

**U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review**

**Breeding algae for long-term stability and
enhanced biofuel production**

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Advanced Algal Systems
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This presentation does not contain any proprietary, confidential, or otherwise restricted information

Quad Chart Overview

Timeline

- Project start date: October 1, 2016
- Project end date: September 30, 2017 (1 year)
- Percent complete: 30% at time of report

Budget

	Total Planned Funding (FY 17-Project End Date)
DOE Funded	\$150,000

Barriers

- Identification of unique genetic markers for each *Chlorella sorokiniana* strain
- Successful induction of gamete formation in multiple *Chlorella sorokiniana* strains
- Successful mating of *C. sorokiniana*

Partners (LANL)

- Dr. Richard Sayre
- Dr. Taraka Dale
- Dr. Norman Doggett
- Dr. Sowmya Subramanian
- Dr. Hajnalka Daligault

1 - Project Overview

The Challenge: Over the last 75 years great strides have been achieved in development of higher yielding and more robust crops. The 7-fold enhancement in corn yields can largely be attributed to the application of modern breeding techniques including linkage analyses of easily tractable traits using molecular markers.

To date these technologies have not been applied to commercial strains of algae for the enhancement and stabilization (inbred hybrids) of strains having reliable biomass production.

The Goals: The major objectives of this proposal are to develop advanced molecular-assisted breeding systems for *C. sorokiniana* so as to breed for more stable (inbred sterile hybrids), robust, and higher yielding algal strains. This proposal addresses the BETO-MYPP Advanced Algal Systems Research and Technical Challenges on Biomass Genetics and Development.

2 – Approach (Management)

- The current project includes three part time staff scientists at LANL, one postdoc, and a part time technician.
 - **Dr. Richard Sayre** is the PI (20% effort) and is responsible for overall management and experimental design
 - **Dr. Taraka Dale** (5% effort) is responsible for managing flow cytometry analysis of genome ploidy levels and optimization of breeding strategies.
 - **Dr. Norman Doggett** (5% effort) is responsible for identification and mapping of genetic markers and development of PCR protocols for high throughput analyses.
 - **Dr. Sowmya Subramanian** is a postdoc (50% effort, reduced to 30% due to leave) on the project and carries out mating experiments and mapping of genetic markers
 - **Dr. Hajnalka Daligault** (15% effort) is generating CFP- and YFP-tagged transformants
 - **Dr. Olga Chertov** (5% effort) bioinformatics

