



DOE Hydrogen Program

# Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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**Project ID # PD036**

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



# Overview

## Timeline

- **Start: 01-Dec-2004**
- **End: 30-Nov-2011**
- **Completion: 85%**

## Budget

- Total Project Funding  
**DOE: \$1.2 M, UCB: \$300 k**
- Funding for FY2011  
**DOE: \$140 k, UCB: \$75 k**

## Barriers addressed

- **Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size (Barrier X).**

## Partners

- **None: Sole Source Effort**



# Objectives and Approach

**Objective:** Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(Identify and characterize genes that regulate the Chl antenna size in the model green alga *Chlamydomonas reinhardtii*. Apply these genes to other green algae, as needed.)

**Approach:** Interfere with the molecular mechanism for the regulation of the chlorophyll antenna size.

(Employ DNA insertional mutagenesis and high-throughput screening to isolate tagged green algae with a smaller Chl antenna size.)





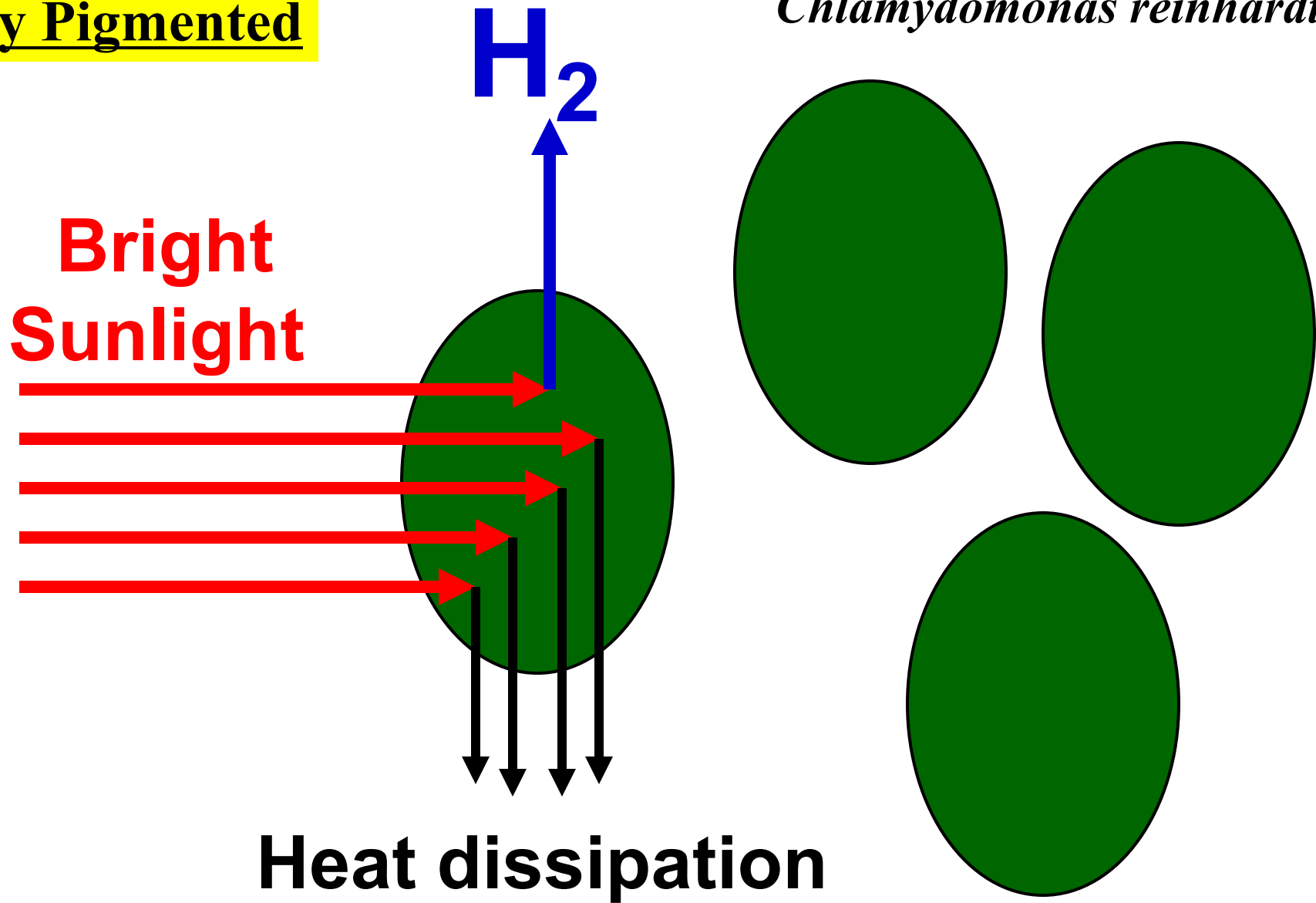
**Hydrogen production  
in a backyard**

*Chlamydomonas reinhardtii* mass culture



**Example:**  
**Fully Pigmented**

The green algae  
*Chlamydomonas reinhardtii*

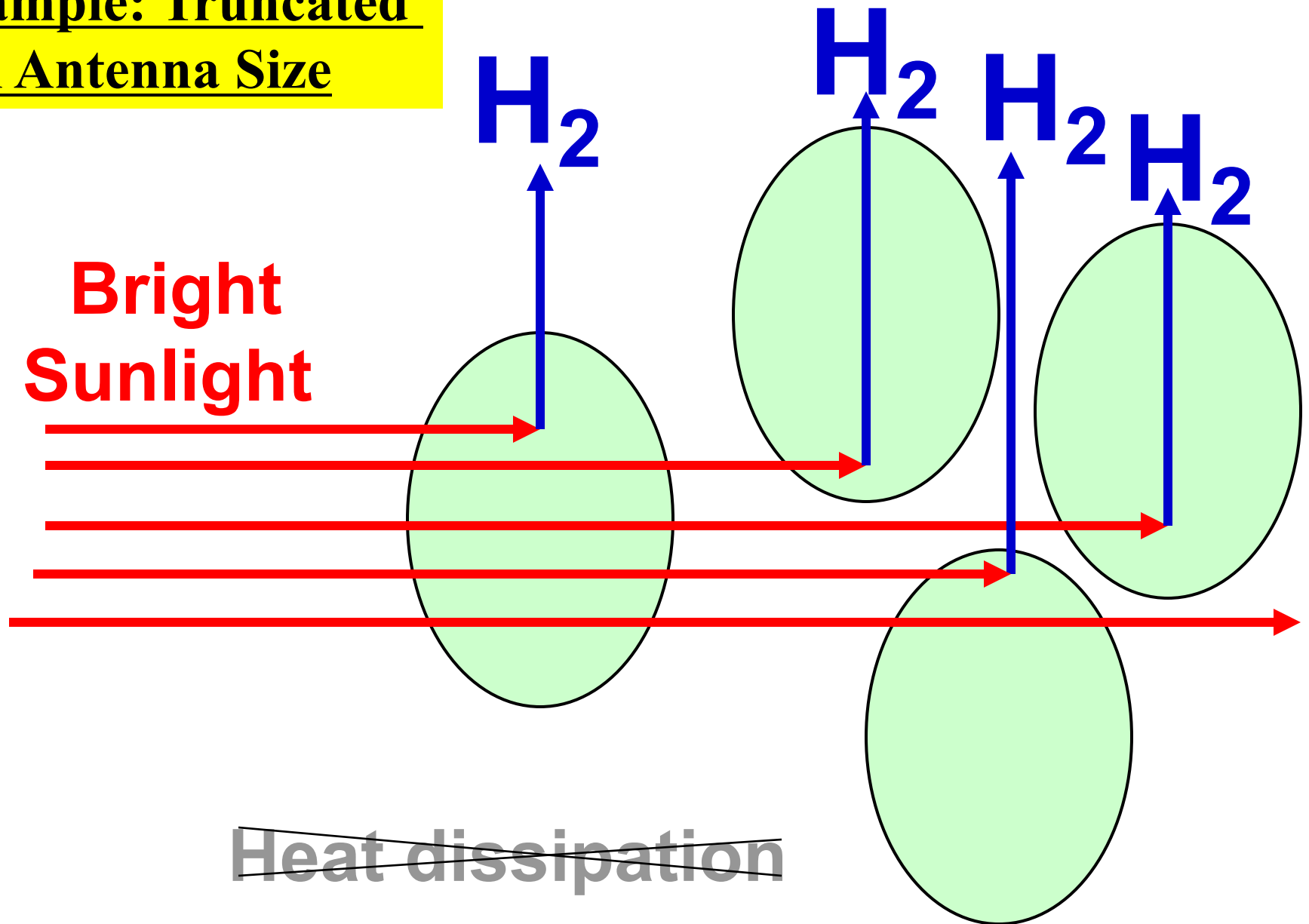


Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.





## Example: Truncated Chl Antenna Size



Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.



