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: 31-Jan-2014
- Completion: 95%

Barriers

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

Budget

- **Total Project Funding**
- DOE: $1.74M
- UCB: $ 675K
- Swedish Res Council: $55.2K (**FY11**)
- **Funding received in FY12: $150K**
- **Planned funding for FY13: $150K**

Partners

- NREL
- Swedish Research Council

Relevance

The TLA concept

(TLA = Truncated Light-harvesting Antenna):

Minimize the light-harvesting antenna size of the photosystems to prevent the early light-saturation of photosynthesis and the associated wasteful dissipation of absorbed sunlight.

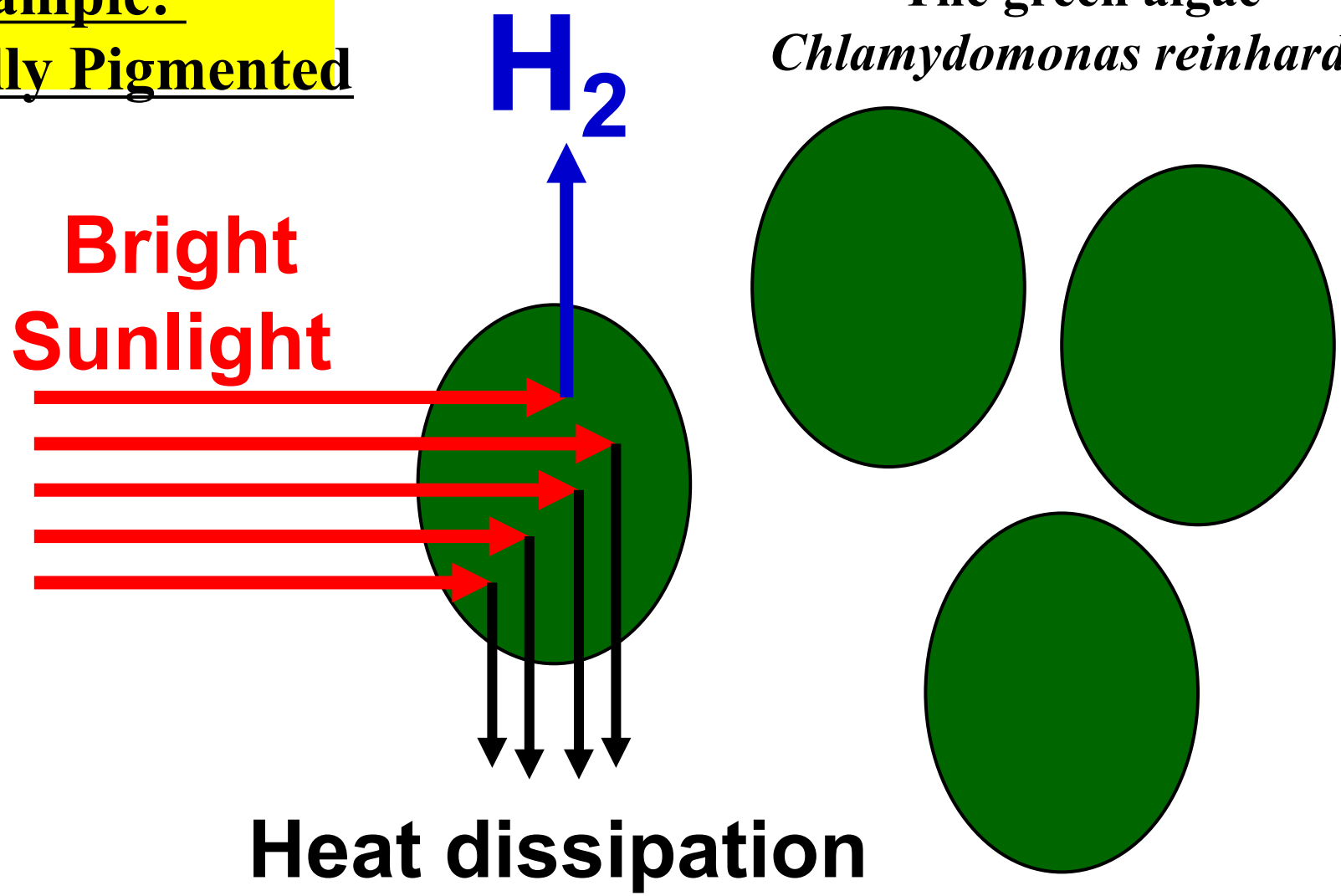
Relevance

Improve, the sunlight-utilization efficiency of photosynthesis in microalgae by up to 300%, which will improve H_2 or fuels production in microalgae and cyanobacteria by about the same percentage.

The work links with effort both at NREL and at the J. Craig Venter Institute, where H_2-production technologies in microalgae and cyanobacteria are being developed.