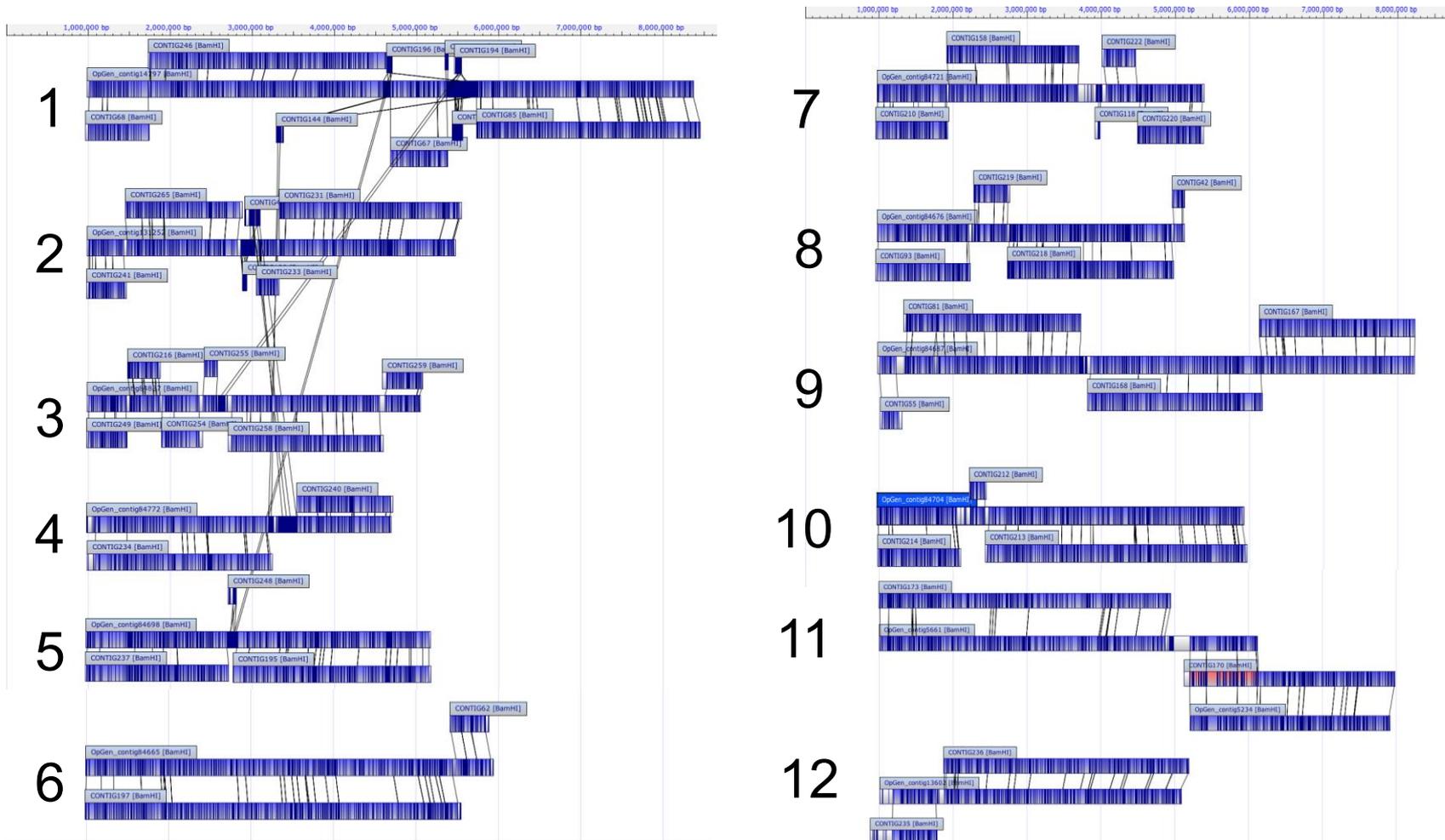
2 – Approach (Technical)

- Use LANL *in silico* bioinformatics tools to identify unique genetic markers for coarse-grain tagging of the 12 chromosomes from *Chlorella sorokiniana* (Cs) strains 1228 and 1230.
- Select an initial set of genetic markers at ~1 Mb intervals across all chromosomes and one each for the cpDNA and mtDNA based on alignment to the final ordered and oriented sequence assemblies for the Cs-1228 and Cs-1230 genomes.
- PCR amplification of unique markers for Cs-1228 and Cs-1230 DNA.
- Deploy marker-assisted tagging (CFP and YFP) in breeding experiments to demonstrate genetic exchange between Cs-1228 and Cs-1230.

