DOE Hydrogen Program

Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Project ID # PD036

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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.)

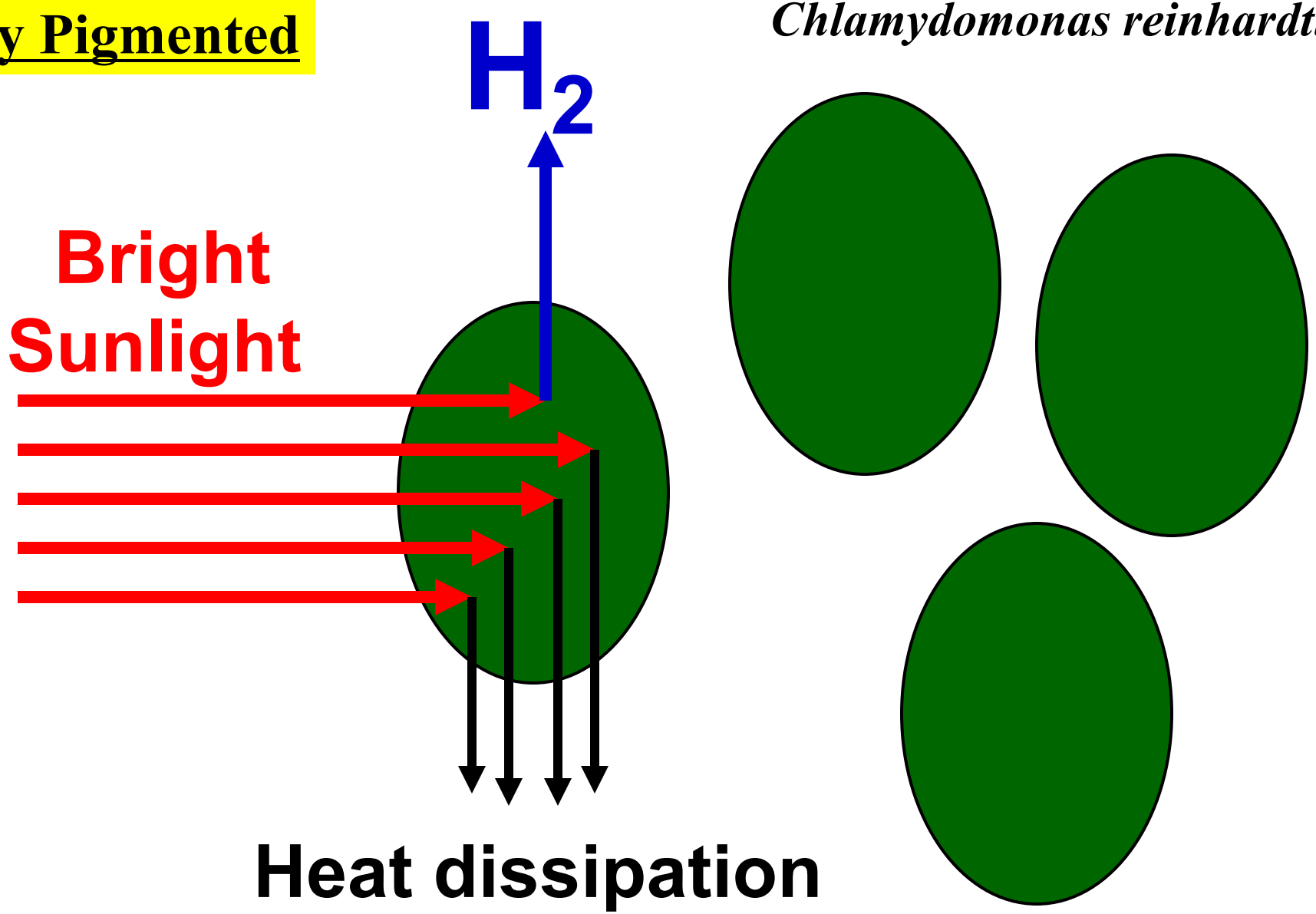
Approach: Interfere with the molecular mechanism for the regulation of the chlorophyll antenna size.