Relevance

Example:
Fully Pigmented

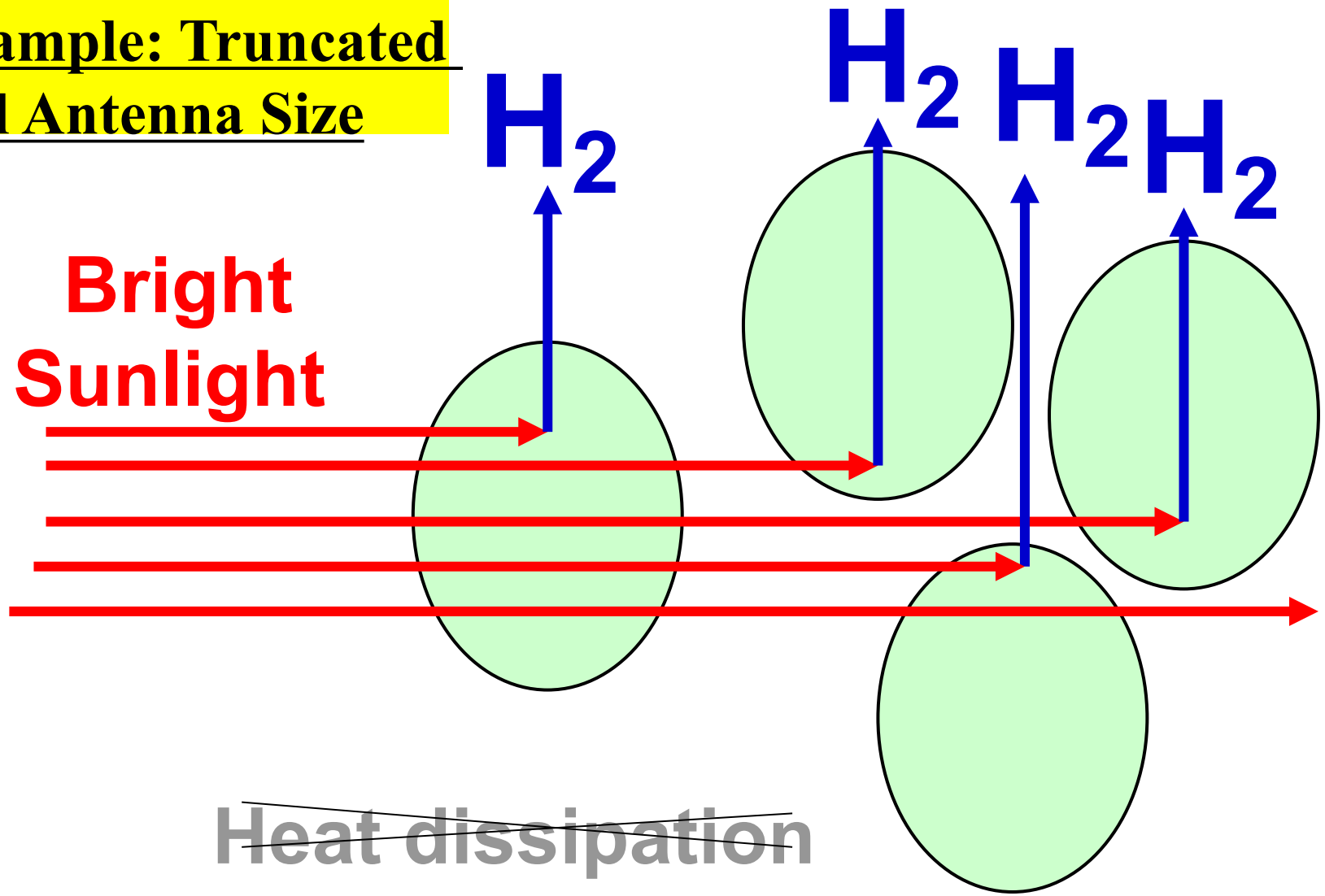


The green algae
Chlamydomonas reinhardtii

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

Relevance

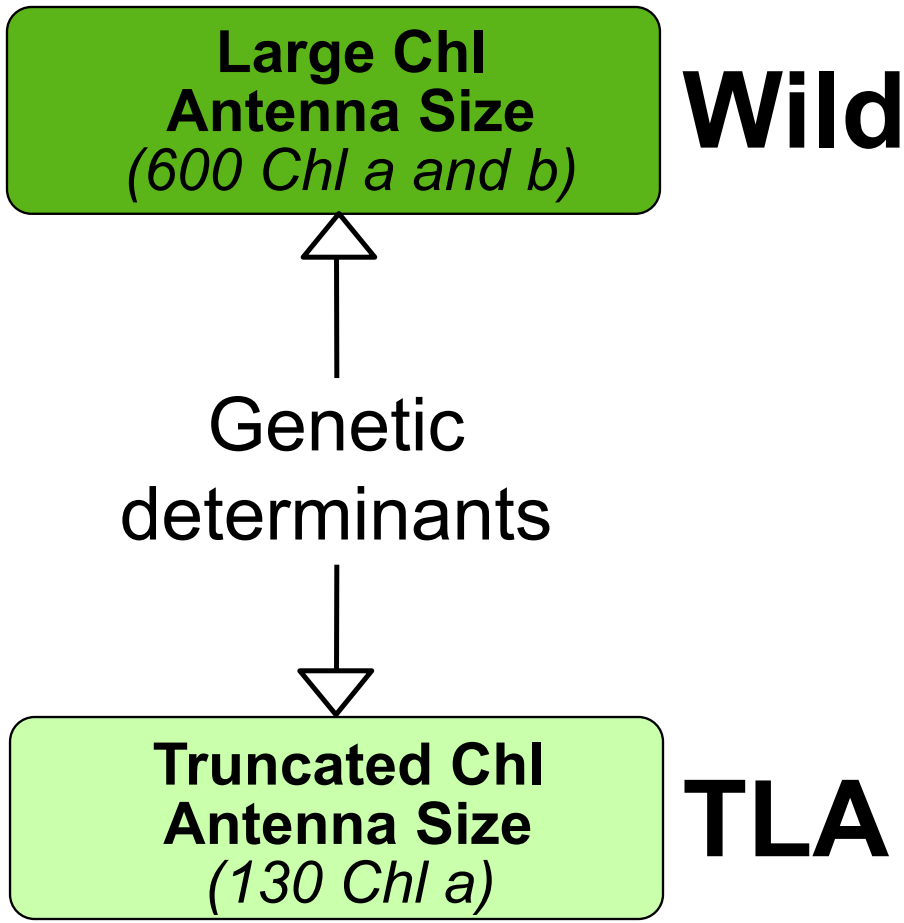
Example: Truncated Chl Antenna Size



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

Objectives and Approach

Chlorophyll *a* and *b* antenna size in microalgae



Identify genes and molecular mechanisms to enable a truncated antenna size in microalgae and cyanobacteria.

Objectives and Approach

Objectives:

Identify genes and associated molecular mechanisms that confer a *T*runcated *L*ight-harvesting *A*ntenna (TLA property) in the ***tla3*** strains of *Chlamydomonas reinhardtii.*

Develop protocols for the targeted truncation of the light-harvesting antenna size in cyanobacteria.

Approach:

(a) Cloning of the genes responsible for the **TLA3** phenotype in *Chlamydomonas reinhardtii*. (b) Identification of genes to be interrupted or deleted in cyanobacteria. (c) Functional analysis of the transformants (Berkeley expertise).

