain improvement evaluated</u>

1) Lower light energy dissipating & higher light-harvesting carotenoids was promising
   - Substantial increase in neutral lipids and protein
   - Higher protein = higher value co-product

2) Lower all photosynthetic pigments was not promising
   - Induces stress but not higher lipid or protein

3) Random mutagenesis & selection – needs additional work
   - Reversion is a problem
   - Breeding or many cycles of selection are options to explore
   - Additional cycle testing in progress, one strain did achieve higher lipids in outdoor testing (46% vs 36%), but the productivity was lower

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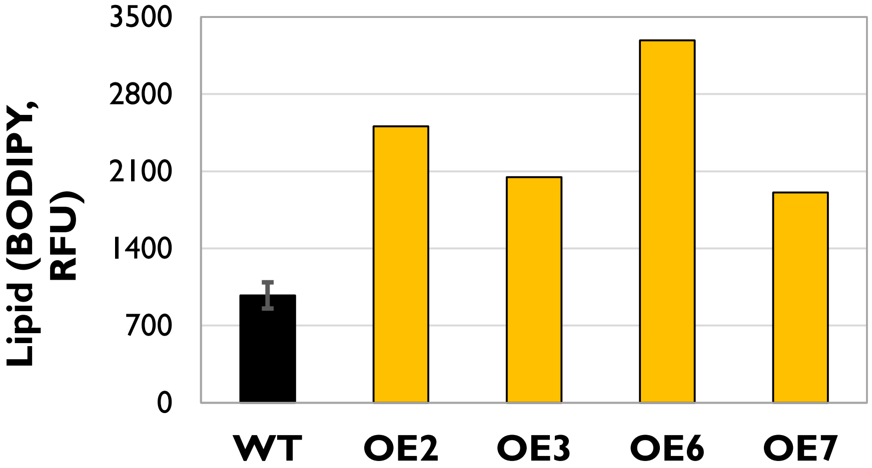
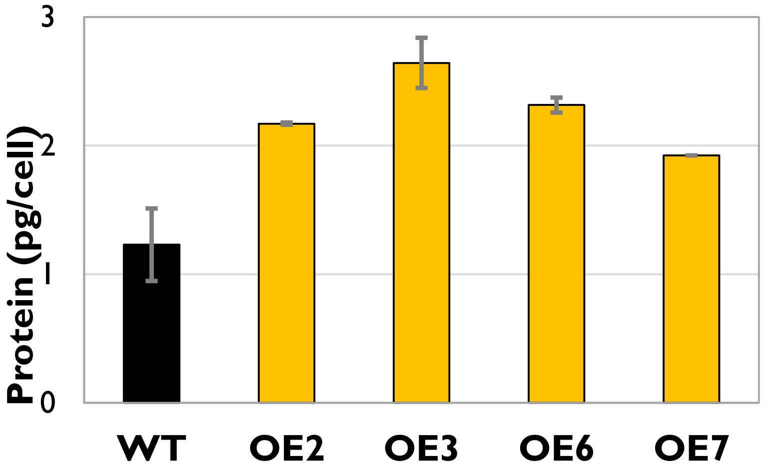
# Increase light harvesting carotenoids
# VDL2 Overexpression (Exponential Growth)

Neutral Lipid: up to 3.4X more




Protein: up to 2X more




No change in carbohydrate content

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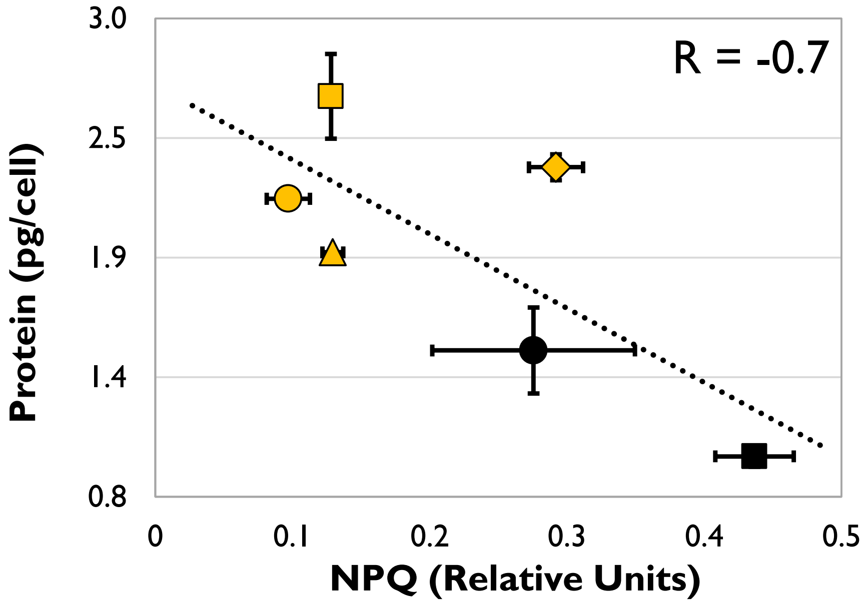
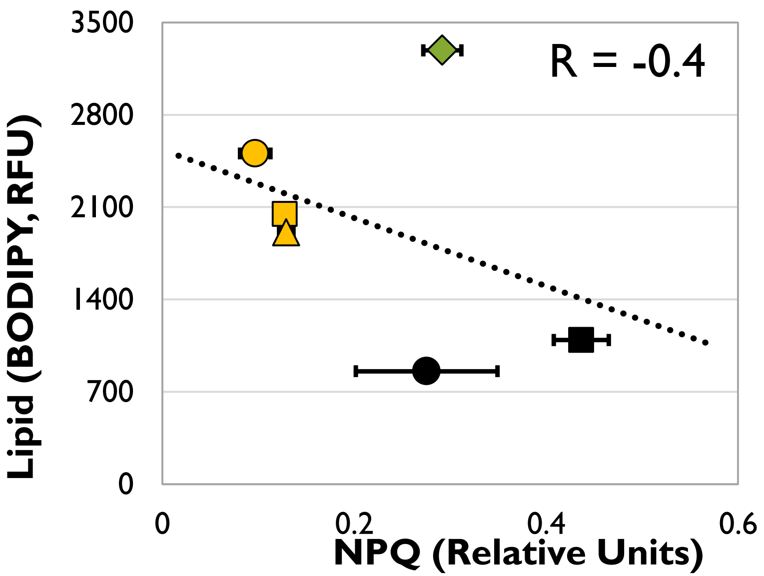
# Lower Non-photochemical Quenching (NPQ) = Lower Light Energy Dissipation