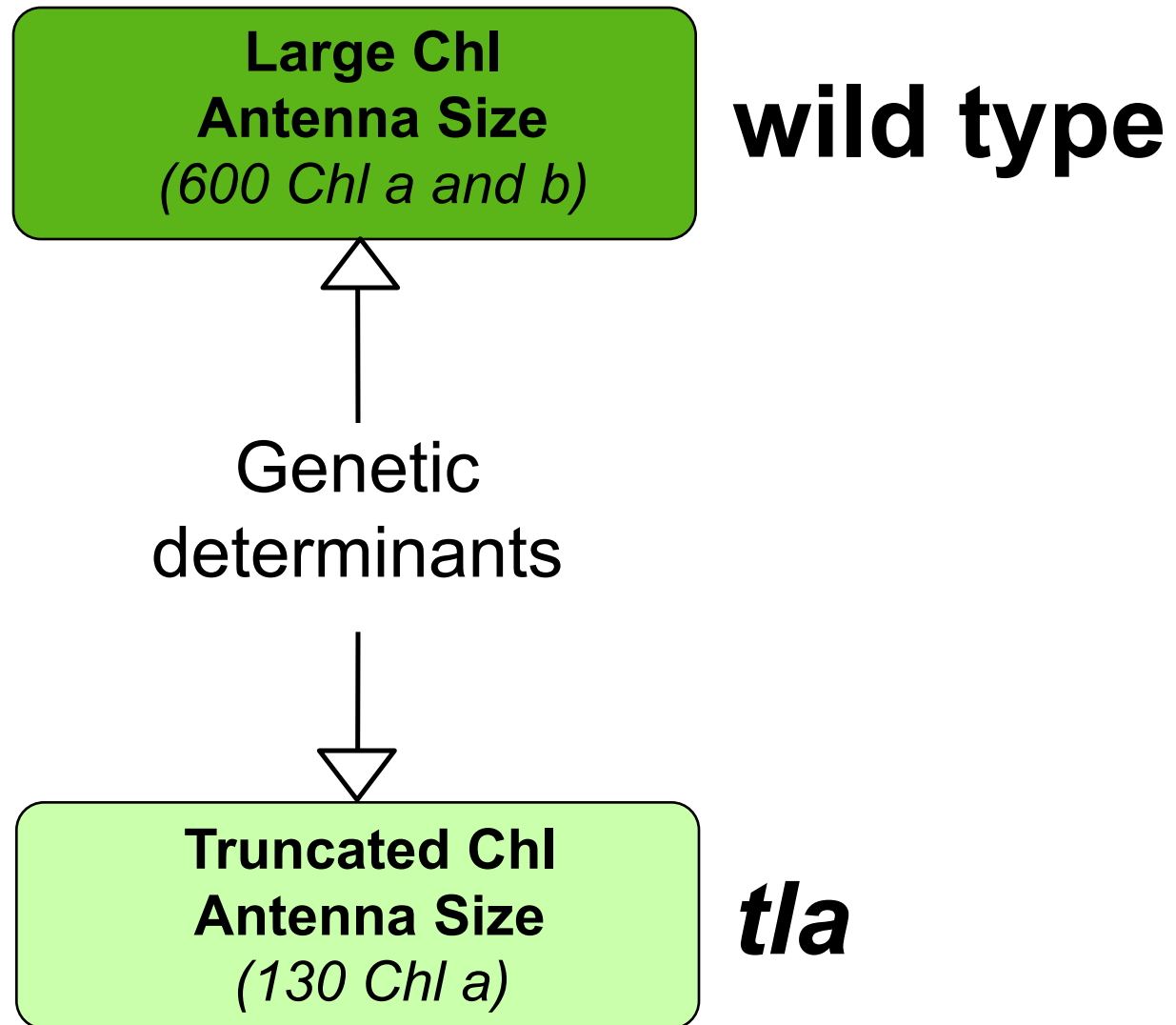
# The *Tla* concept

(*Tla* = Truncated light-harvesting antenna)

**Minimize the chlorophyll antenna size of photosynthesis to prevent the early light-saturation effect.**



# Regulation of the Chl antenna size



Interference with the genetic mechanism for the regulation of the Chl antenna size, to derive a permanently truncated Chl antenna size, is the goal of this R&D.





# Approach

- Identify genes that confer a truncated antenna in a model organism.
- Apply these genes to other organisms of interest.
- Improve photosynthesis, hydrogen, or fuels production by up to 300%.



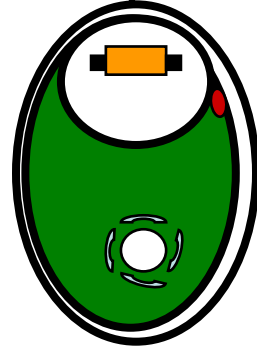
# DNA insertional mutagenesis and screening

**ARG7**

Plasmid DNA

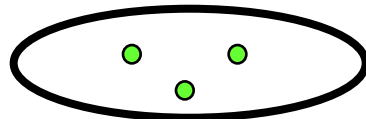
**TRANSFORMATION**

(Random insertion of plasmid DNA  
in the nuclear genome)