PART 1

Green Microalgae

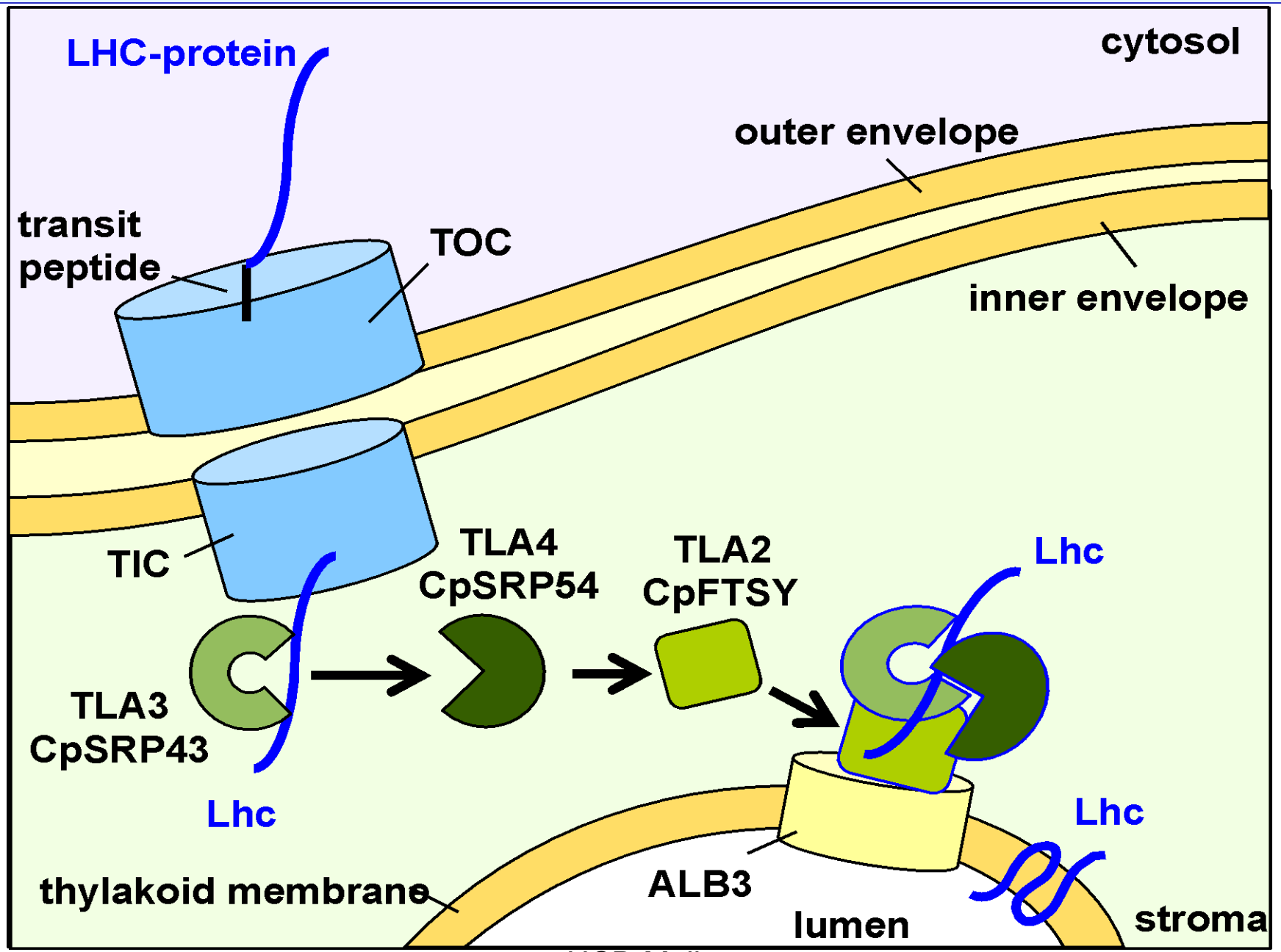
Microalgae Accomplishments and Progress

Sunlight Utilization Efficiency, % of Incident Solar Energy (maximum possible = 30%)

	2000	2003	2005	2007	2008	2010	2011	2012	2015
Targets (Light utilization efficiency)	3%	10%				15%			20%
Tla strain with the highest efficiency identified	3% (WT)	10% *TLA1*	15% *TLA2*		25% *TLA3*				
Gene cloning from the TLA strains				TLA1: Mov34 MPN			TLA2: FTSY	TLA3: SRP43	