Less light energy dissipated = more carbon fixed

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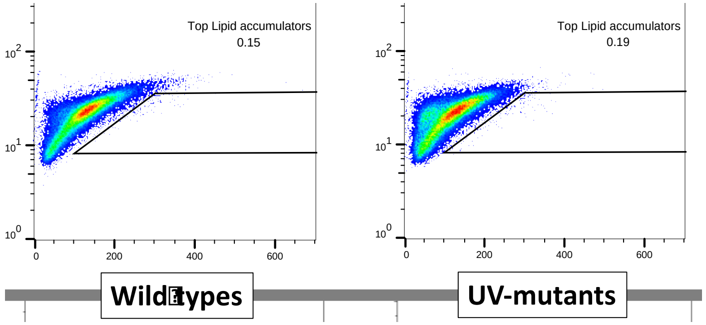
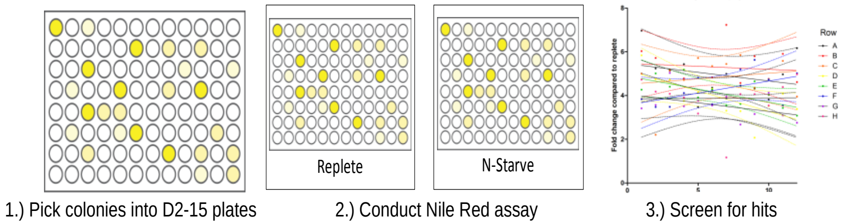
# Strain Improvement – Lipid Formation
# University of California, San Diego

1. UV Mutagenesis

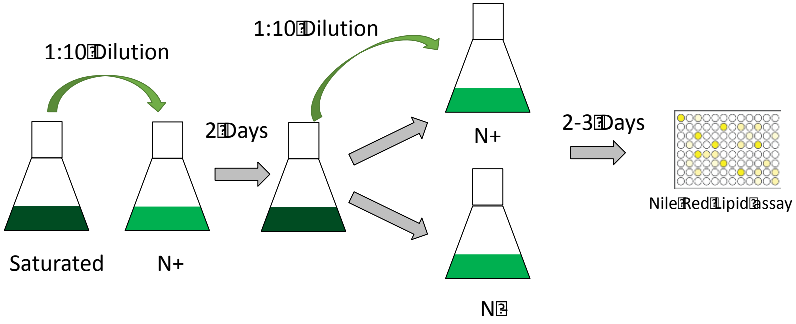
2. FACS for high-lipid strains




3. Screening individuals in microplates




4. Flask-scale experiments




- Screen 1,344 individuals recovered colonies for GAI-247 mutants
- Best two mutants from two rounds were sent to Hawaii
- **All mutants reverted to WT**

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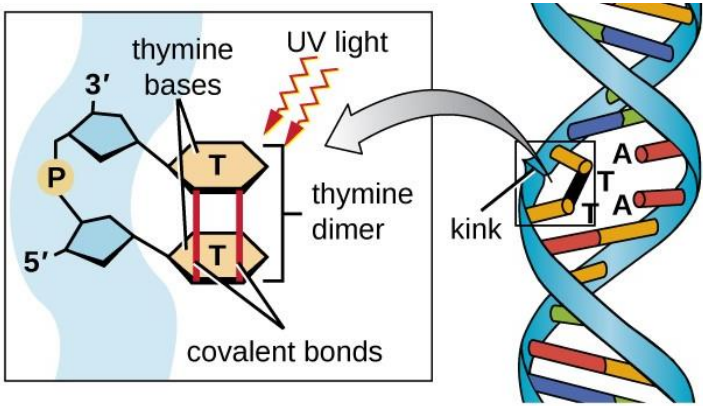
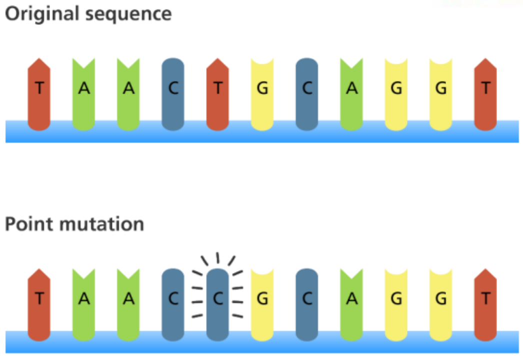
# Different types of mutagenesis yield different types of mutations