3 – Technical Accomplishments/ Progress/Results

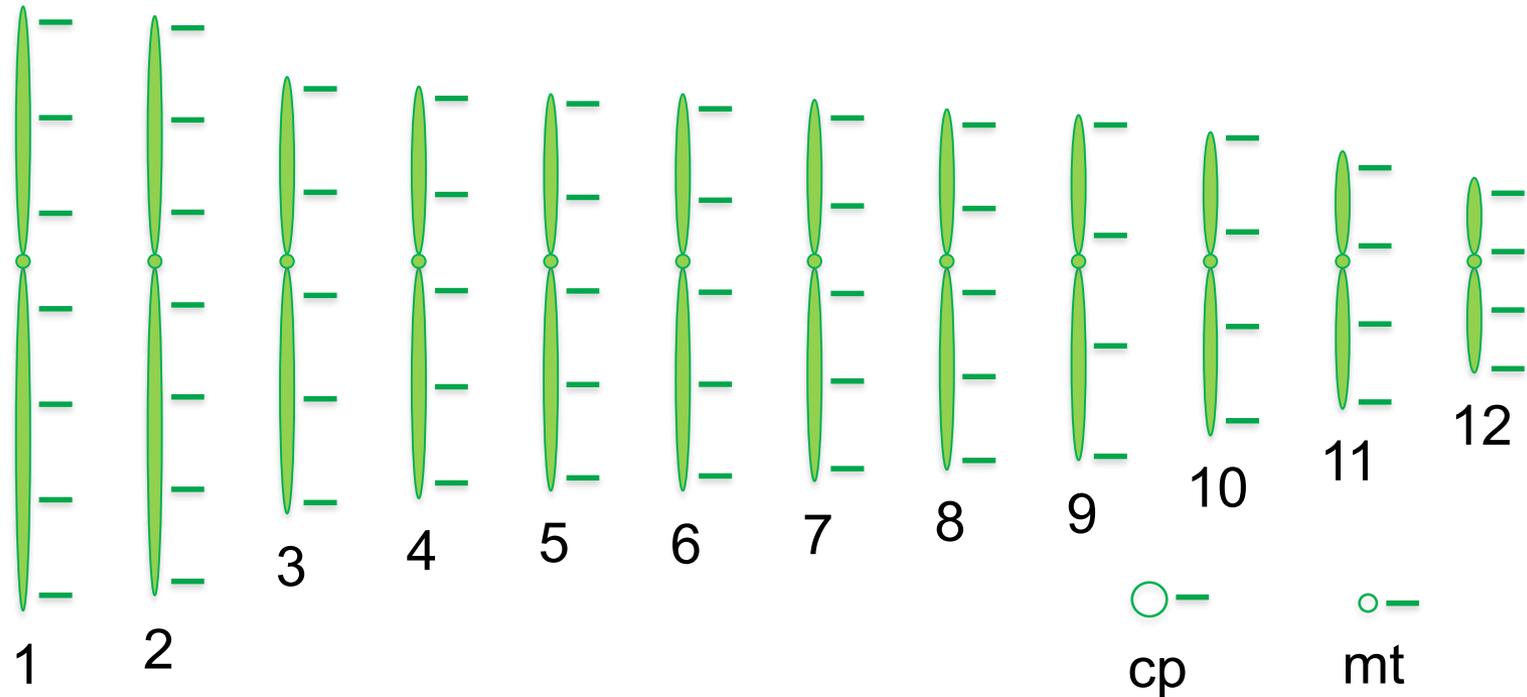
- Unique PCR markers have been identified across the Cs-1228 and Cs-1230 genomes at an average density of 37kb between markers!
- A subset of PCR marker assays have been selected and ordered at 1Mb resolution across the Cs-1228 and Cs-1230 genomes.
- Highly purified Cs-1228 and Cs-1230 has been prepared. PCR marker assay testing is underway
- Meiosis and mating genes identified in Cs
- Cs-1230 was transformed with the YFP-vector by biolistic particle bombardment method.
- For the first time ever, flagellar structures were observed in Cs-1228 following induction of gametogenesis under minus nitrogen/dark treatment.

High quality PacBio sequence assembly of Cs-1228 and Cs-1230 genomes



Unique PCR markers have been identified across the Cs-1228 and Cs-1230 genomes at an average density of 37kb between markers!