(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

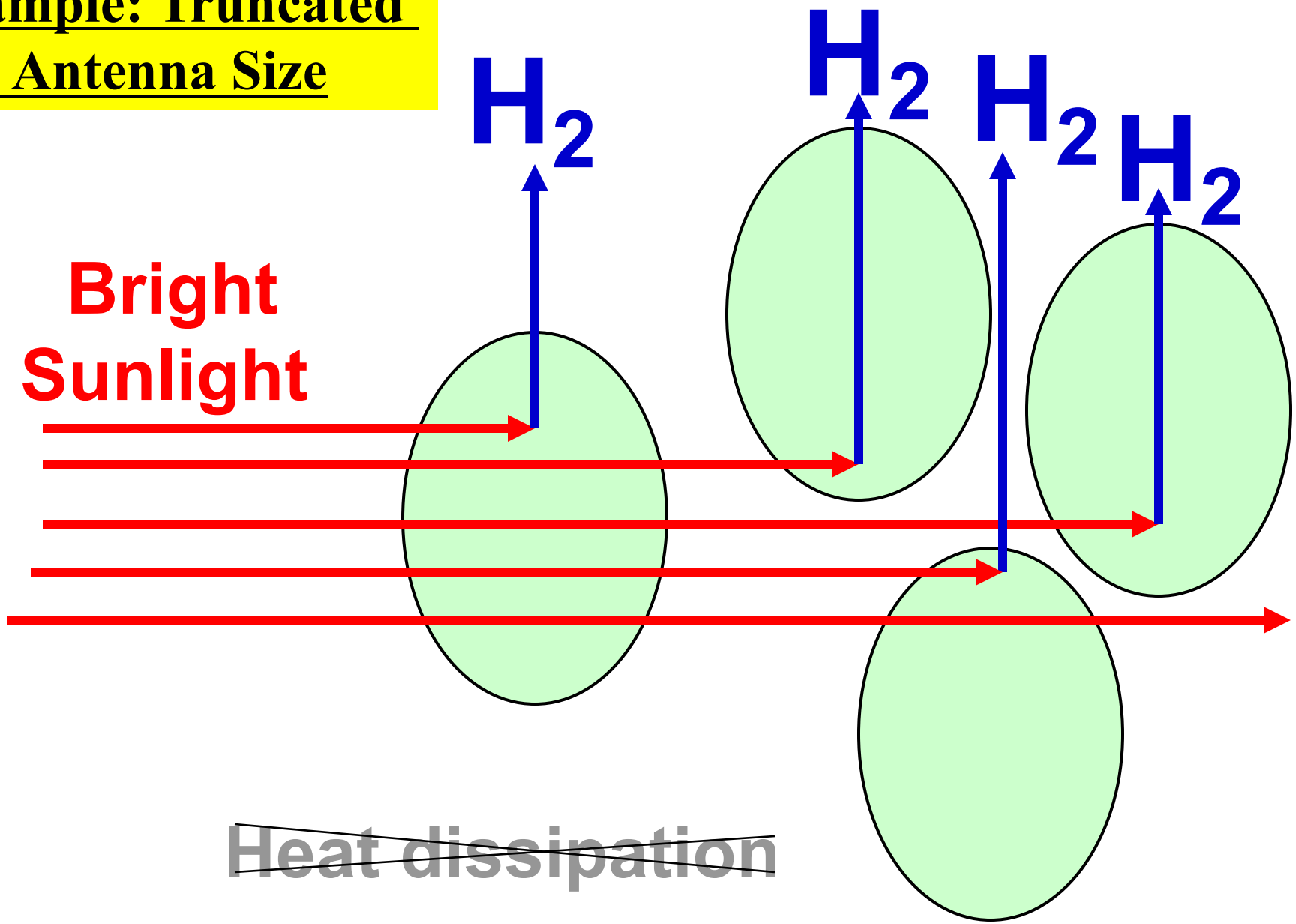
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



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

Example: Truncated Chl Antenna Size

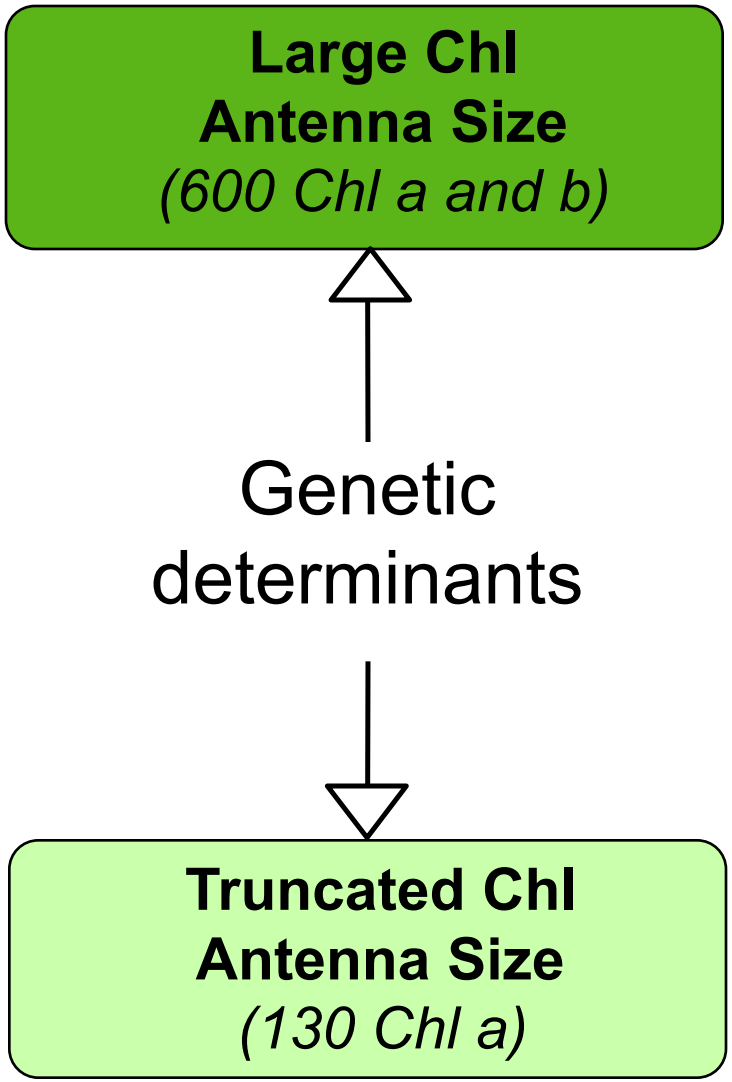


Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.

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.