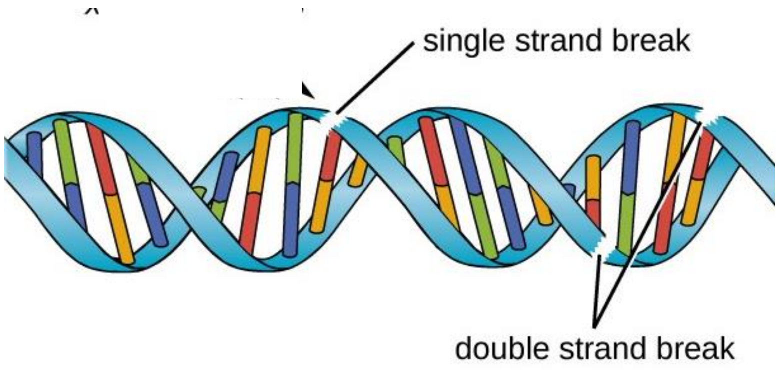
## UV-light

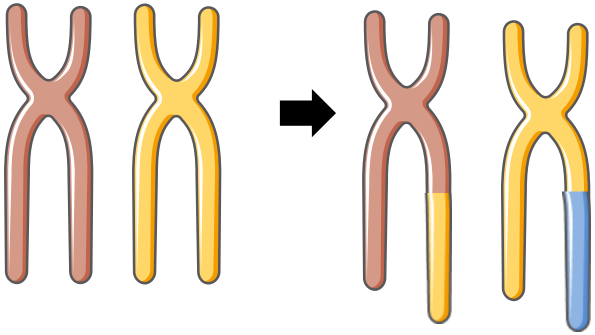



- Results in mostly point mutations in DNA sequence

## Zeocin



- Acts like ionizing radiation causing DNA breaks.
- Chromosome rearrangements

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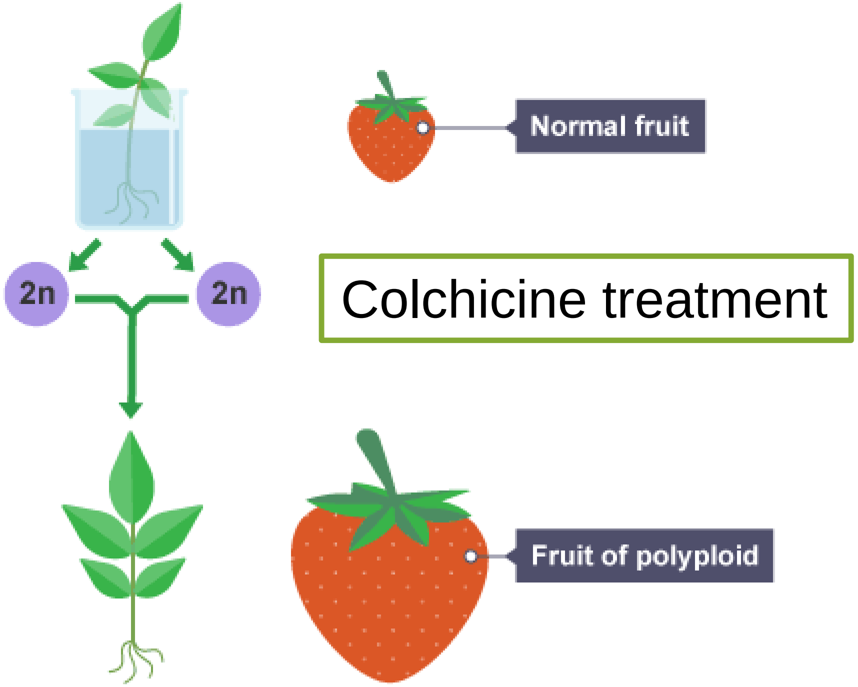
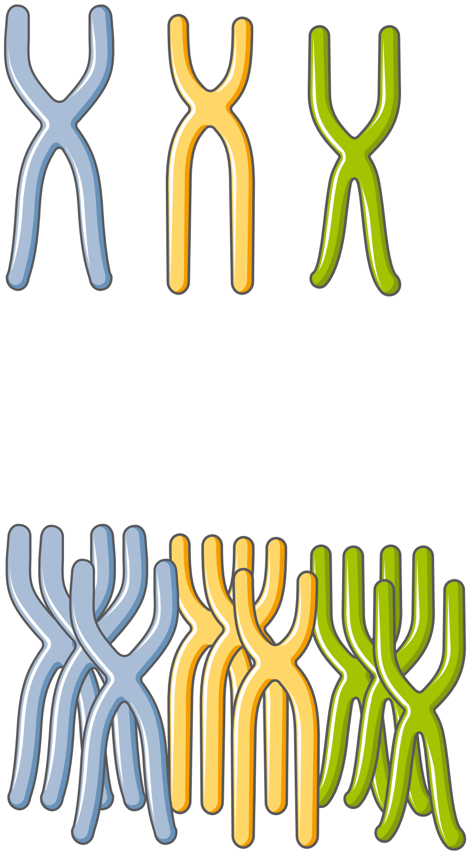
Modified from: https://www.yourgenome.org/facts/what-types-of-mutation-are-there