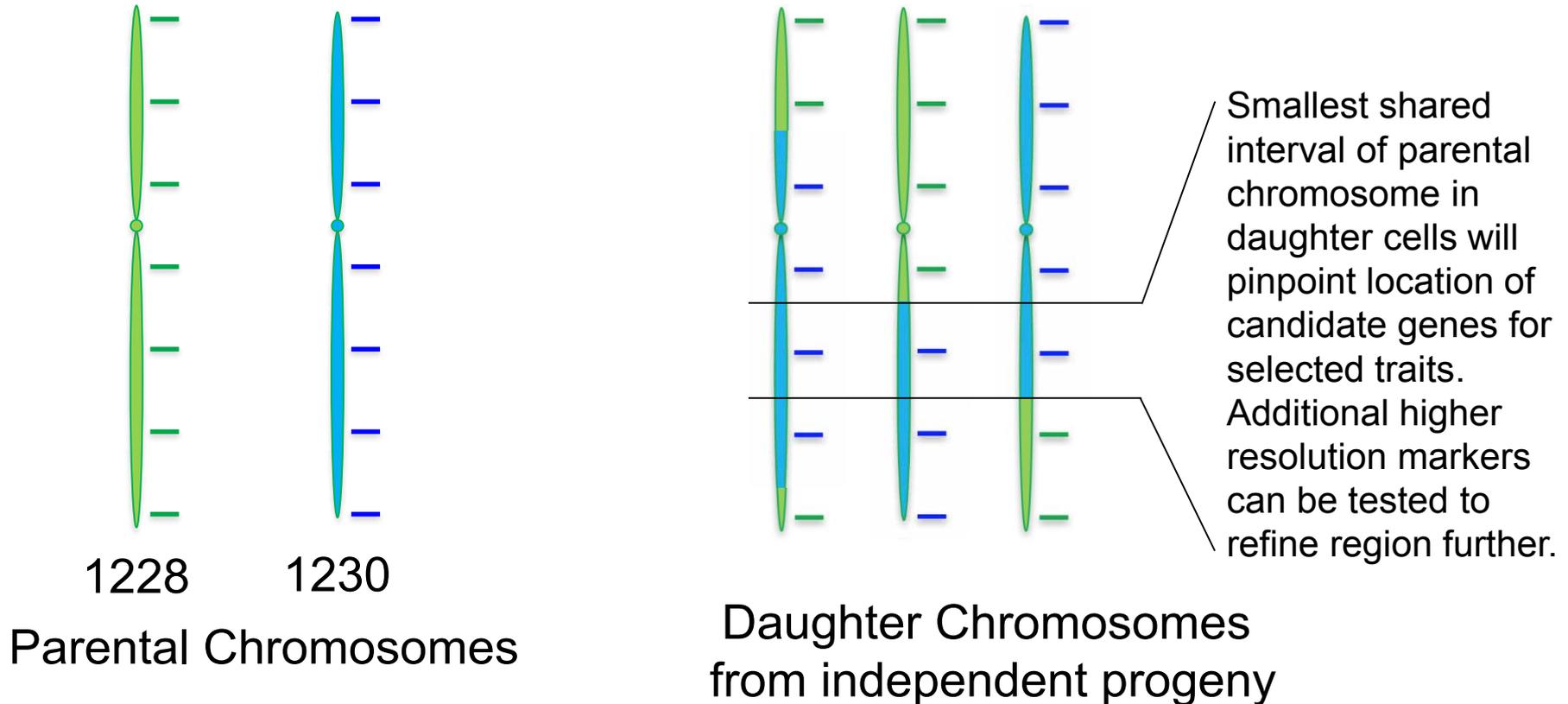
Chlorella sorokiniana unique genetic markers



62 unique PCR markers for strain Cs-1228 and 63 unique markers for strain Cs-1230 have been developed at approximately 1 Mb intervals across each of the 12 chromosomes.

Markers have also been developed for cpDNA and mtDNA.

Using genetic markers to map traits



With the development of unique genetic markers for each chromosome, we now have the tools to map genetic traits selected through genetic breeding of *Chlorella sorokiniana*.