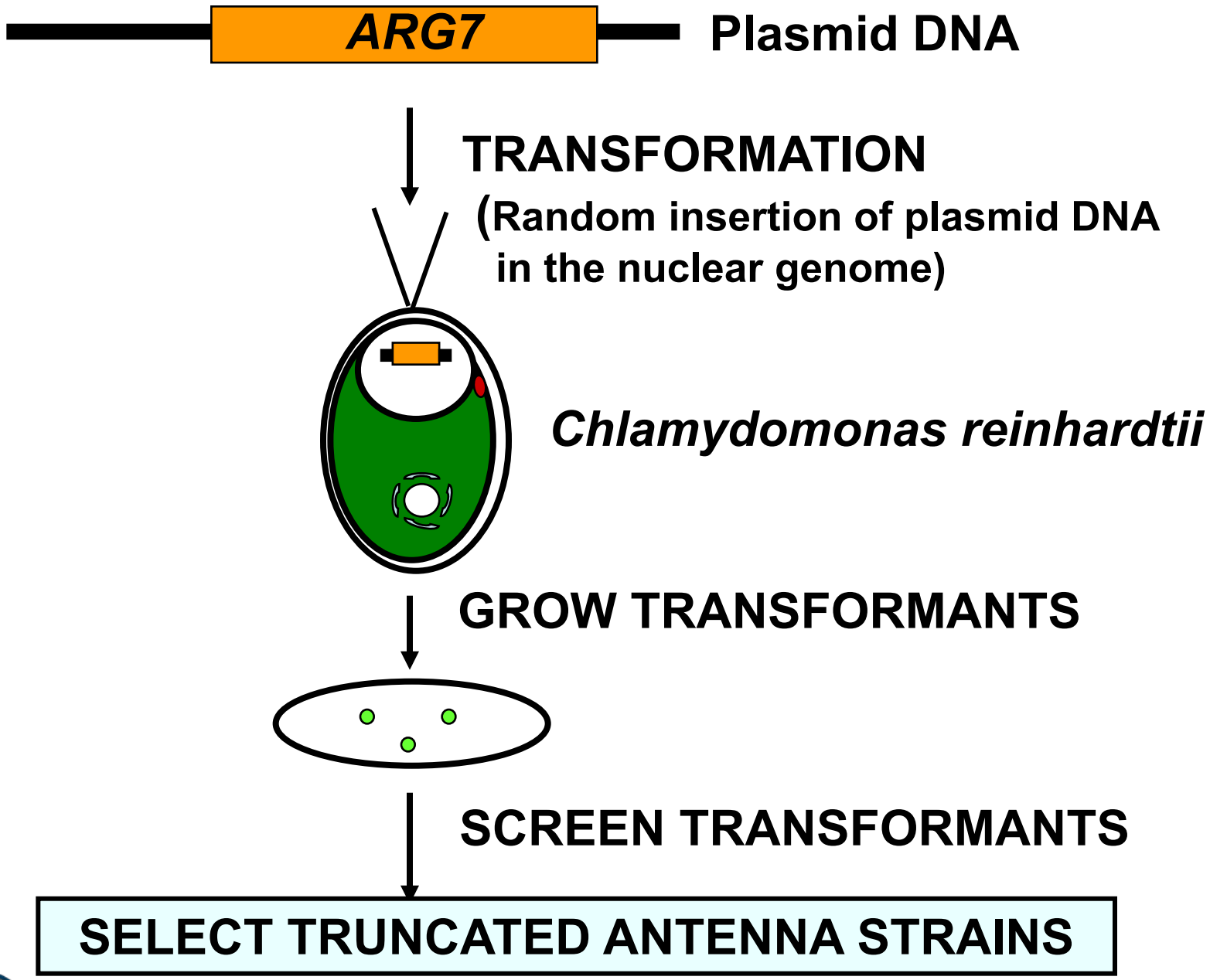
Approach

- Identify genes that confer a truncated antenna in a model organism.

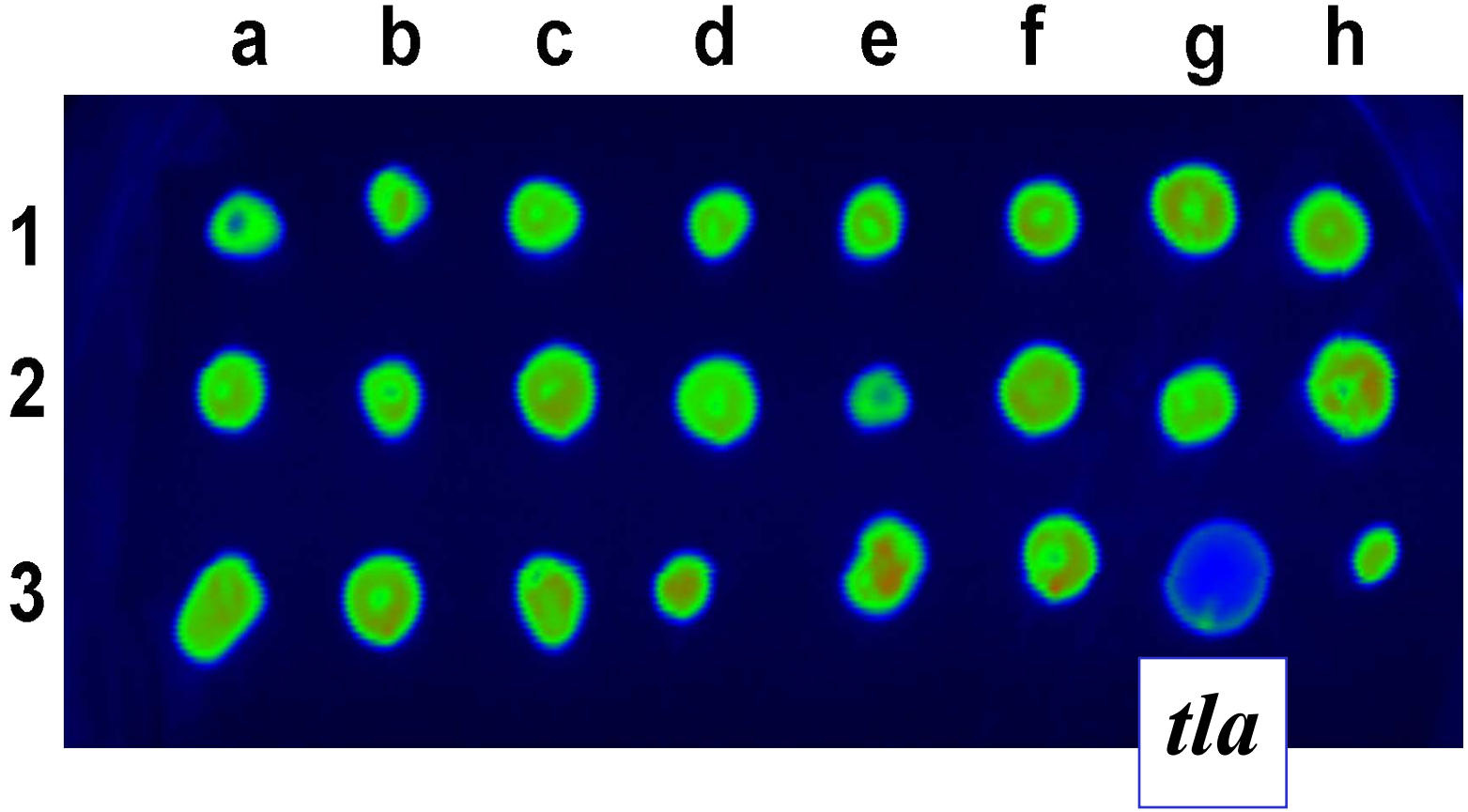
- Apply these genes to other organisms of interest.