*Chlamydomonas reinhardtii*

**GROW TRANSFORMANTS**

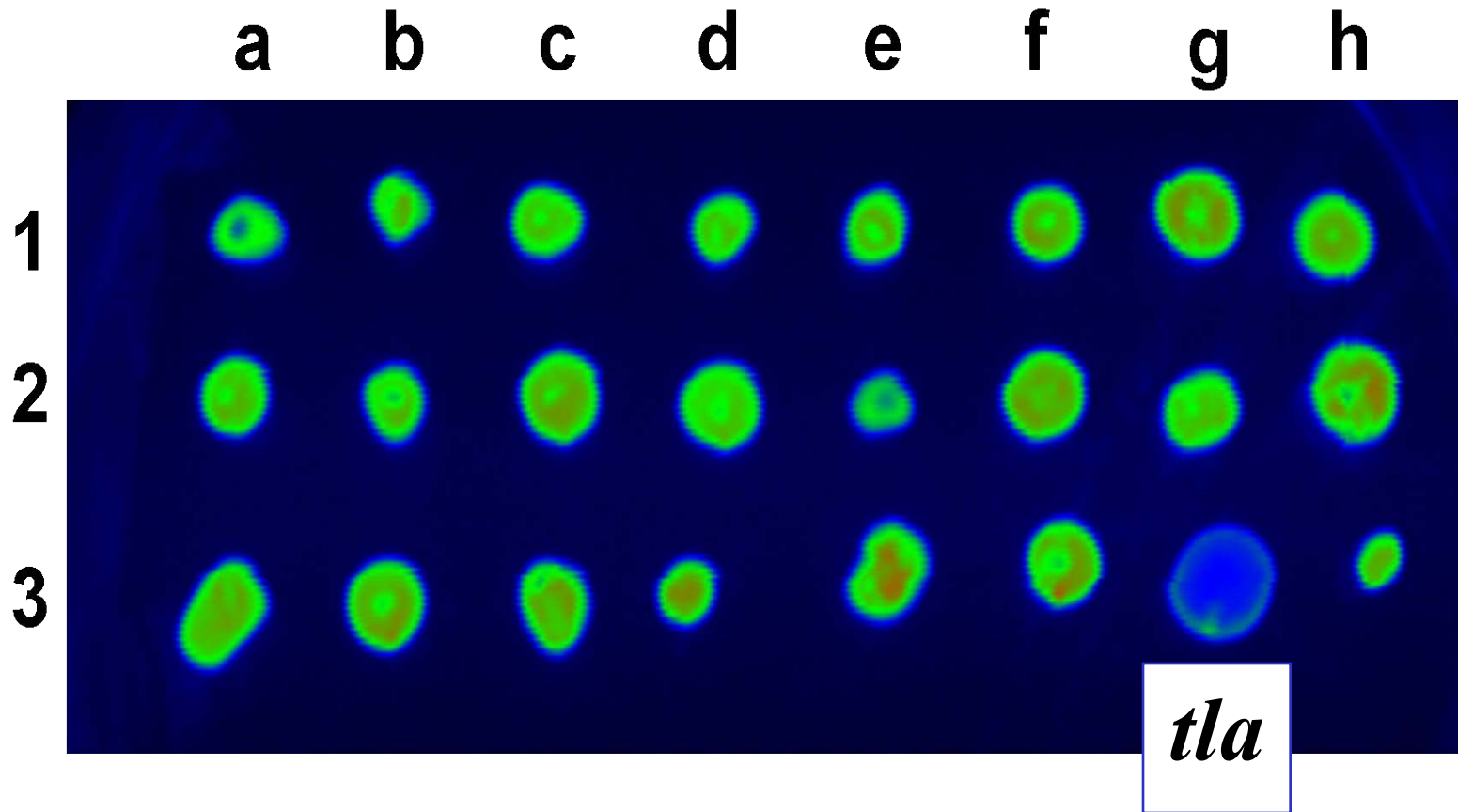


**SCREEN TRANSFORMANTS**

**SELECT TRUNCATED ANTENNA STRAINS**



# DNA insertional mutagenesis and screening



low

control

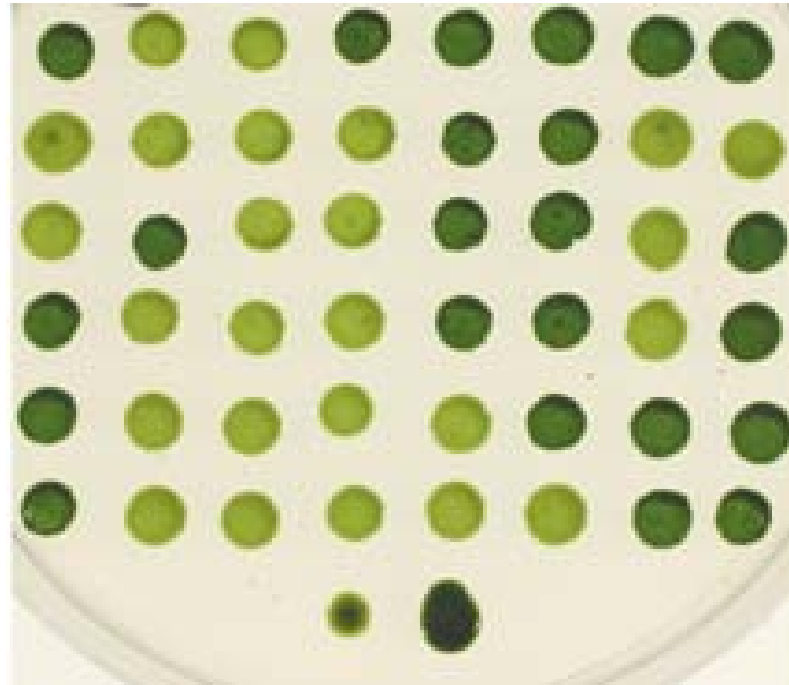
high

## Chlorophyll *a* fluorescence imaging analysis

UCB-Melis

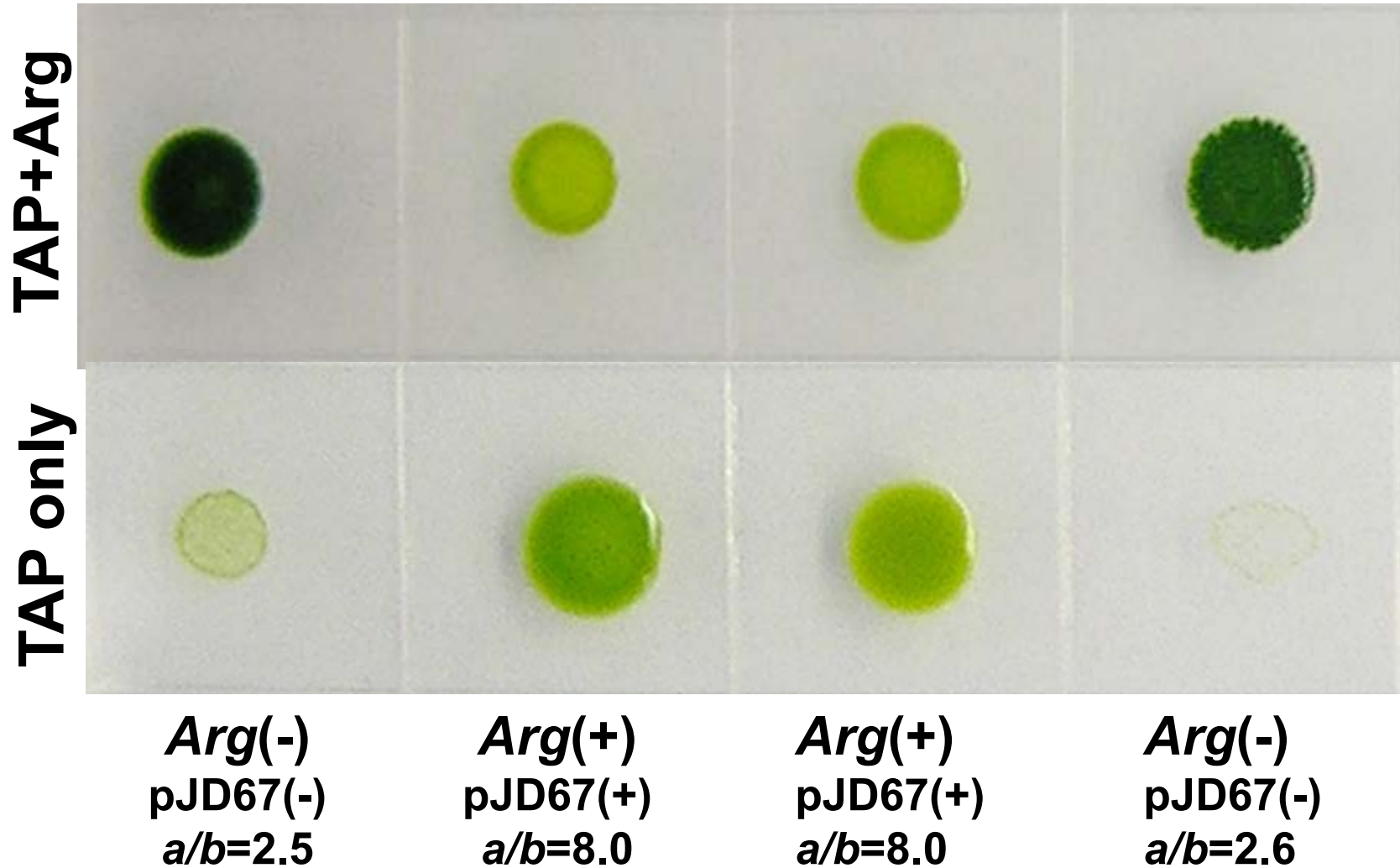


# DNA insertional mutagenesis and screening for *truncated light-harvesting antenna (tla)* mutants



**Dot-Spot Colonies of  
*Chlamydomonas reinhardtii***