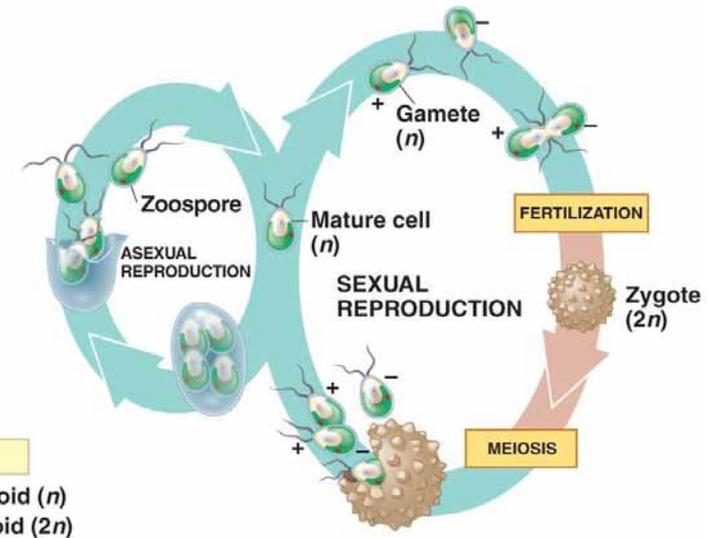
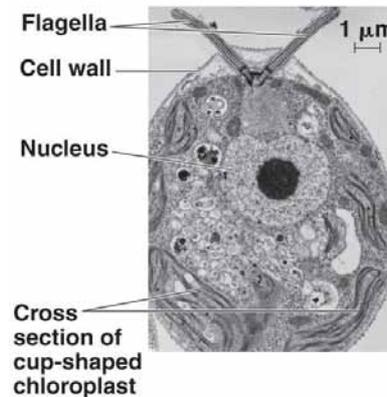
BLASTP analysis of Cs genomes indicates presence of meiosis and flagellar genes as well as strain-specific variations in their sequences

	<i>C.reinhardtii</i>	Cs 1230 E value	Cs 1228 E value	
Meiotic recombination protein	SPO11A	e-166	e-121	
Minichromosome maintenance protein	Mcm8	2e-91	2e-91	
Hsp70-Hsp90 organizing protein	Hop1	e-165	e-170	
DNA mismatch repair protein	MutS	8e-44 2e-43	8e-46 2e-44	CmMsh5 GsMsh4
Axon outer dynein arm protein	AOIDA-1c	2e-54	2e-54	
Meiotic Nuclear division protein	MND1	6e-31	---	
Fusion protein	HAP2/GCS1	3e-84	7e-94	
Gamete specific protein (similar to HAP2)	FUSM	2e-81	2e-92	
Cohesion complex subunit	CCS	e-29	2e-35	
Glycogen Synthase Kinase	GSK3	e-166	e-166	

Some Cs proteins had significant homology to *C. reinhardtii* proteins, while some like MutS had no significant homology. Surprisingly, Cs genes share more homology with proteins from the red algae *Cyanidioschyzon merolae* (Cm) and *Galdieria sulphuraria* (Gs).

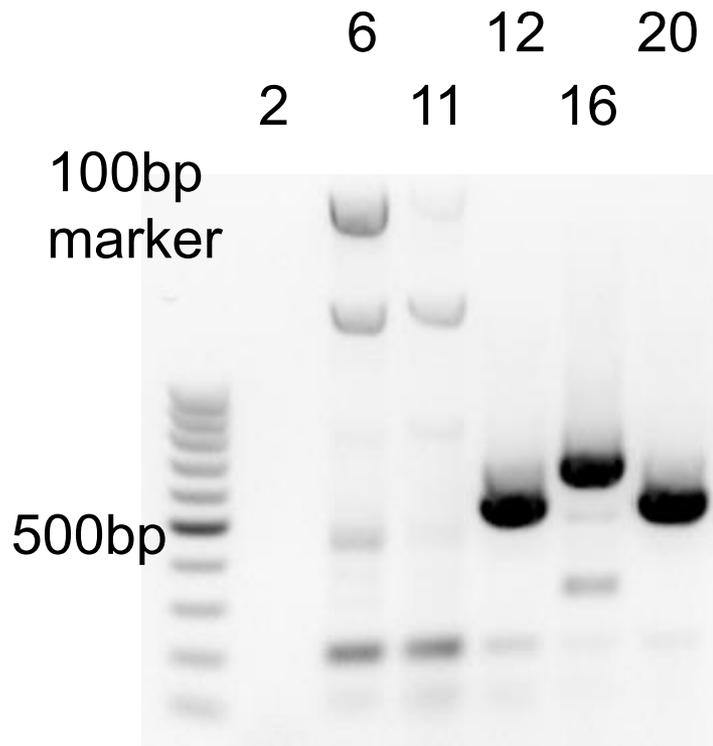
A brief SOP for induction of gametogenesis in *Chlorella sorokiniana*