- Improve photosynthesis, hydrogen, or fuels production by up to 300%.

DNA insertional mutagenesis and screening

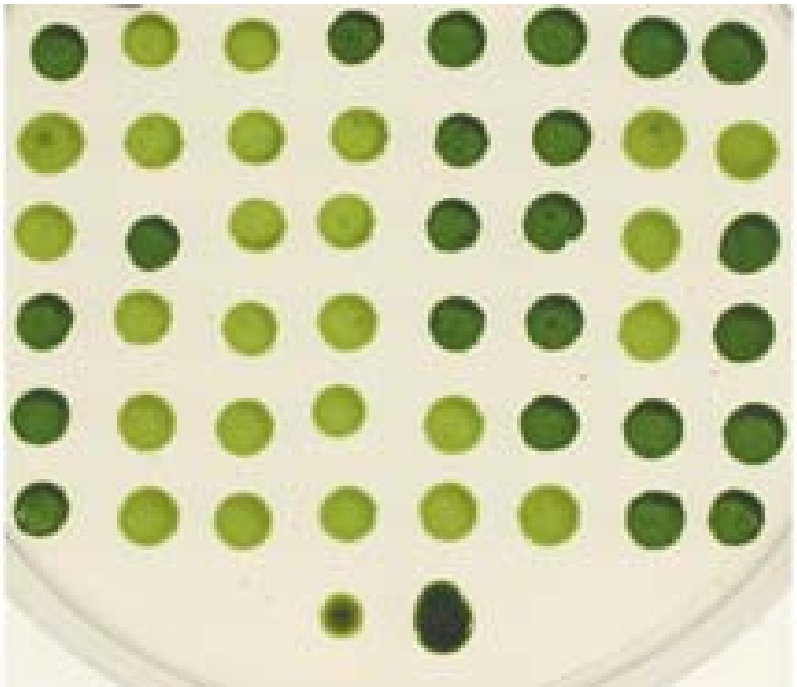


DNA insertional mutagenesis and screening



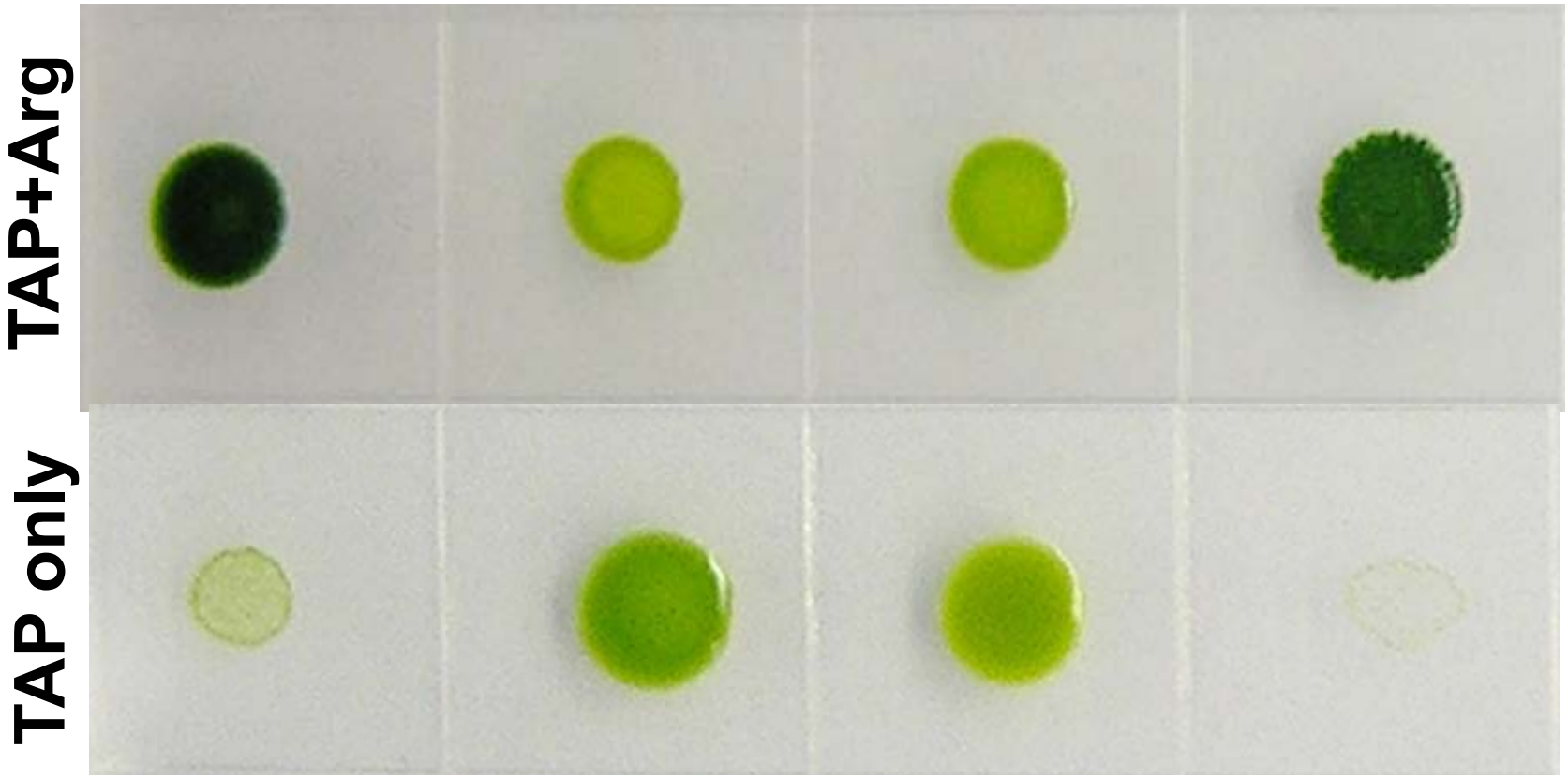
Chlorophyll *a* fluorescence imaging analysis