Microalgae Accomplishments and Progress

Mechanism of TLA2 and TLA3 Function in LHC-protein assembly

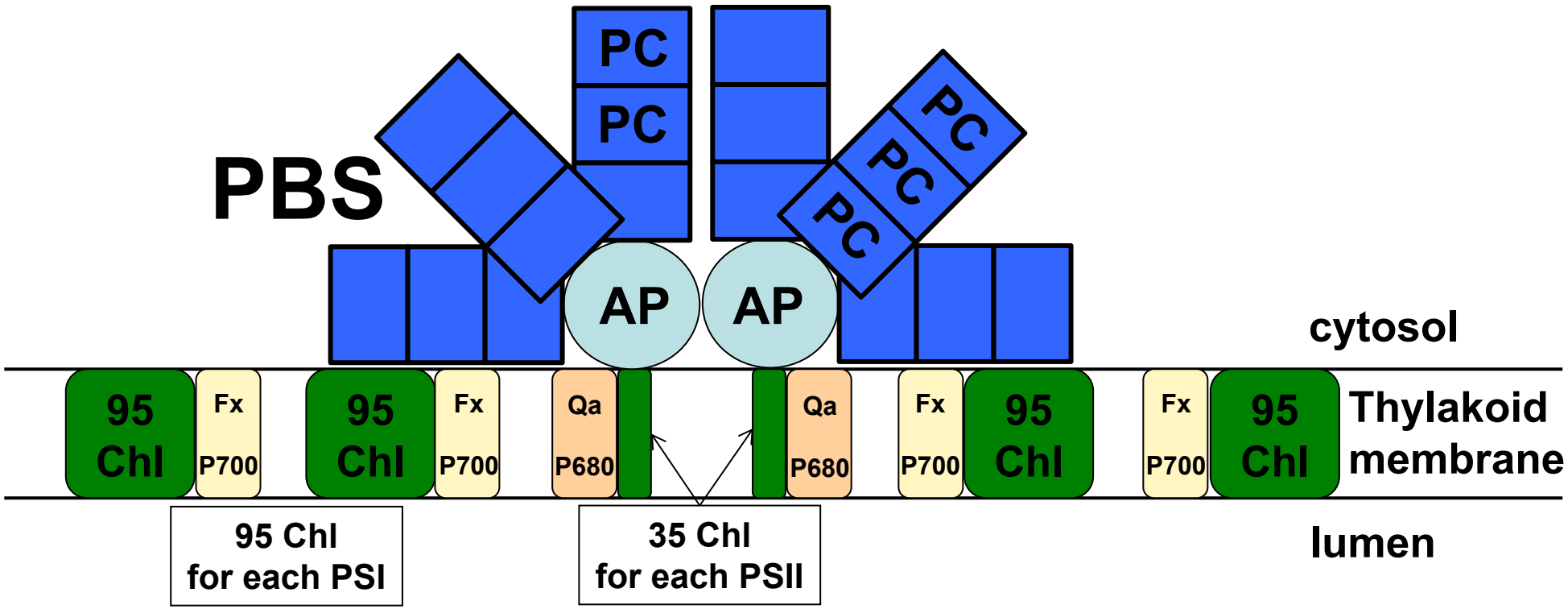


PART 2

Cyanobacteria

Cyanobacterial Approach

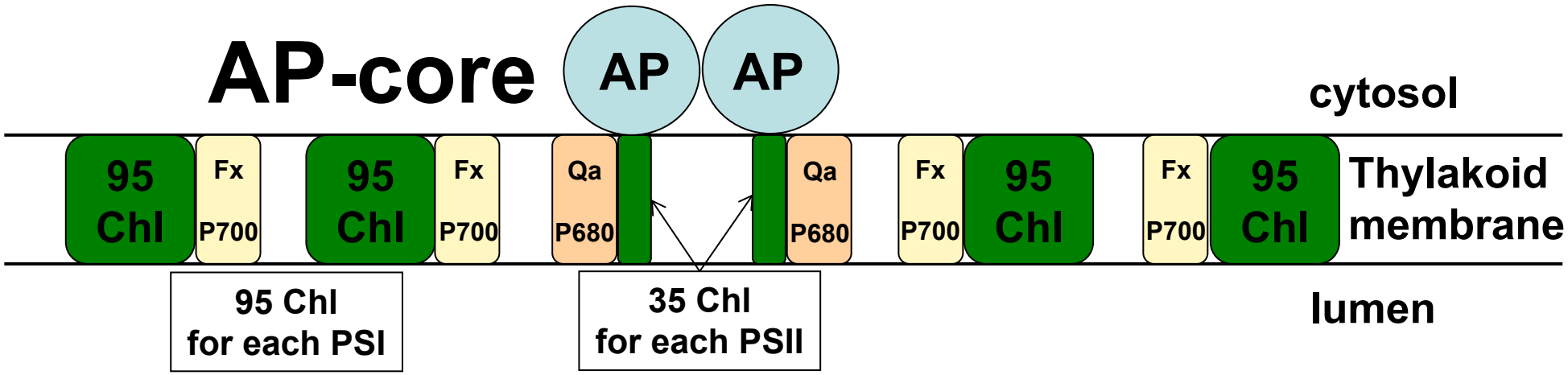
Unit photosynthetic apparatus and cyanobacterial antenna organization



Photosystem stoichiometry and phycobilisome-chlorophyll antenna organization in the thylakoid of cyanobacteria. Cyanobacteria may possess up to **850** phycocyanin (PC), allophycocyanin (AP), and chlorophyll (Chl) molecules per unit photosynthetic apparatus. *Phycobilisome (PBS) schematic adapted from Glazer and Melis 1987*.

Cyanobacterial Approach

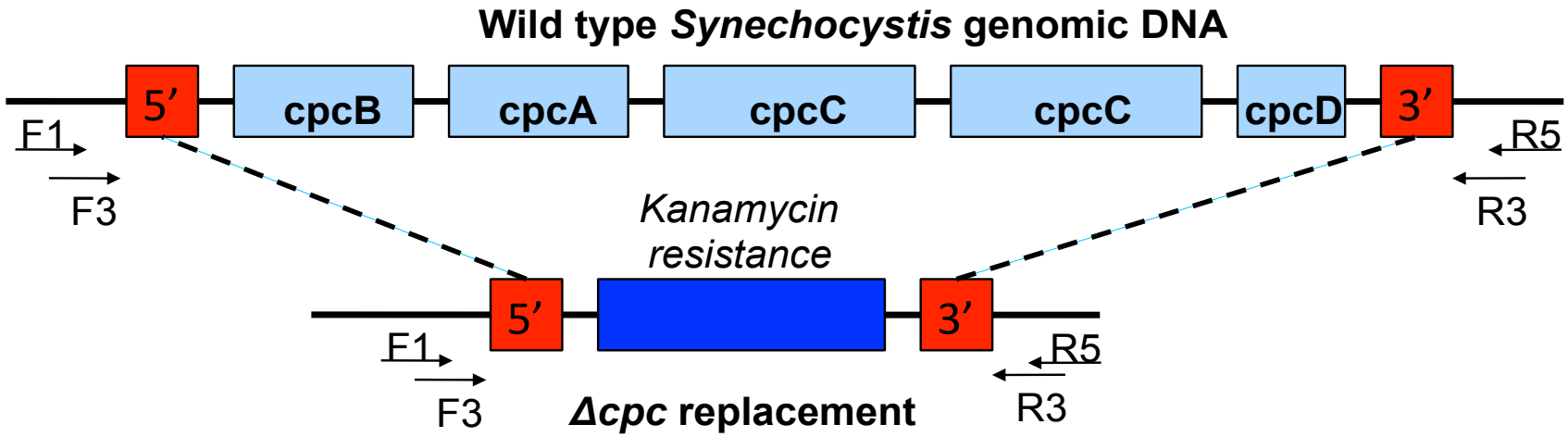
Unit photosynthetic apparatus and cyanobacterial TLA organization
The model shows the anticipated antenna size in TLA cyanobactaria



Go/No-Go Decision: Present evidence at the **molecular** and **biochemical** levels to demonstrate proof-of concept and capability of gene replacement in cyanobacteria, as the method of choice for the generation of TLA mutants in these photosynthetic microorganisms.