# “Tetrad Analysis” of Progeny from a Single *tla2xArg(-)* Zygosporangium



$Arg^- = AGI \times 3.24 = 4A(mt^-)Arg(-)$



# Secondary screening for *tla* mutants

## Criteria:

- Functional photosystem antenna size *smaller than wild type*
- Number of photosystems per chloroplast should be *the same or greater than wild type*
- High quantum yield of photosynthesis is maintained
- Photosynthesis & productivity per chlorophyll: *inversely proportional to antenna size*

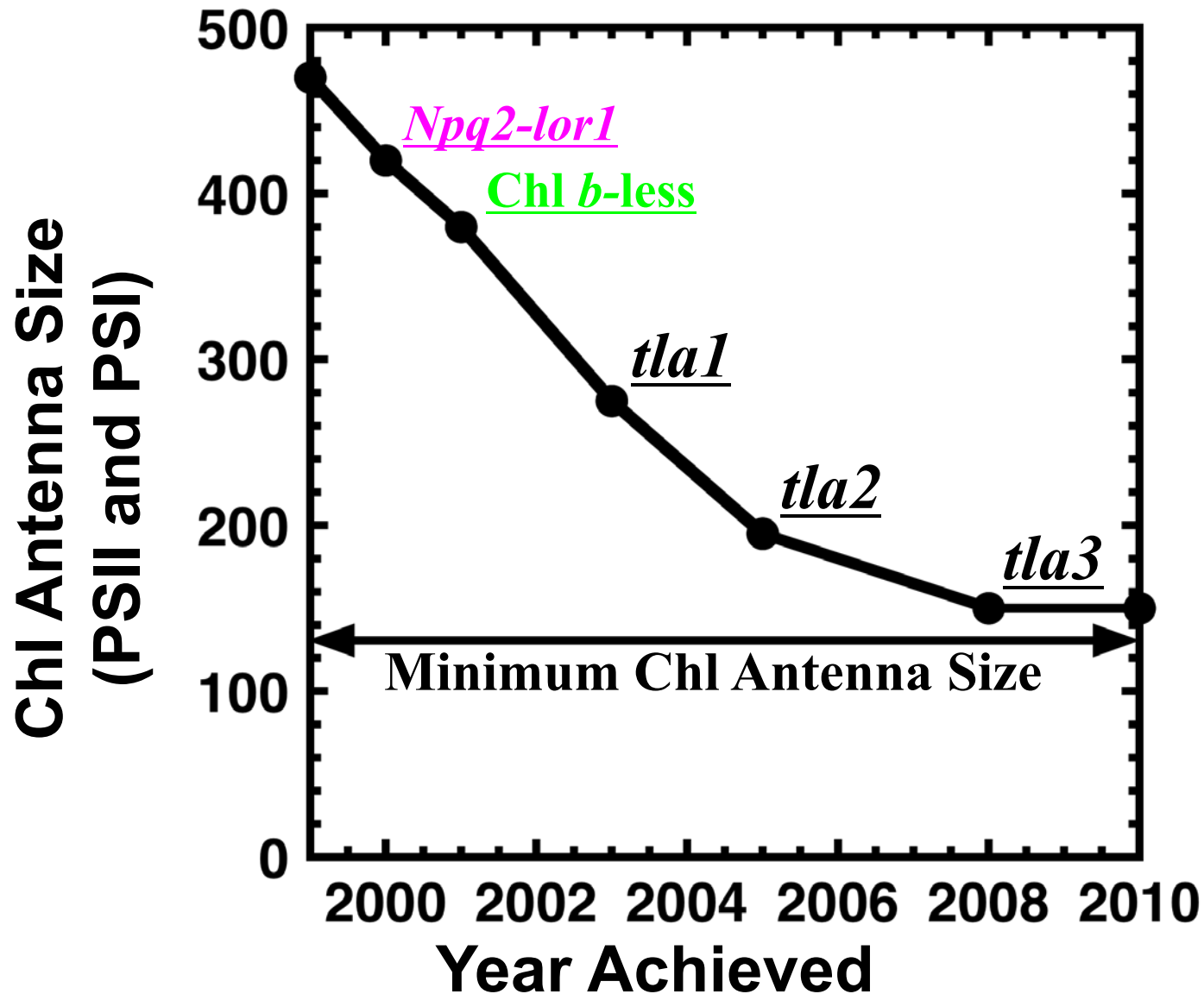
## Objective:

- Identify “true positive” *tla* mutants with **improved sunlight utilization efficiency**.