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(-)* Zygospore



***Arg*(-)**	***Arg*(+)**	***Arg*(+)**	***Arg*(-)**
pJD67(-)	**pJD67(+)**	**pJD67(+)**	**pJD67(-)**
***a/b*=2.5**	***a/b*=8.0**	***a/b*=8.0**	***a/b*=2.6**

Arg^- = AGIx3.24 = 4A(mt-)Arg(-)

Secondary screening for *tla* mutants

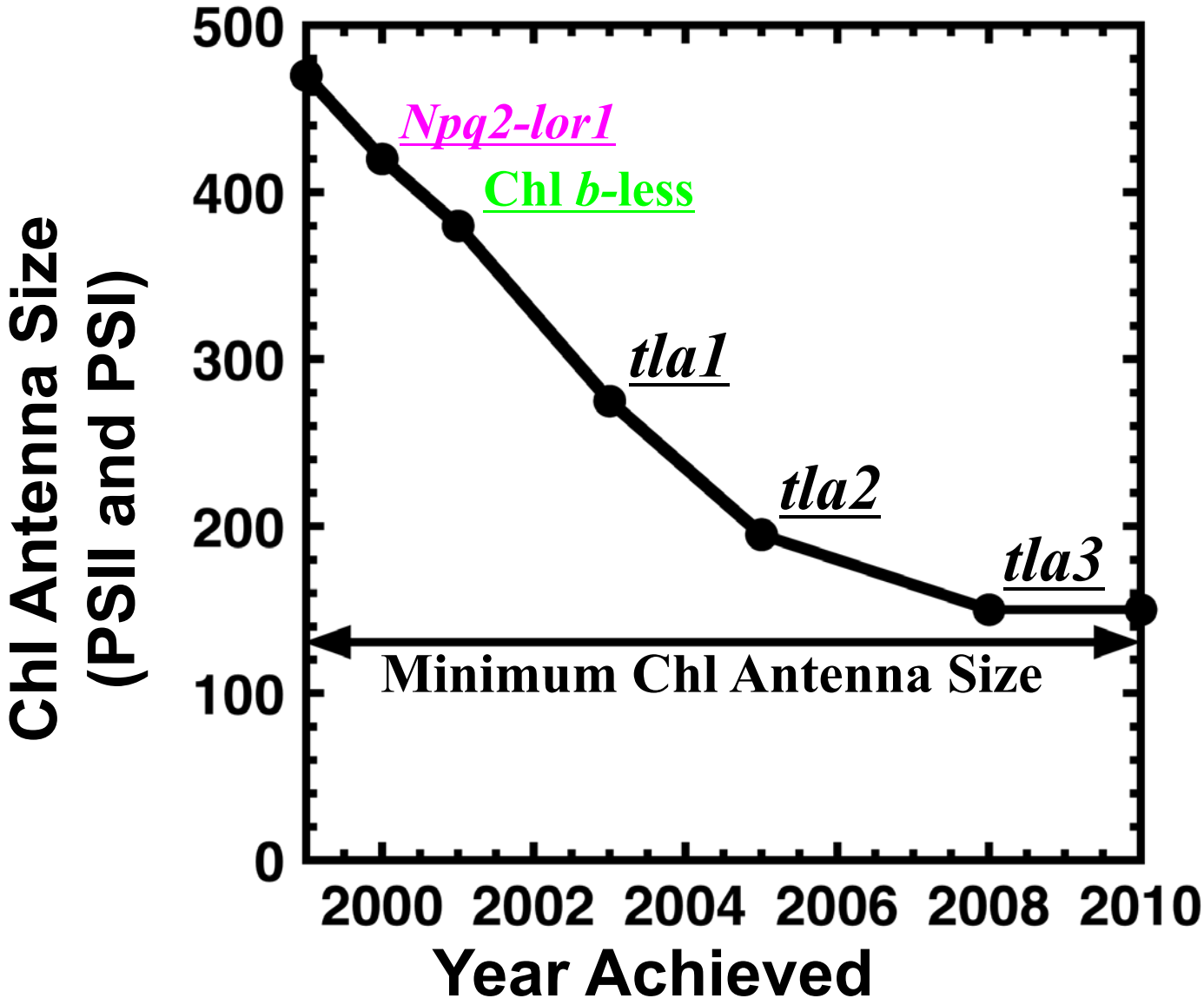
Criteria:

- **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
Targets *(Chl Antenna size)*	600 (WT)		300		200	150
Progress Achieved	600 (WT)	275 *tla1*	195 *tla2*	150 *tla3*		

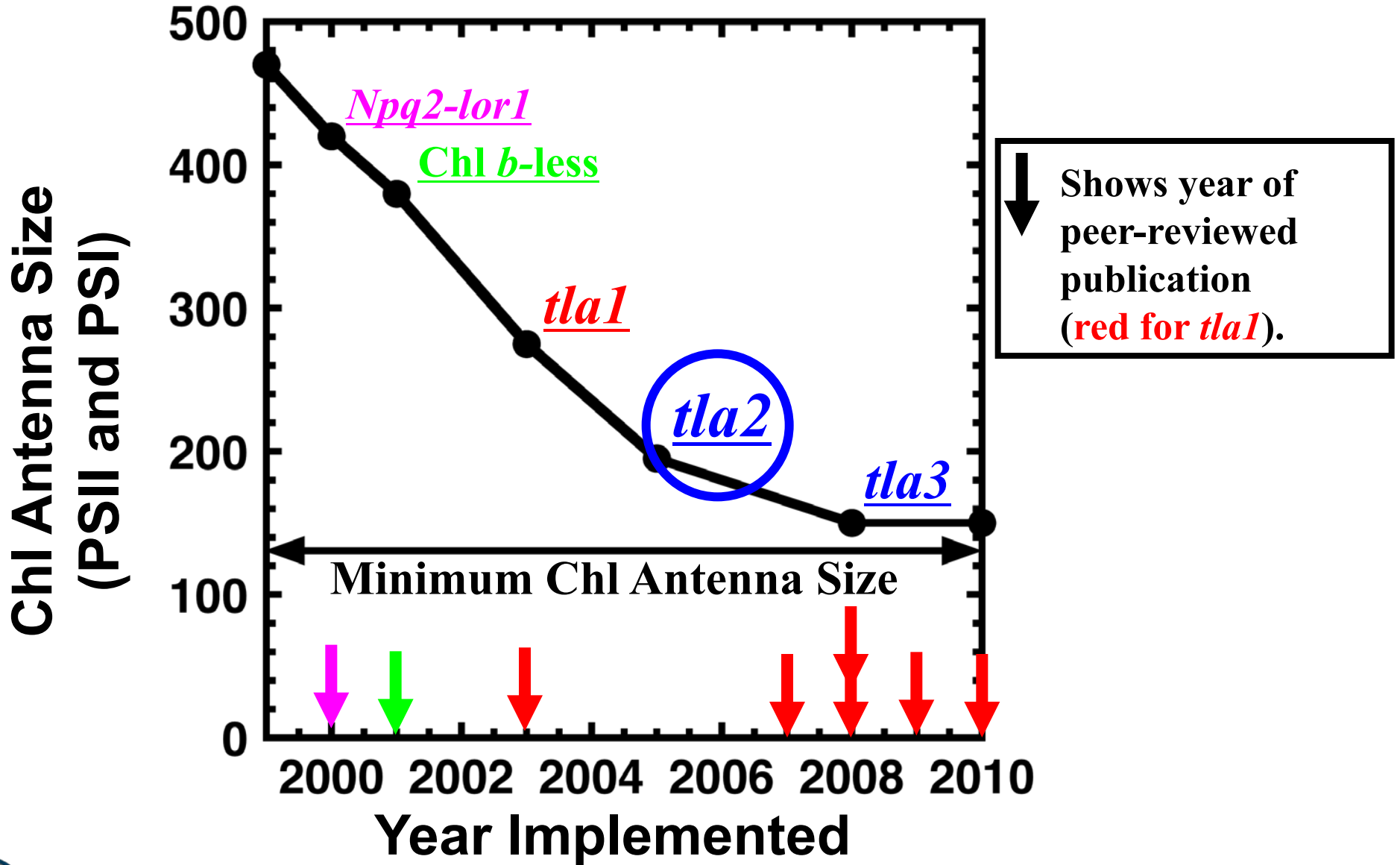
Progress achieved *vs* the DOE targets

Utilization Efficiency of Incident Solar Light Energy, E_0xE_1, %

(maximum E_0xE_1 possible = 30%)