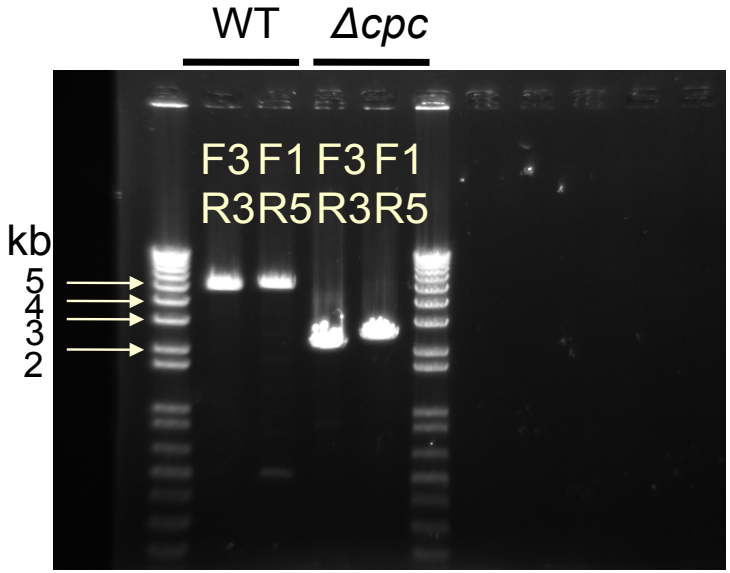
Cyanobacterial Approach

Replacement of the phycocyanin-encoding operon (Go/No-Go Decision)



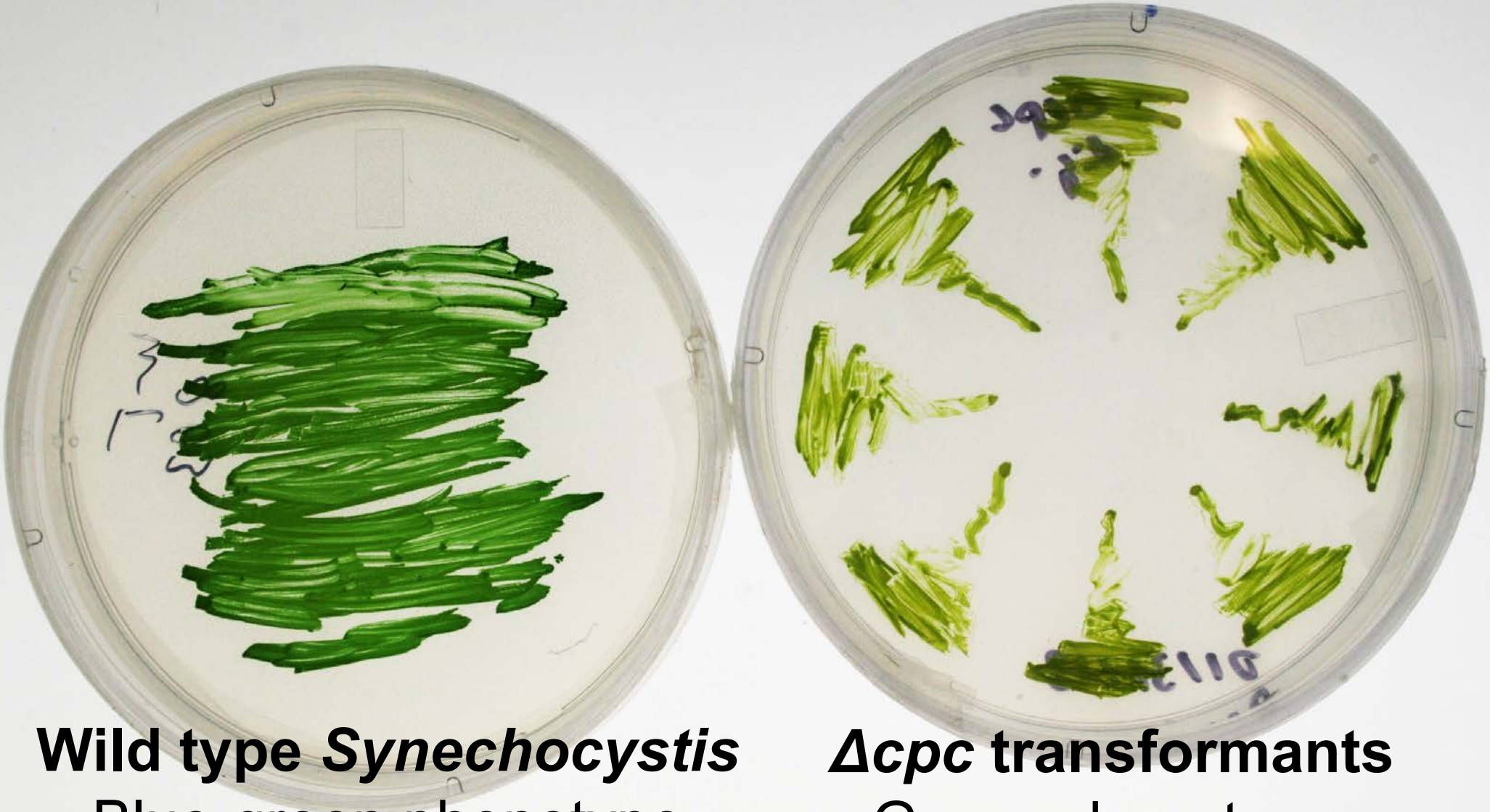
Expected PCR product size:

	WT [bp]	*Δcpc* [bp]
F3R3	4683	2126
F1R5	4973	2416



Cyanobacterial Approach

Molecular and Physiological Evidence of Transformation (Go/No-Go Decision)