- Collect light/dark cycle synchronized algae cultures 2 hours before solar dawn
- Harvest and wash one half of cells with HS media (+ N = control) and one half with HS media (- N = meiosis induction)
- Re-suspend cells in HS (+N) or HS (-N) and return to ePBR in darkness.
- Collect samples for analysis of DNA ploidy level at various time intervals



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Introduction of YFP and CFP markers into Cs-1230 and Cs-1228, respectively, to track successful matings

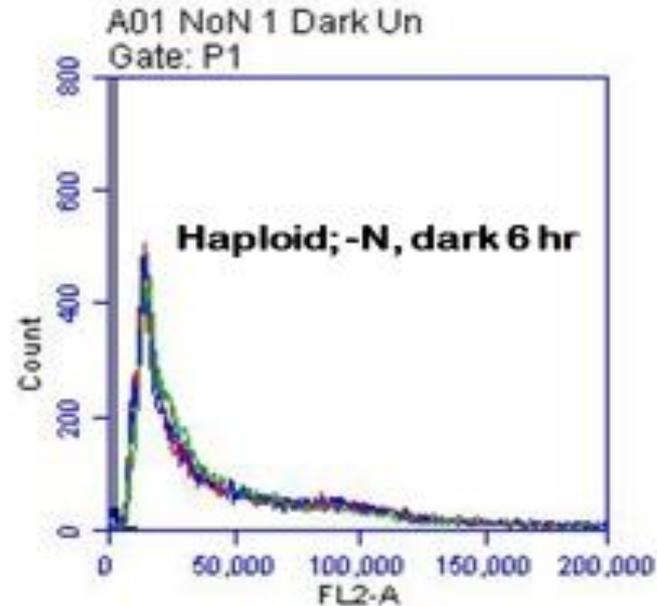
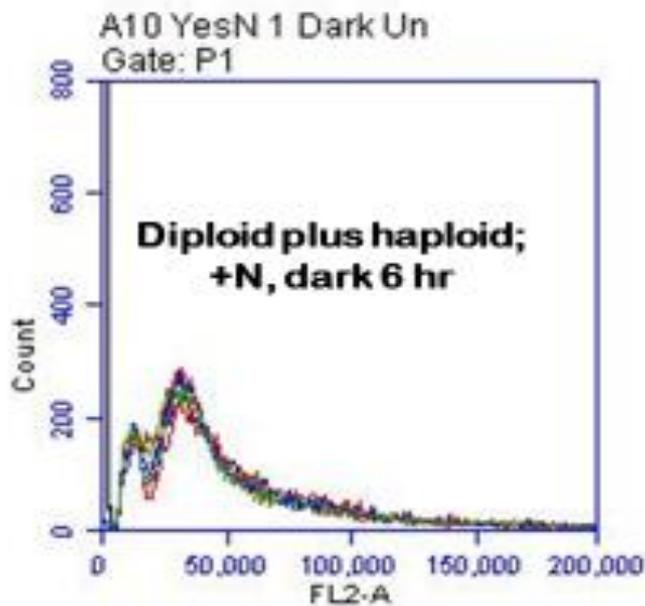


PCR amplification of *psaD* promoter/YFP construct used to transform algae. Expected size: 542bp