# Colchicine to induce polyploidy

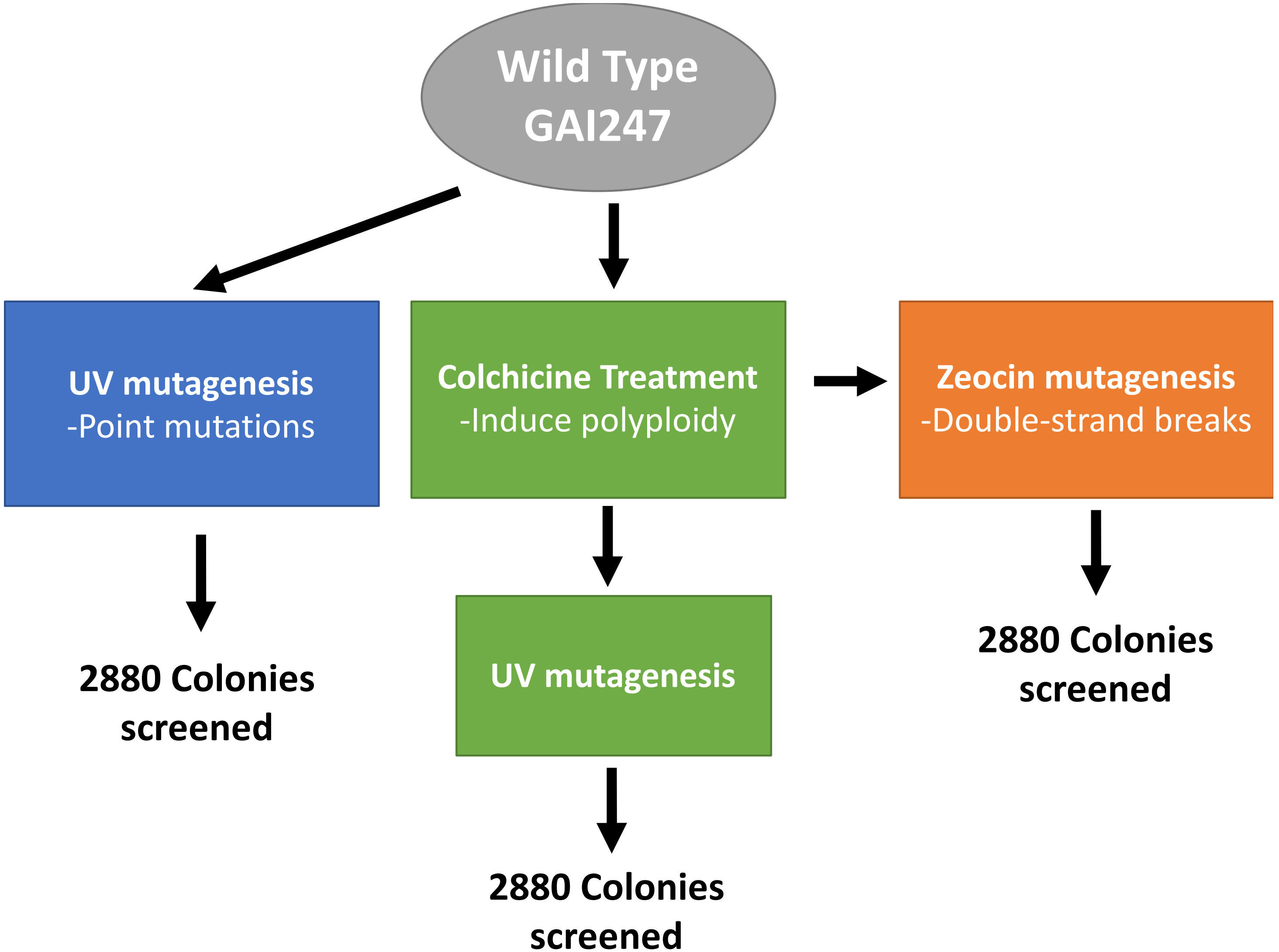
Inspired by generation of conventional cultivars of fruits and vegetables through inducing polyploidy

Colchicine inhibits mitosis and separation of chromosomes (microtubule poison).