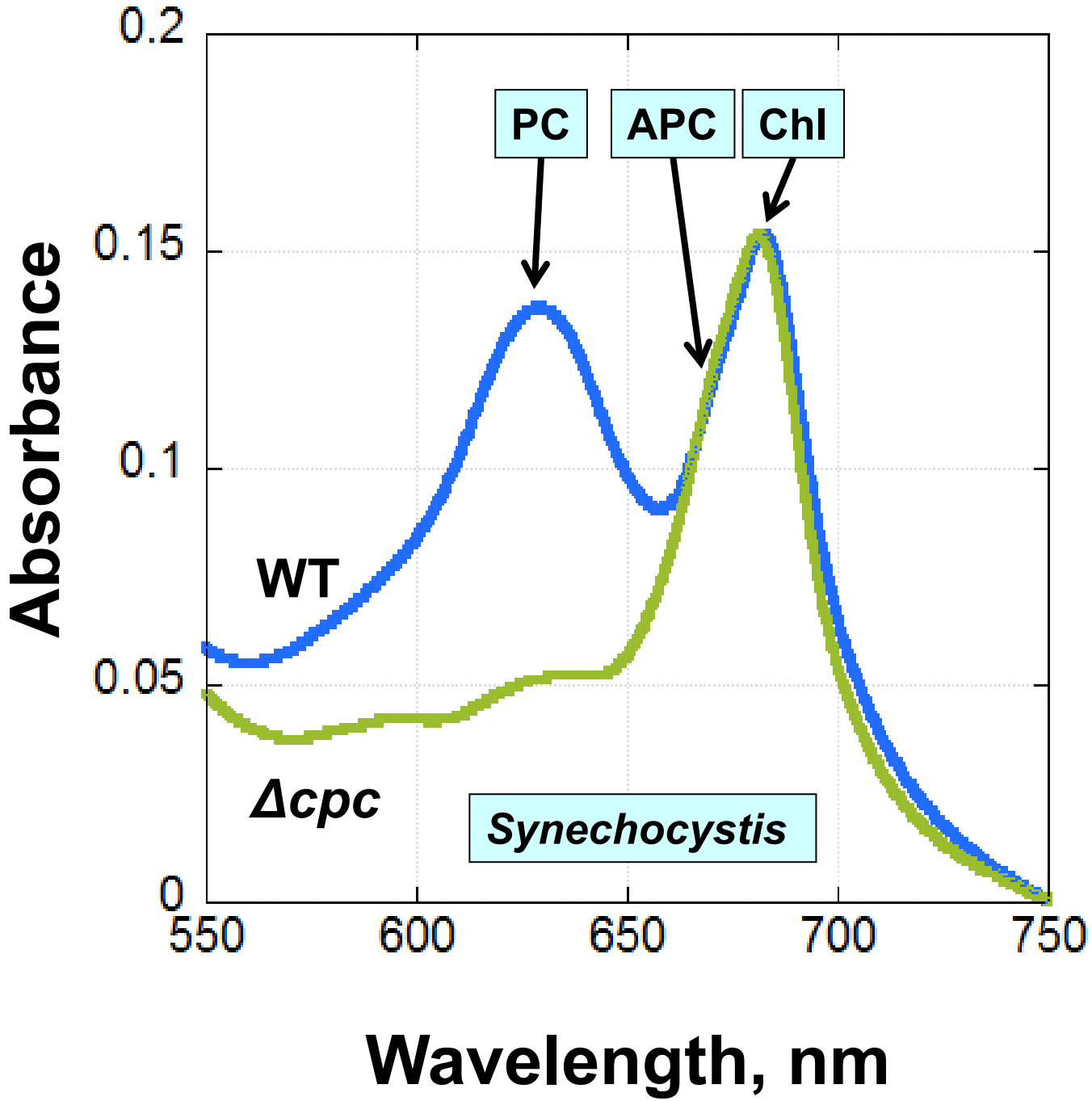


Wild type *Synechocystis*
Blue-green phenotype
due to PC and Chl pigments

***Δcpc* transformants**
Green phenotype
indicating loss of PC

Cyanobacterial Approach

Biochemical Evidence of Transformation (Go/No-Go Decision)



Accomplishments and Progress

Evidence is presented at the genetic and molecular levels to demonstrate successful double homologous recombination for replacement of the ***cpc*** DNA operon in cyanobacteria.

Further evidence is presented at the biochemical level to show the ensuing absence of phycocyanin light-harvesting pigments from cyanobacteria (***Δcpc*** strains), seen by the green coloration of the latter, as opposed to the blue-green coloration of the wild type and by the absence of phycocyanin from the absorbance spectra of the ***Δcpc*** strains.

Accomplishments and Progress

Peer reviewed publications:

Mitra M, Dewez D, García-Cerdán JG, Melis A (2012) Polyclonal antibodies against the TLA1 protein also recognize with high specificity the D2 reaction center protein of PSII in the green alga *Chlamydomonas reinhardtii.* Photosynth Res 112:39-47

Kirst H, Garcia-Cerdan JG, Zurbriggen A, Ruehle T, Melis A (2012) Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the *TLA3-CpSRP43* gene. Plant Physiol. 160(4):2251-2260

Mitra M, Kirst H, Dewez D, Melis A (2012) Modulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii* by *TLA1* gene over-expression and RNA interference. Phil. Trans. R. Soc. B 367:3430-3443

Accomplishments and Progress

- **Successfully completed the TLA3 project, deposited strains in the *Chlamydomonas* library**
- **Successfully applied the TLA concept to cyanobacteria.**
- **Potential of enhancing photosynthetic efficiencies and hydrogen production of cyanobacteria under mass culture conditions.**

Collaborations, Applications, and Impact of the R&D

→ **The *TLA* concept is applied at:**

NREL and by the Brisbane (Australia)-Bielefeld (Germany) groups in *Chlamydomonas* for H_2 production and by the U-Wageningen (The Netherlands) for biomass production, and by two companies in the private sector for commercial production of polyunsaturated fatty acids (PUFAs) in *Nannochloropsis*.

→ **TLA strains were requested from the *Chlamydomonas* library and acquired by universities (x29), industry (x14), government labs (x4), research institutions (5), and high schools (x6).**

→ **(A total of 58 strains were shipped since 2010, most of them in 2012.)**

→ **Collaboration was offered in the form of advising some of the above groups in the use of the TLA strains.**

Proposed Future Work

Compete the analysis of the TLA cyanobacteria, including:

(i) Assessment of stability and fitness of the Δcpc transformants;

(i) Organization of the photosynthetic apparatus (measurement of PS stoichiometry and functional antenna size in the Δcpc transformants);

(ii) Efficiency and productivity of photosynthesis measurements from the light-saturation curve); and