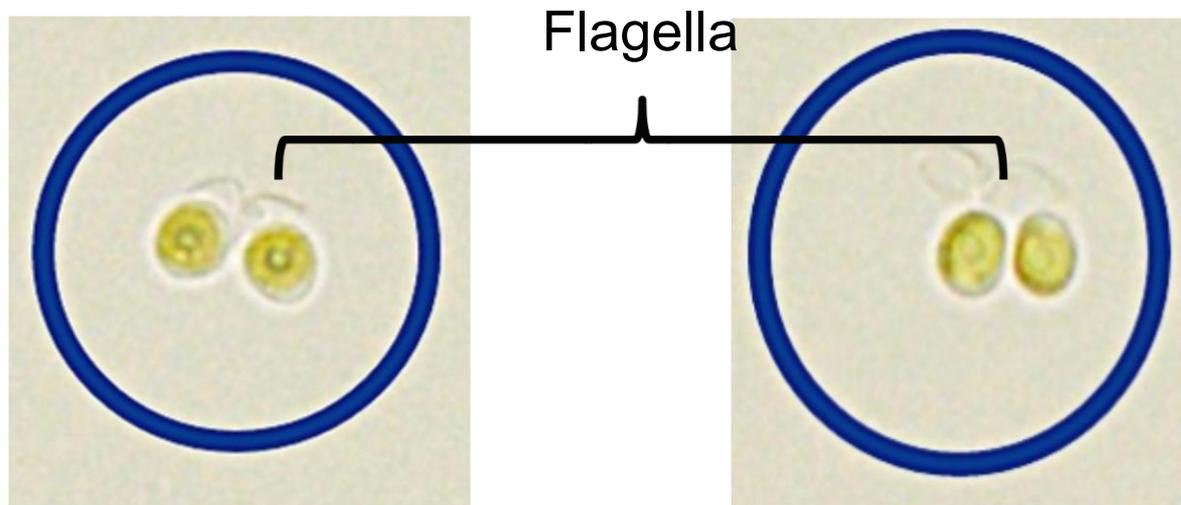
Induction of gametogenesis (haploidy) in Cs-1228 following nitrogen starvation in dark (treated)

Induction of haploidy in *Chlorella sorokiniana*

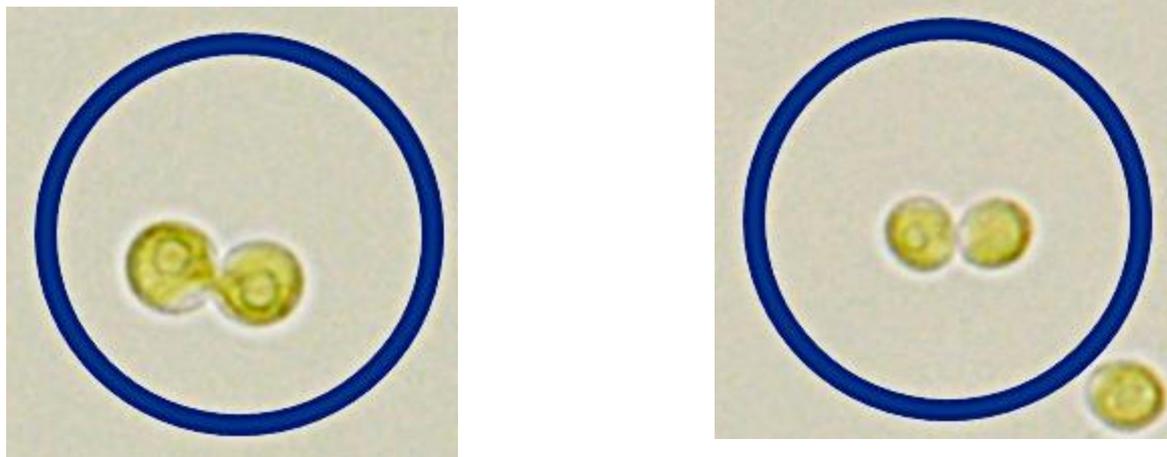
Fluorescence intensity (ploidy level) versus cell number for DNA staining dye



Light microscopic images of Cs-1228 forming gametes (flagellated) and mating 5 hours after induction