# Project Timeline

## Chlorophyll Antenna Size in Chlamydomonas





# Progress achieved vs the DOE targets

Chlorophyll antenna size in wild type and mutants

(minimum possible = 130 Chl molecules)

	2000	2003	2005	2008	2010	2015
<b>Targets</b> <i>(Chl Antenna size)</i>	<b>600</b> <b>(WT)</b>		<b>300</b>		<b>200</b>	<b>150</b>
<b>Progress Achieved</b>	<b>600</b> <b>(WT)</b>	<b>275</b> <b><i>tla1</i></b>	<b>195</b> <b><i>tla2</i></b>	<b>150</b> <b><i>tla3</i></b>		



# Progress achieved vs the DOE targets

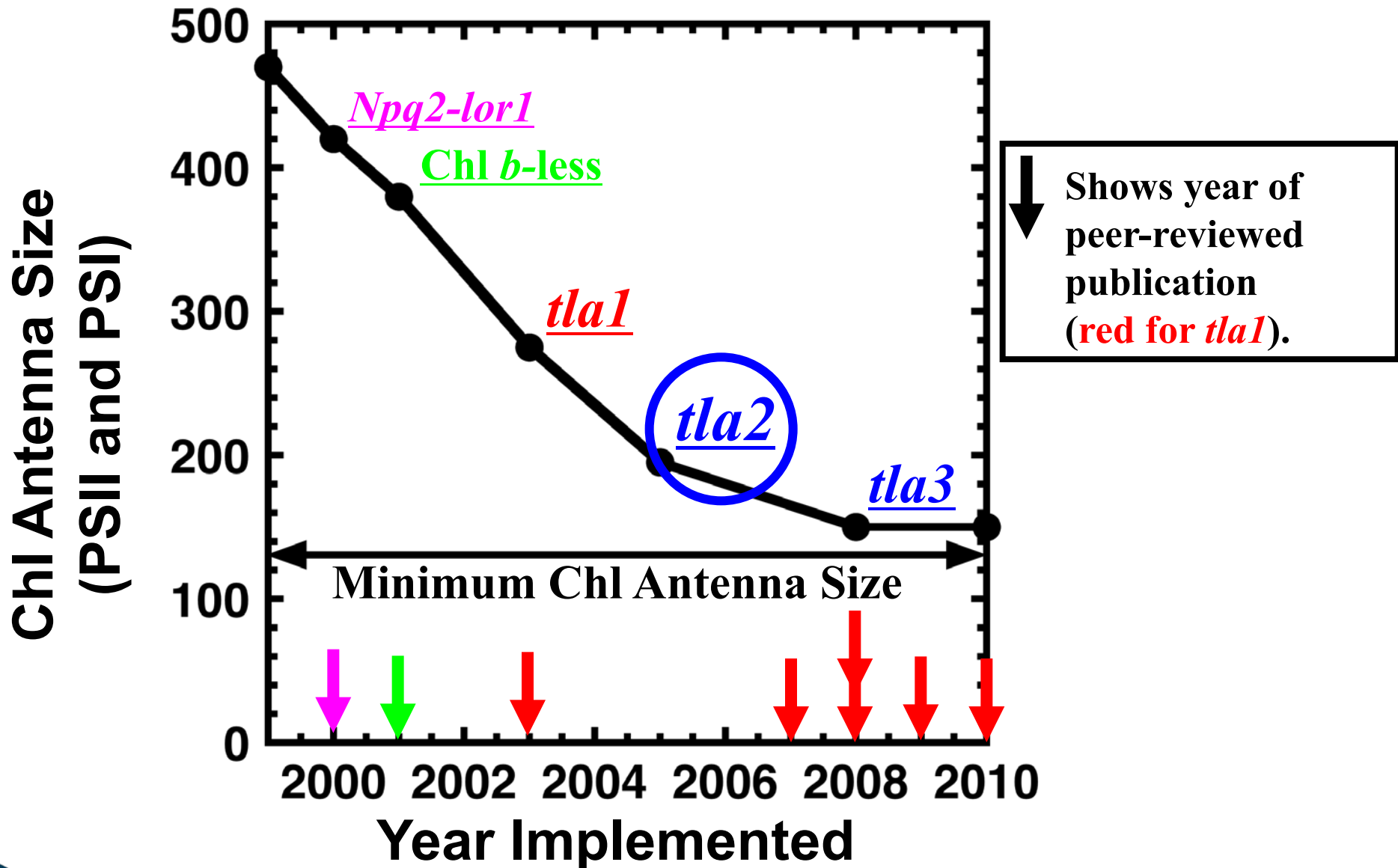
Utilization Efficiency of Incident Solar Light Energy,  $E_0 \times E_1$ , %  
(maximum  $E_0 \times E_1$  possible = 30%)

	2000	2003	2005	2008	2010	2015
Program Targets	3%	10%			15%	20%
Progress	3%	10% <i>tla1</i>	15% <i>tla2</i>	25% <i>tla3</i>		