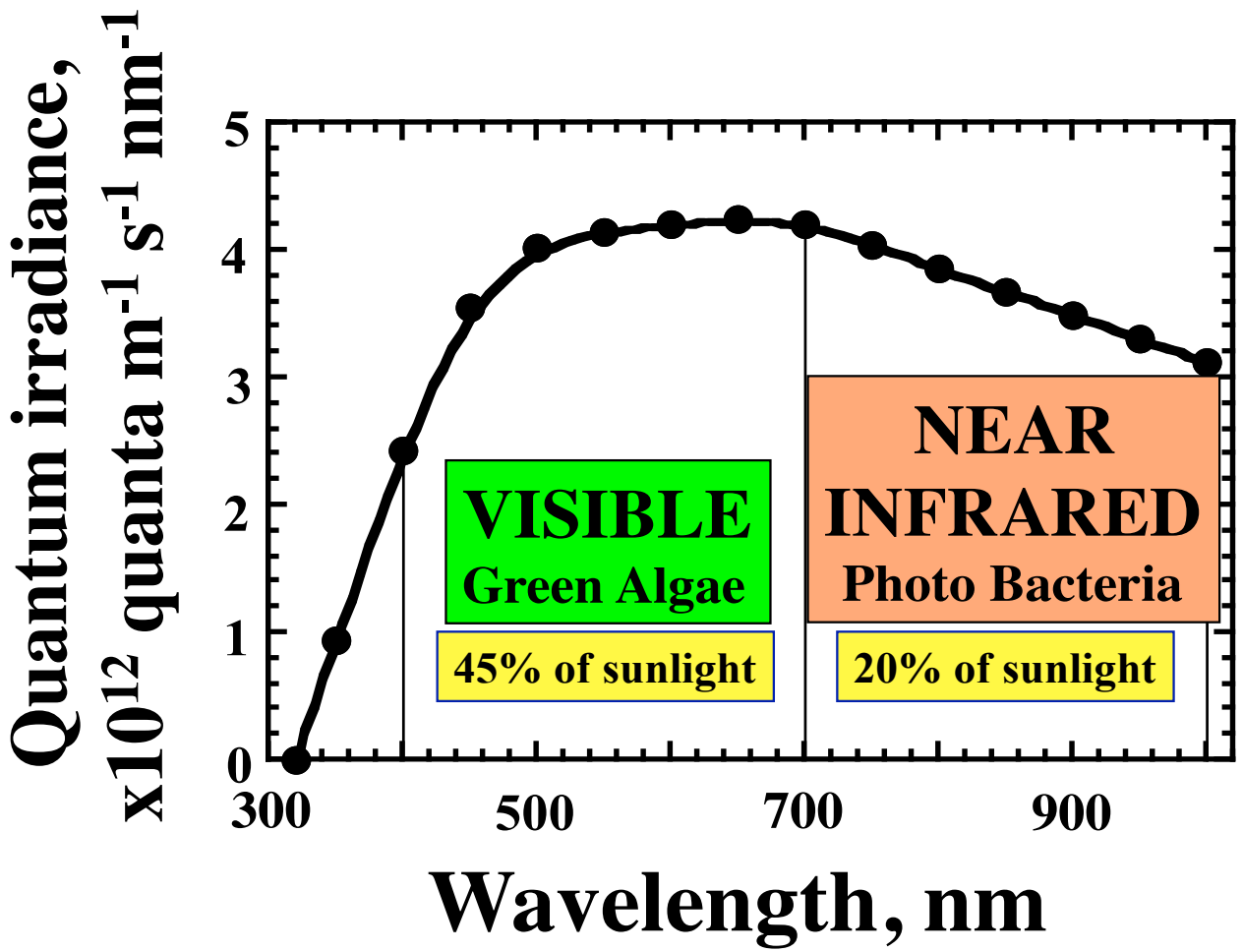
(iii) Measurements of Δcpc productivity under mass culture conditions. (Currently in progress.)

Advance exploration of the "*extended photosynthetically active radiation*" (ePAR) concept.

(Proprietary molecular genetic design not disclosed. Please see next slide for a concept explanation, to be further discussed during the presentation.)