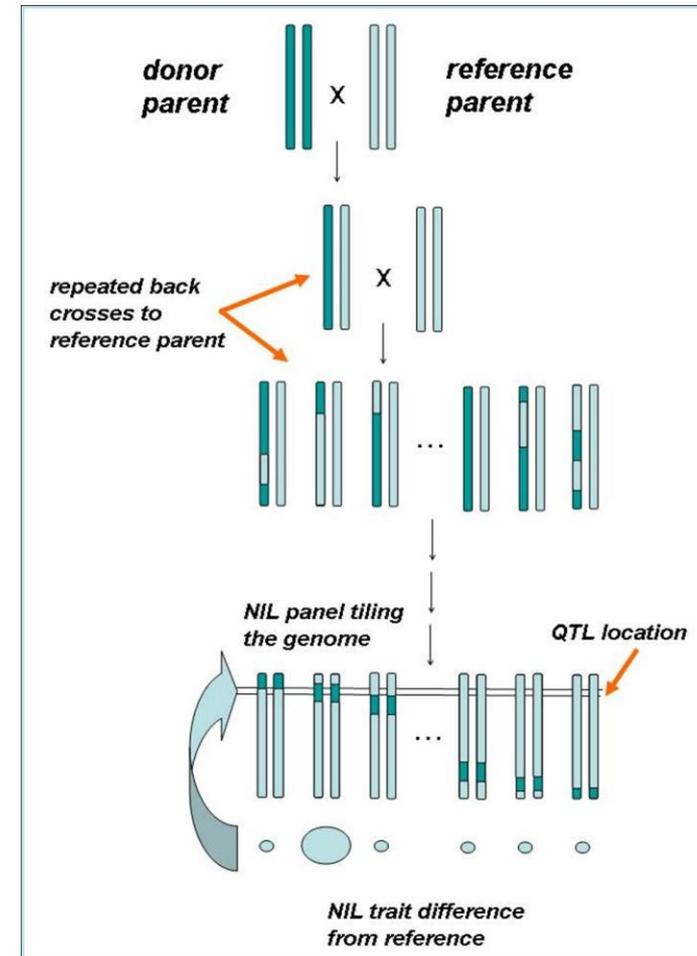
Snapshot of mating in Cs-1228



4 – Relevance

Goal: Maximizing stable biomass yields while minimizing resource use.

- Develop advanced molecular-assisted breeding systems for *C. sorokiniana* to breed for more stable (homozygous), robust, and higher-yielding hybrid algal strains.
- Utilize Marker-Assisted Selection (MAS) and Quantitative Trait Locus (QTL) mapping to breed and identify genes for enhanced biomass yields consistent within the BETO Multi-Year Program Plan.
- By making publicly available standardized mating procedures complemented with strain-specific genetic markers, we will help accelerate the development of advanced inbred hybrid algal strains for the industry.



5 – Future Work

Immediate objectives:

- Identify and characterize the YFP transformants in Cs-1230 and CFP transformants in Cs-1228 to use as markers to optimize transformation efficiencies (Select for progeny with both CFP and YFP).
- Generate homozygous, in-bred lines of algae with greater yield and stress tolerance traits.

Key Milestones and Go/No-Go:

- **Demonstration of unique genetic markers for Cs-1228 and Cs-1230**
- Generation of fluorescent tagged Cs-1228 and Cs-1230 strains and co-expression in hybrids
- Crossing of Cs-1228 and Cs-1230 to generate hybrids followed by backcrossing to develop in-bred lines
- The major Go/No-Go decision will be demonstration of cross-breeding within and between CS-1228 and Cs-1230 using the CFP and YFP tagged lines.
- Budget expenditures are on schedule and sufficient to complete proposed work over the next 6 months.

Summary

- 1. Major objectives:** To develop advanced molecular-assisted breeding systems for *C. sorokiniana* to breed for stable (homozygous), robust, and higher yielding hybrid algal strains
- 2. Approach:** 1) Identification and saturation of genomes with unique genetic markers to map traits, 2) Development of robust mating protocols, 3) Demonstration of hybrid strains.
- 3. Accomplishments:** 1) Strain-specific markers identified from genome across all 12 chromosomes, 2) Induction of gametes and breeding achieved, and 3) Tagging of lines with CFP and YFP markers to demonstrate sexual crosses in progress
- 4. Relevance:** Demonstration of marker-assisted breeding tools will allow for selection and fixation of advanced traits in breeding populations
- 5. Future work:** Selection for enhanced starch accumulation lines and identification of genes that control this process. Development of sterility systems to fix genotypes.