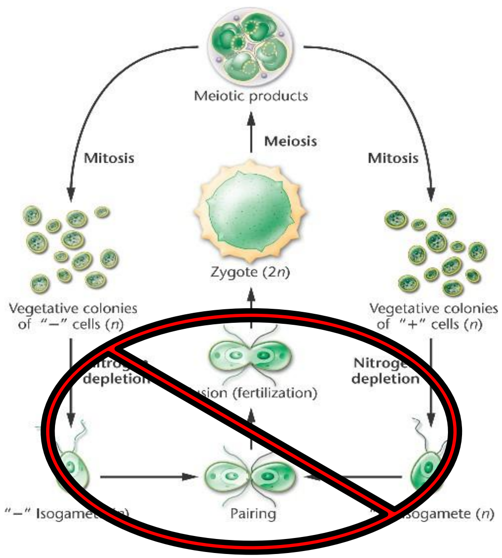
# Revised approach testing in progress



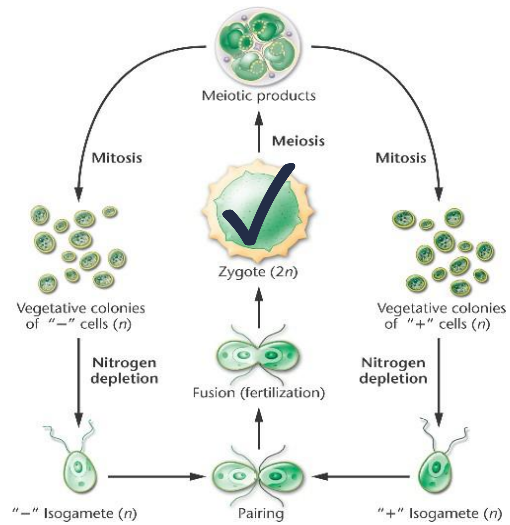
# Strain Improvement - Growth
# University of California, San Diego

## Adaptive evolution of *C. reinhardtii* for growth in bicarbonate media

Mating of C. reinhardtii strains was impossible in bicarb containing media due to loss of flagella as a response to elevated pH and salt. (Flagella are required for Zygote fusion)




By shifting back to fresh water we were able to successfully mate strains together and create robust hybrids




**Cycling between adaptive evolution & shifting to fresh water to mate strains created robust hybrids that were capable of growth in bicarbonate media**

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# Risk Reduction – Isolated more strains

## Adaptive evolution of San Diego strains

- 8 additional strains isolated in San Diego were selected for adaptation to GAI media
- 3 of the 8 were capable of growing in GAI media
  - *C. moewusii*
  - *C. vulgaris*
  - *D. armatus*

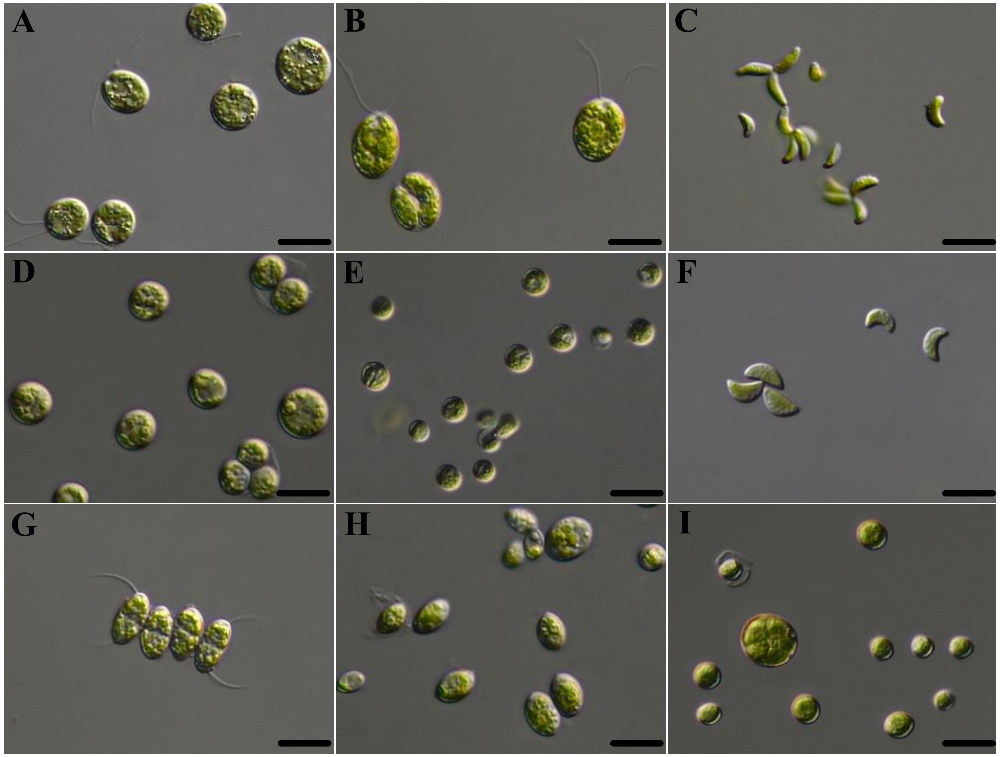


**Figure 2** Light microscope images of *Chlamydomonas reinhardtii* (A), *Chlamydomonas moewusii* (B), *Tetranephris sp.* (C), *Coelastrella sp.* (D), *Chlorella vulgaris* (E), *Monoraphidium sp.* (F), *Desmodesmus armatus* (G), *Acutodesmus obliquus* (H), and *Parachlorella kessleri* (I). Scale bars are 10 µm.