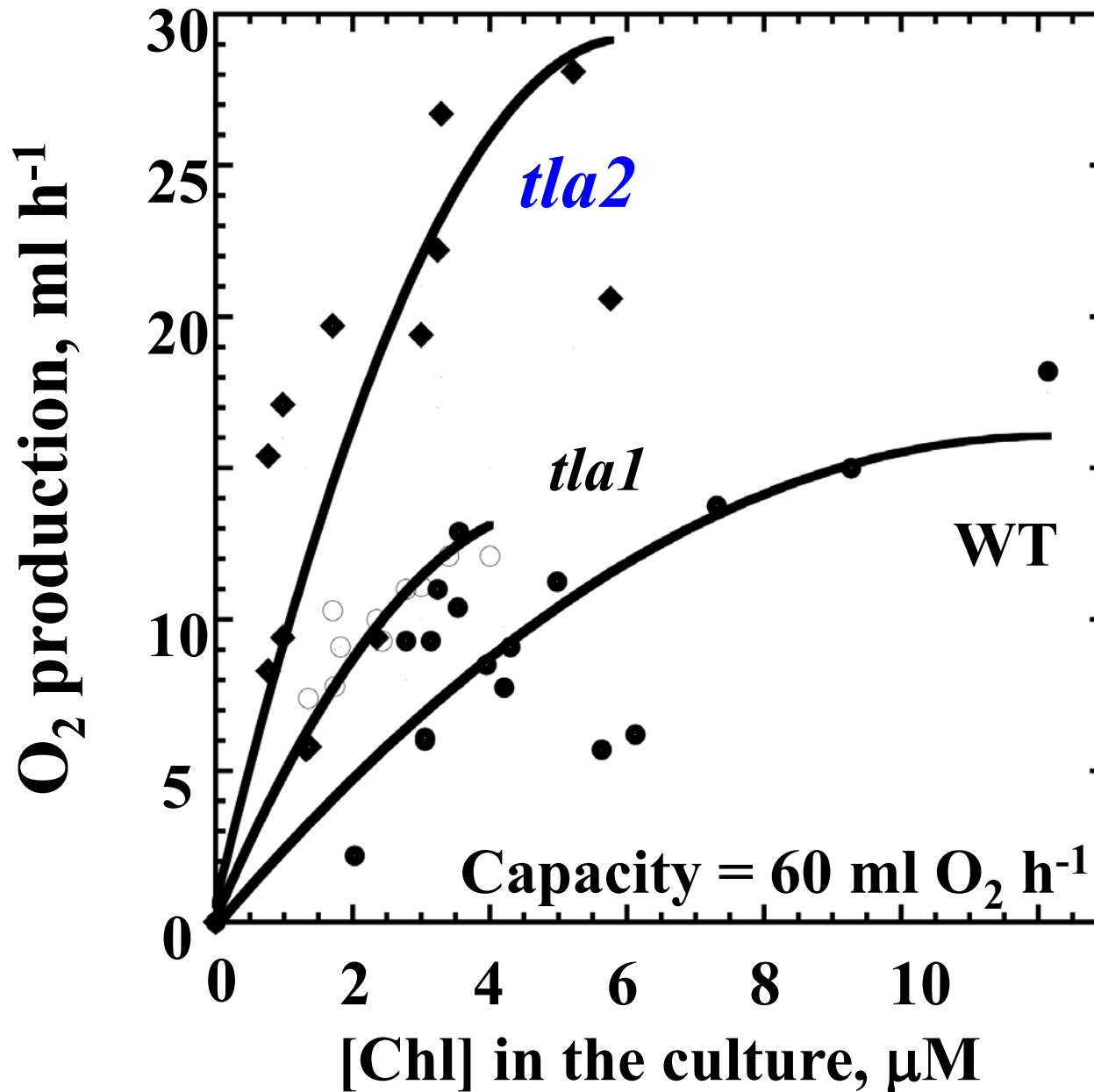
# Project Timeline

## Chlorophyll Antenna Size in Chlamydomonas

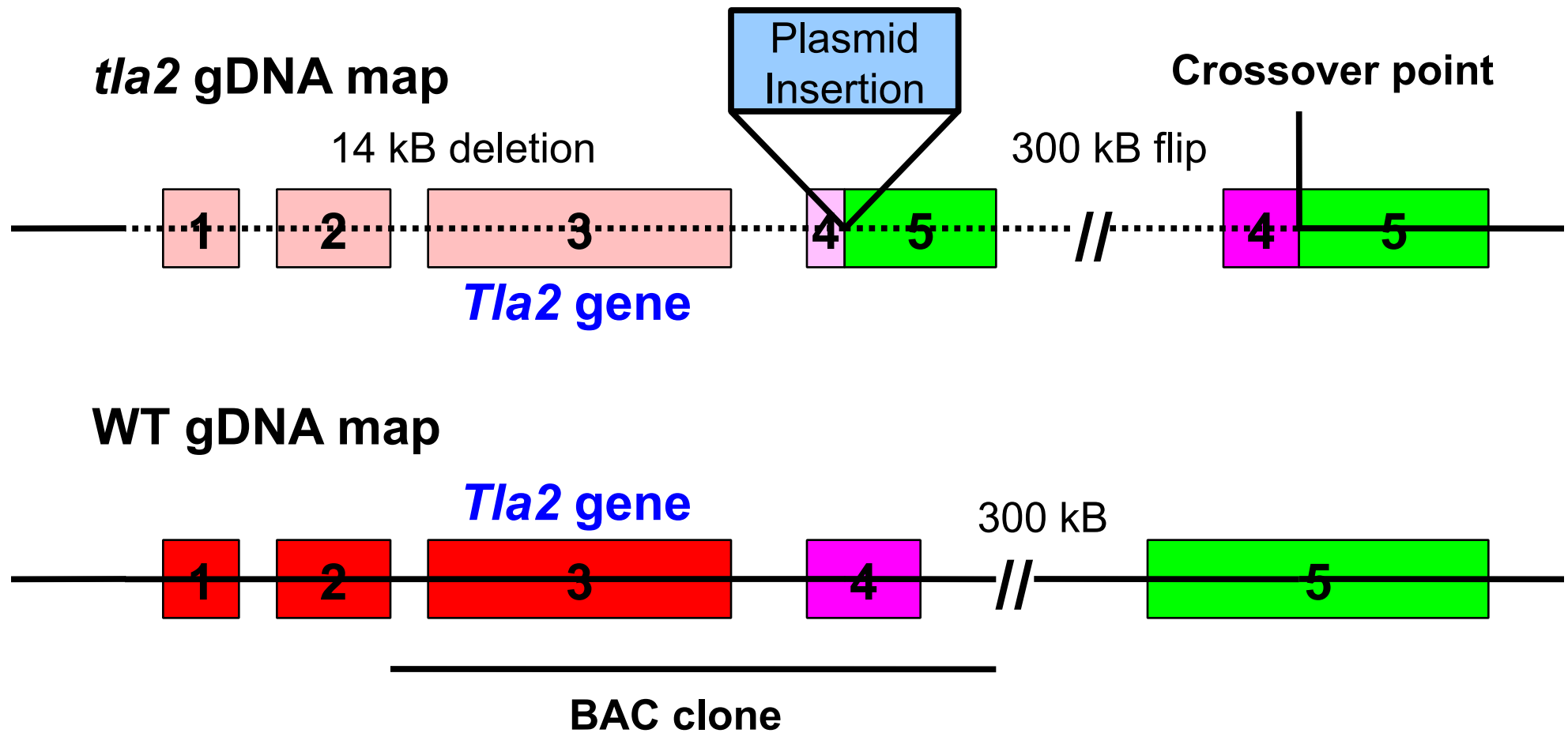


# Productivity in Scale-up of Cultures

(*tla2* outperforms both wild type and *tla1* strains)