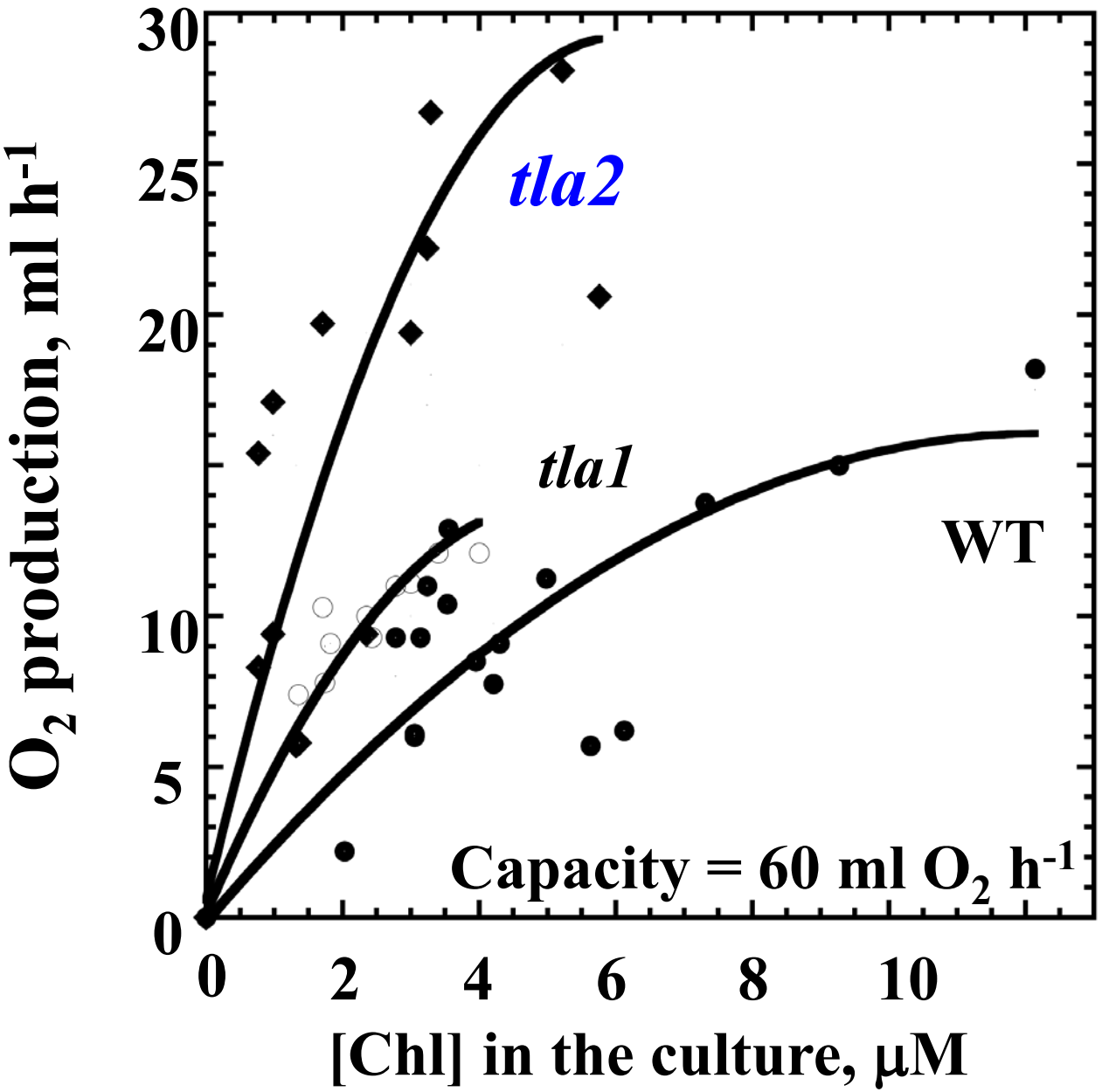
	2000	2003	2005	2008	2010	2015
Program Targets	3%	10%			15%	20%
Progress	3%	10% *tla1*	15% *tla2*	25% *tla3*		