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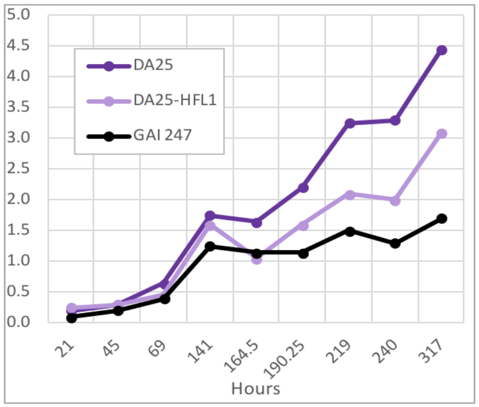
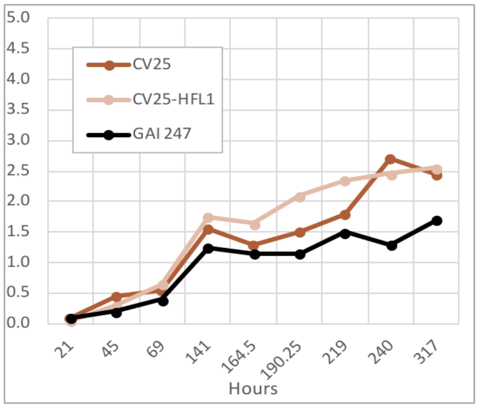
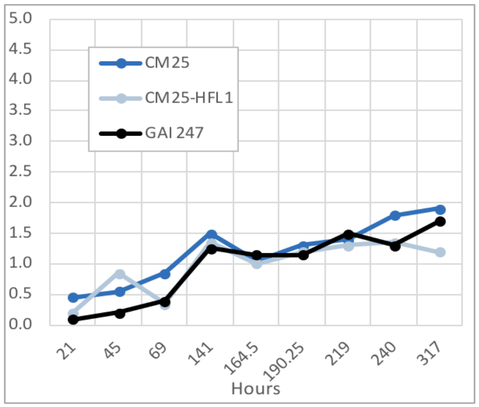
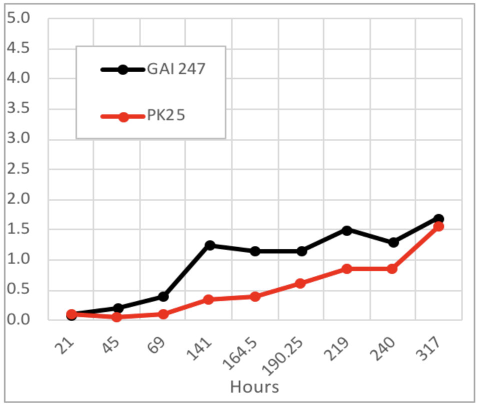
# Initial comparison of top mutants to baseline species, GAI-247

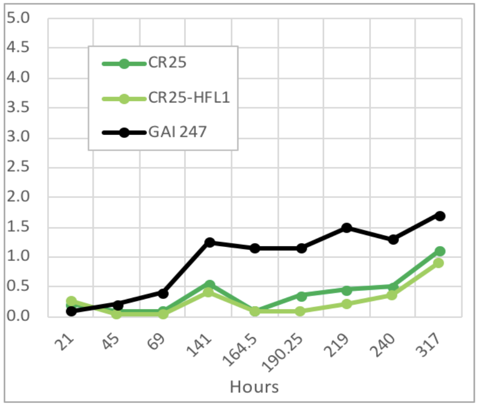
## Biomass (g/L) vs Time




GAI 247 = *Coccomyxa, sp.*
CV = *Chlorella vulgaris*
CM = *Chlamydomonas moewusii*
CR = *Chlamydomonas reinhardtii*
DA = *Desmodesmus armatus*
PK = *Parachlorella kessleri*

**DA wild type and mutant achieved in the best biomass production**
CV Better than baseline, CM, PK same as baseline, and CR below baseline

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# Technical Progress - Strain Development
# Global Algae Innovations

Directed Evolution

GAI-247 Max Temperature = 36-37° C

GAI-285 Max Temperature = 39-40° C

| Test Months | Productivity ($gm^2d$) |
|---|---|
| ABY1 Feb – June | 23 |
| ABY1 June - July | 14 |
| ABY2 May | 24 |
| ABY2 July | 29 |
| ABY2 Aug | 25 |
| ABY2 Nov | 28 |