# *tla2* gDNA Map and Complementation



# Summary of Accomplishments

- **Successfully cloned the *Tla2* gene and fully elucidated its function. Manuscript and disclosure in preparation.**
- **Successfully cloned the *Tla3* gene. Currently, conducting biochemical analyses and are in the process of elucidating the *Tla3* gene function.**



# Significance of Work

- **First-time identification and documentation of three different genes (*Tla1*, *Tla2*, and *Tla3*) that regulate the chlorophyll antenna size in photosynthesis.**
- **Findings are applied to increase biomass production and (soon) photobiological hydrogen production.**





# Current and Future Work

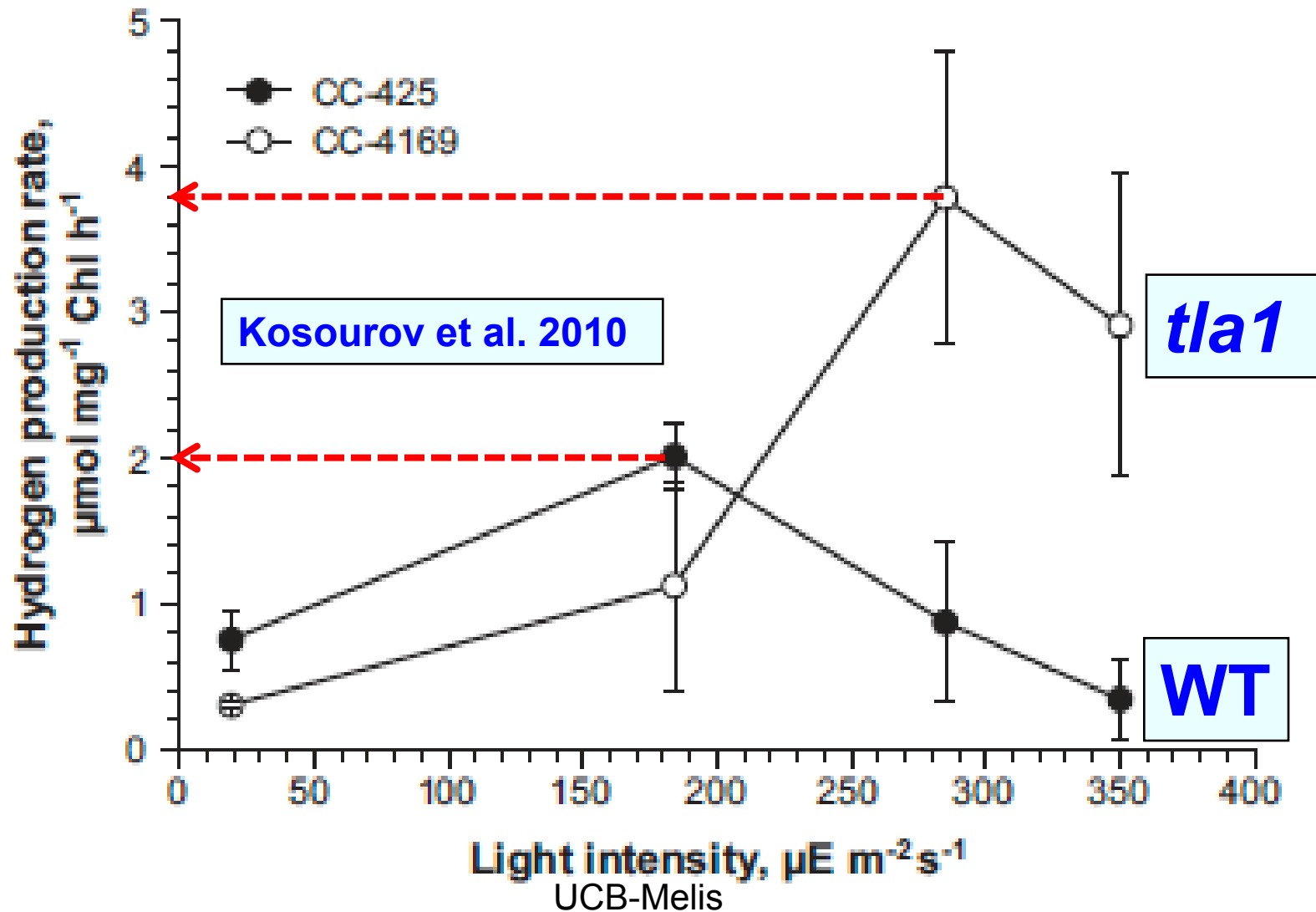
Complete manuscript and disclosure on *Tla2*.

Complete biochemical analyses and process elucidation for the *Tla3* gene.



# Collaborations & Applications

Light intensity-dependent rate of H<sub>2</sub>-production by immobilized wild type (CC-425) and *tla1* antenna mutant (CC-4169)



# Collaborations & Applications

- The *Tla* concept is commercially applied in green microalgae:
  - *Chlamydomonas* for biomass production; and
  - *Nannochloropsis* for commercial production of polyunsaturated fatty acids (PUFAs).
- The *tla1 mutant* strain was requested and acquired by universities (x6), industry (x5), and government labs (x4).
- Successful application of the *Tla1* gene at NREL for enhanced H<sub>2</sub>-production.