Project Timeline
Chlorophyll Antenna Size in Chlamydomonas

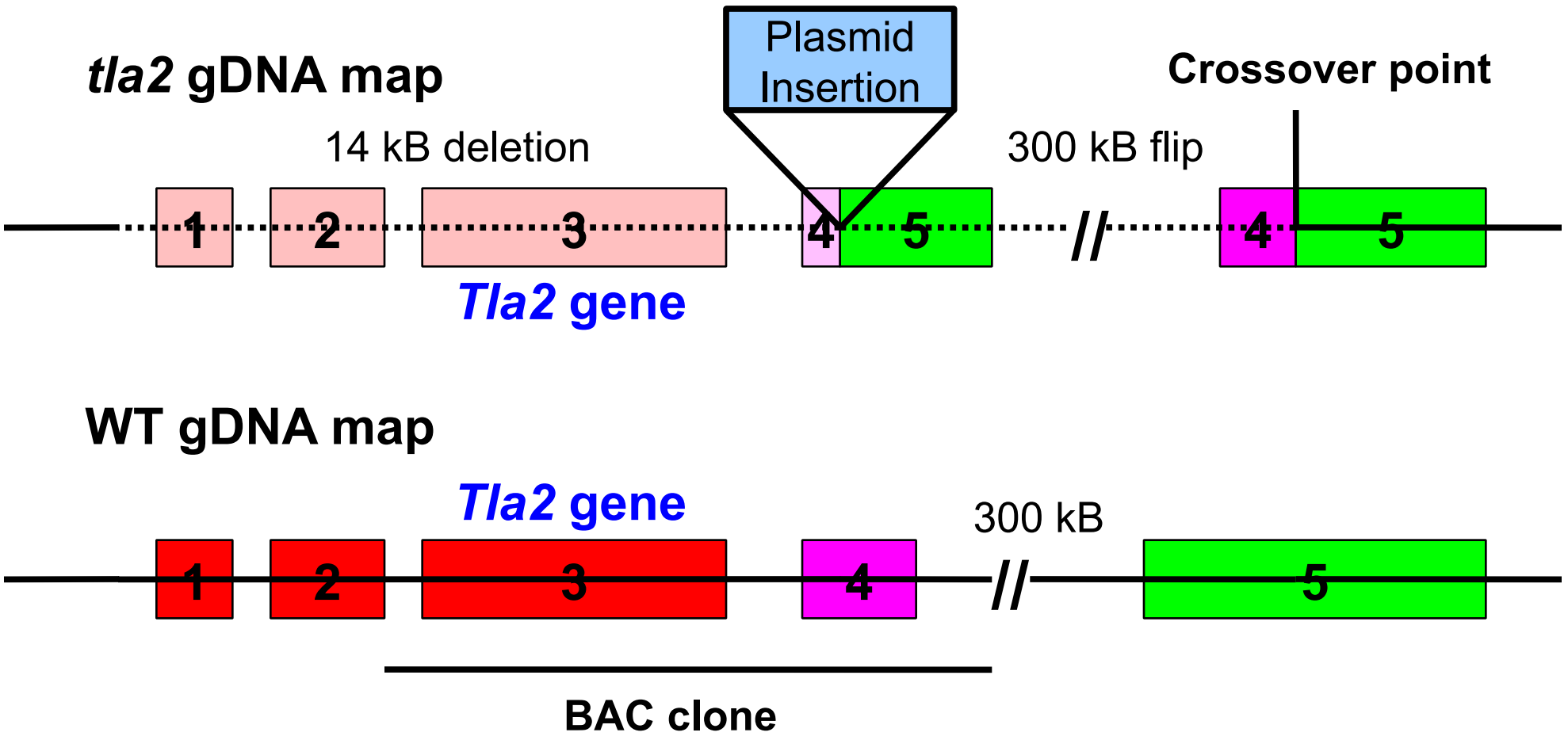


Productivity in Scale-up of Cultures

(*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.**

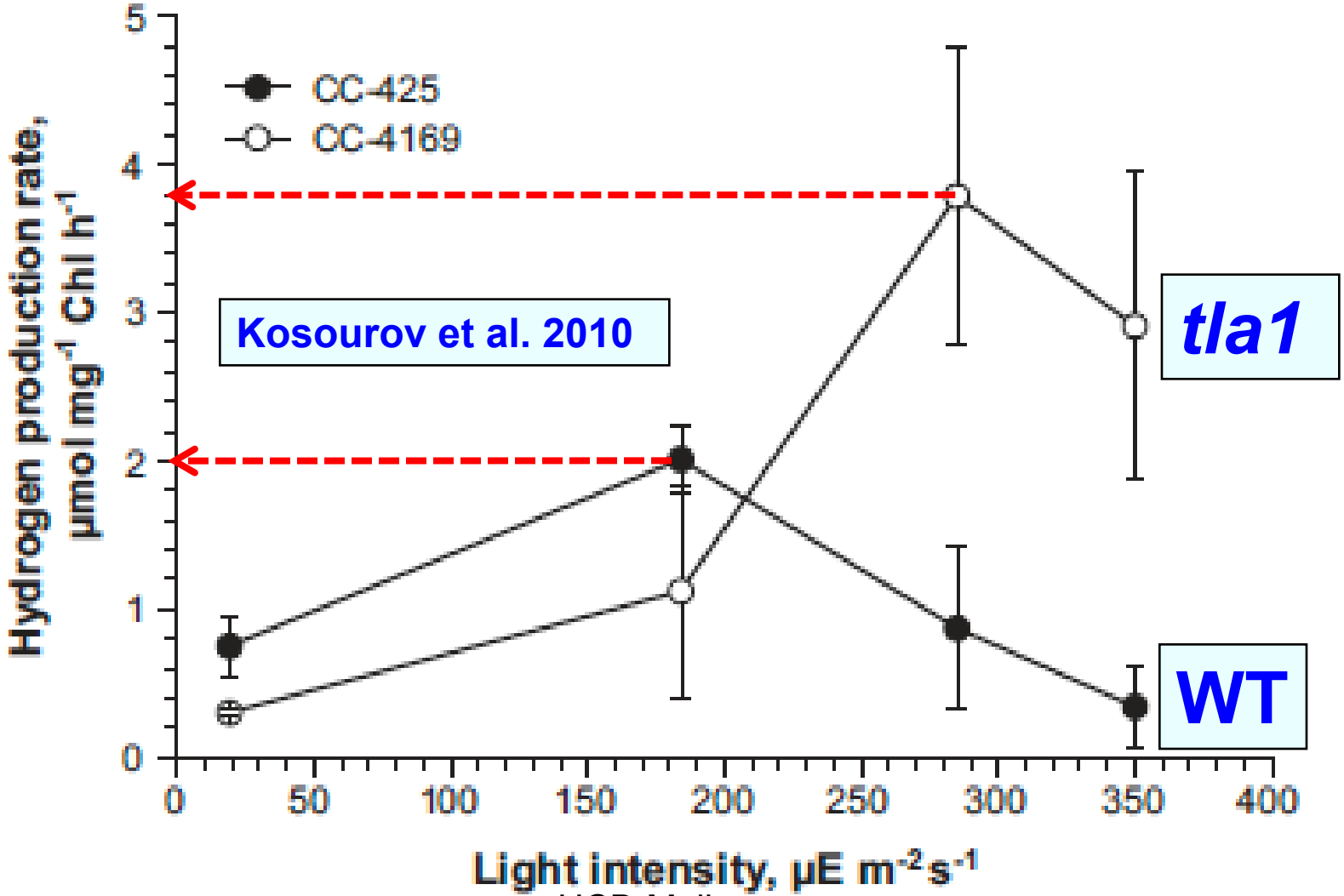
Current and Future Work

Complete manuscript and disclosure on *Tla2*.

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

Collaborations & Applications

Light intensity-dependent rate of H_2-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_2-production.**