Targeted strain collection & selection

GAI-220 Protein is 72% of dry weight

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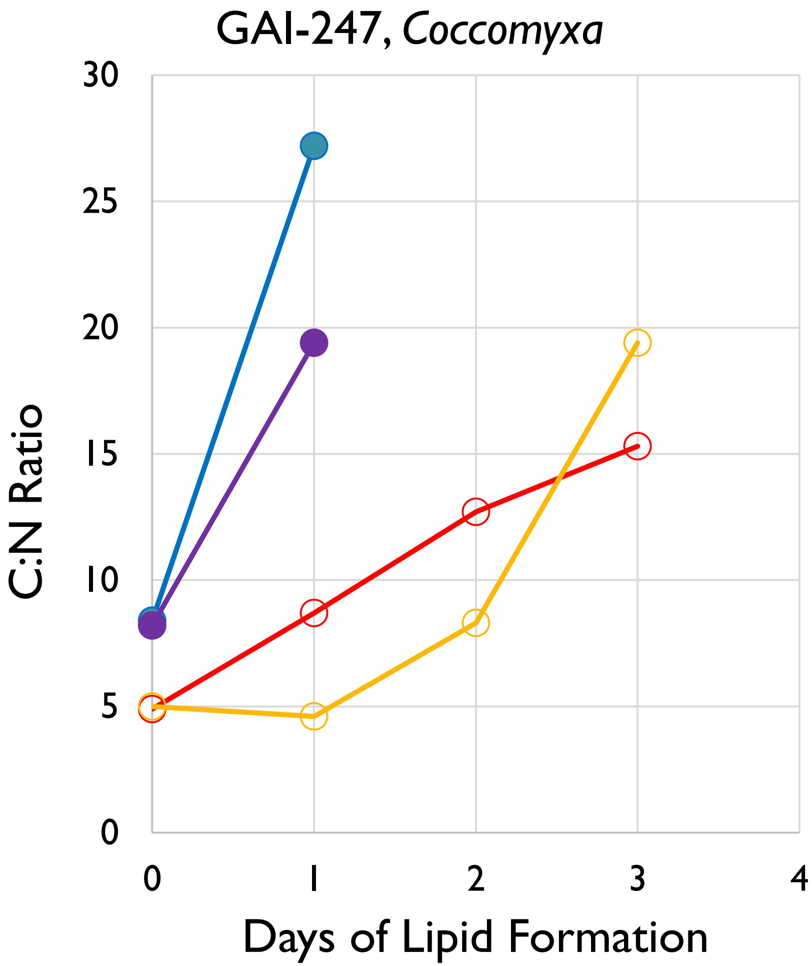
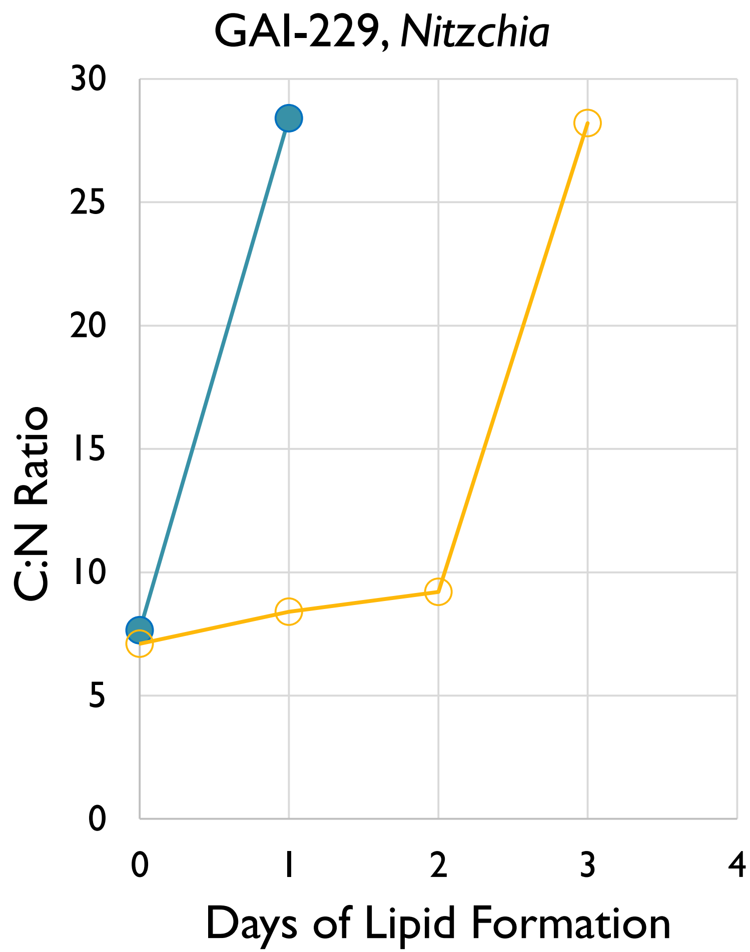
# Technical Progress – Cultivation

**3x faster lipid formation**

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# Summary of Lipid Productivity

| | Growth ($g/m^2d$) | Lipid ($g/m^2d$) | Lipid (days) | Lipid Area | Average ($g/m^2d$) | Lipid Content | Productivity (gal/ac-yr) |
|---|---|---|---|---|---|---|---|
| ABY1 | 27 | 6 | 4 | 58% | 15 | 35% | 2200 |
| 1-d formation | 28 | 8 | 1 | 25% | 23 | 36% | **3500** |
| | | | | | | | |
| Higher Prod | 28 | 12 | 1 | 25% | 24 | 36% | 3700 |
| Higher Cont. | 23 | 8 | 2 | 40% | 20 | 44% | 3700 |

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# Technical Progress – Processing

<u>Baseline (% of biofuel)</u>
Pre-processing 87%
Drying 57%

| ABY1 | Power (kJ/kg) | Heat (kJ/kg) | Total (kJ/kg | % of Biofuel | Energy Reduc. |
|---|---|---|---|---|---|
| Harvesting | 170 | 0 | 170 | 1.2% | |
| Lysis | 250 | 220 | 470 | 3.4% | |
| Extraction | 50 | 630 | 680 | 4.9% | |
| Total | 470 | 850 | 1320 | 9.6% | 89% |
| | | | | | |
| VCR-dry | 220 | 4570 | 4790 | 35% | 39% |

<u>ABY1</u>
Extruded Collets
Solvent Extraction

| ABY2 | Power (kJ/kg) | Heat (kJ/kg) | Total (kJ/kg | % of Biofuel | Energy Reduc. |
|---|---|---|---|---|---|
| Harvesting | 170 | 0 | 170 | 1.2% | |
| Lysis/Extraction | 80 | 100 | 180 | 1.3% | |
| **Total** | **250** | **100** | **350** | **2.5%** | **97%** |
| | | | | | |
| **Drying** | **30** | **630** | **660** | **4.8%** | **92%** |

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# Technical Progress - Other

- Developed, validated, and applied for patents on Zobi harvester® system improvements that have led to commercial technology
- Completed extensive testing of combined drying & extraction
  - Reduced time for separation from to 1 hr from 24 hrs
  - Found major issue, so eliminated this approach
- Developed 5 new unit operations for new low energy and low cost extraction and drying processes; anticipate multiple patent applications
- Developed and applied for patents on new energy savings and flow dynamics approach for raceways
- Demonstrated improved lipid formation approaches:
  - 33% greater productivity
  - 3x faster lipid accumulation
- Designed and built prototype lab PBR to better simulate outdoor raceways
  - Many options available for increased productivity & lipid formation
  - Faster strain improvement testing
- Development of scale-down for the open raceway cultivation improvements that double the productivity during growth phase
  - Initial approaches inadequately represent the physics below 0.2-acre
  - Designed new 2m$^2$ raceway to overcome these issues

# 4 – Relevance

**Higher yield, lower energy use, lower cost algae biofuel intermediate production in an integrated outdoor facility**

**Directly Supports the BETO mission to** "Develop and demonstrate transformative and revolutionary bioenergy technologies for a sustainable nation."

**Goal aligned with major Algal R&D targets:**

- Higher biofuel intermediate yield
- Lower energy for processing algal biomass
- Lower cost of algal biofuel

**Technology advance objectives**

- Advanced cultivation and strain improvements for a very high productivity system
- Much lower energy and cost for harvesting, extraction, and drying
- Higher productivity, lower cost processing, and protein co-product to enable cost competitive algal biofuel

**Tech Transfer/marketability**

- Incorporating advances into integrated biorefinery project
- Zobi harvester® is commercially available
- Partnering with multiple teams on DOE FOAs
- Building IP portfolio that covers the entire process with dozens of innovations
- Building cultivation and processing database

# 5 – Future Work

- **Strain improvement**
- **Integrated lipid formation and extraction testing**
- **Higher conversion in hydrothermal liquefaction**
- **Update to the technoeconomic model**

# Summary

1. **Overview:** Addresses key BETO targets - yield, energy use, cost
2. **Approach:** Comprehensive cost model and development map
   Early risk retirement with many technology options
   Rapid feedback to accelerate development
   Fully integrated outdoor operations
3. **Technical Accomplishments/Progress/Results:**
   - 60% improvement in lipid productivity over ABY1 (project goal is 70%)
   - Faster lipid formation and higher temperature strains
   - 75% reduction in processing energy use over ABY1 (project goal is 50%)
   - Five new processing unit operations
   - 25% reduction in algal biofuel MSP (project goal is 20%)
4. **Relevance:** Integrated outdoor operation, leading to commercial products
5. **Future Work**
   - Cultivation & strain improvement to reach project goal for lipid productivity

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# Additional Slides

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# Responses to Reviewer Comments

Reviewer comments were accurate

Slight confusion on goals versus targets:

1. Goals are as described on first slide (which is the same as in 2017);
2. Reviewer comments discuss our targets, which are higher than the goals to drive more innovation so that hopefully goals are exceeded
3. Results so far:
   - Lipid productivity near the goal, below the target
   - Processing energy exceeded both the target and the goal
   - Algal biofuel cost exceeded both the target and the goal

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# Patents

Zobi harvester® patent applications:

- US 15/273,552
- US 15/273,558
- US 15/273,575
- PCT/ US2016/ 053203

Cultivation system and methods patent applications

- US 15/590,403
- US 15/590,441
- US 15/590,430
- PCT/US2017/31689

Preparing invention disclosures and patent applications for new cultivation technology, new extraction and drying processes, and improved algal strains

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# Commercialization and technology transfer

Zobi® available commercially

Partnering with multiple DOE teams

- Proposals with 12 different research teams
- Awards with 8 different research

Transferred high protein strain into a USDA project

- Equivalent digestibility and higher protein content than fishmeal
- Good amino acid profile - high content of all essential amino acids

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