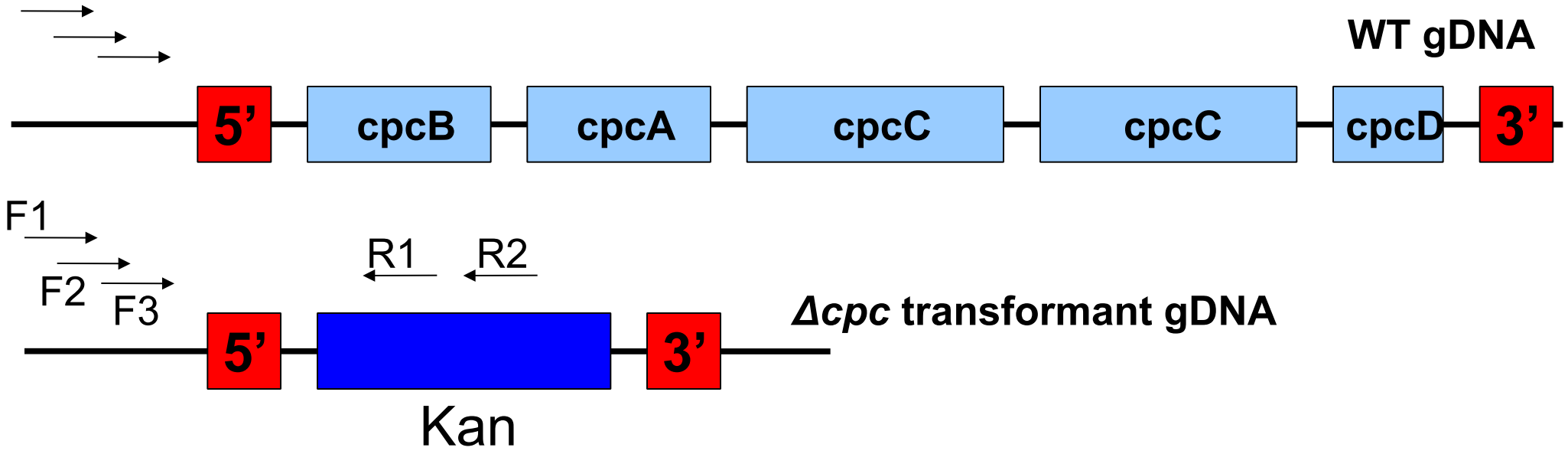
Proposed Future Work

***Extended photosynthetically active radiation*" (ePAR) concept**



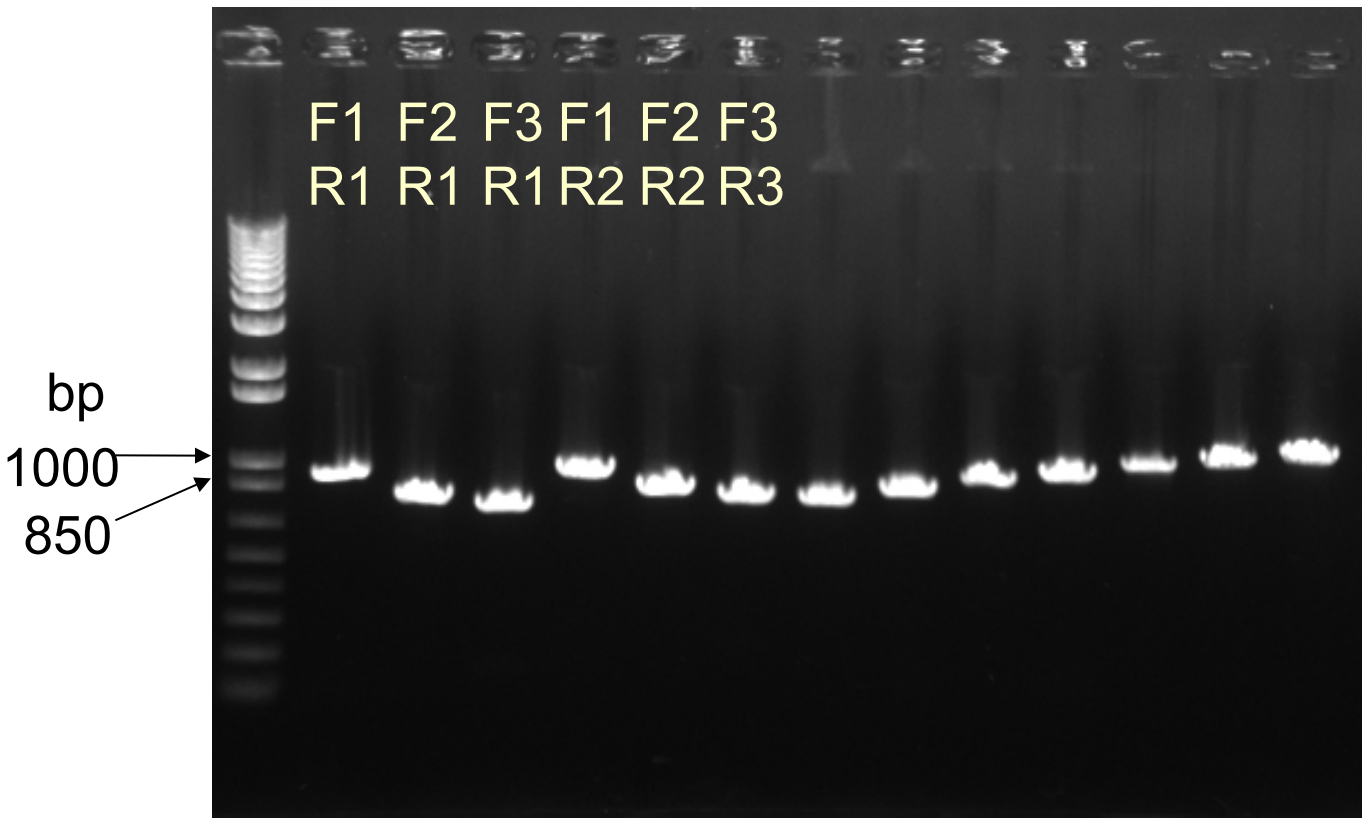
Technical Backup Slides

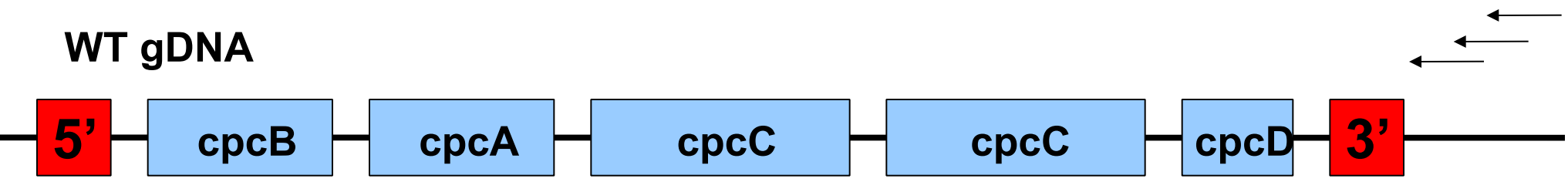
Additional Exploratory PCR Analyses Undertaken for Wild type and *Δcpc* transformants

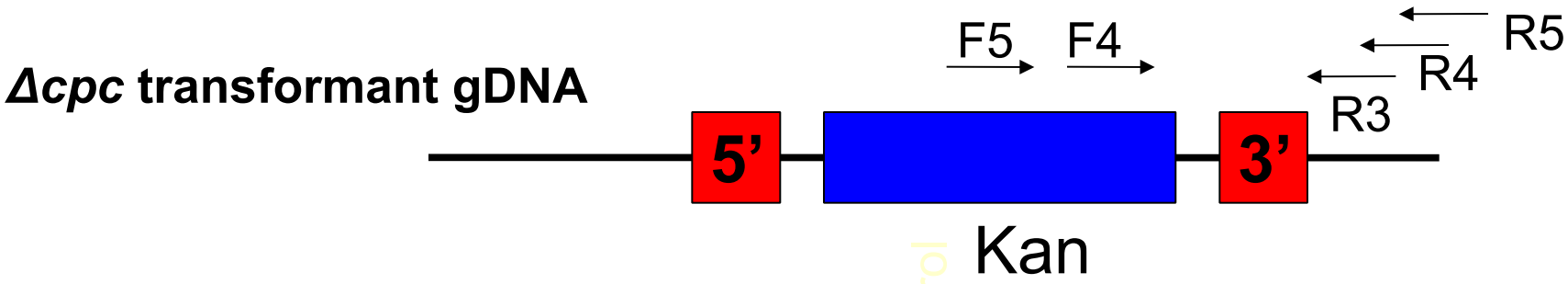


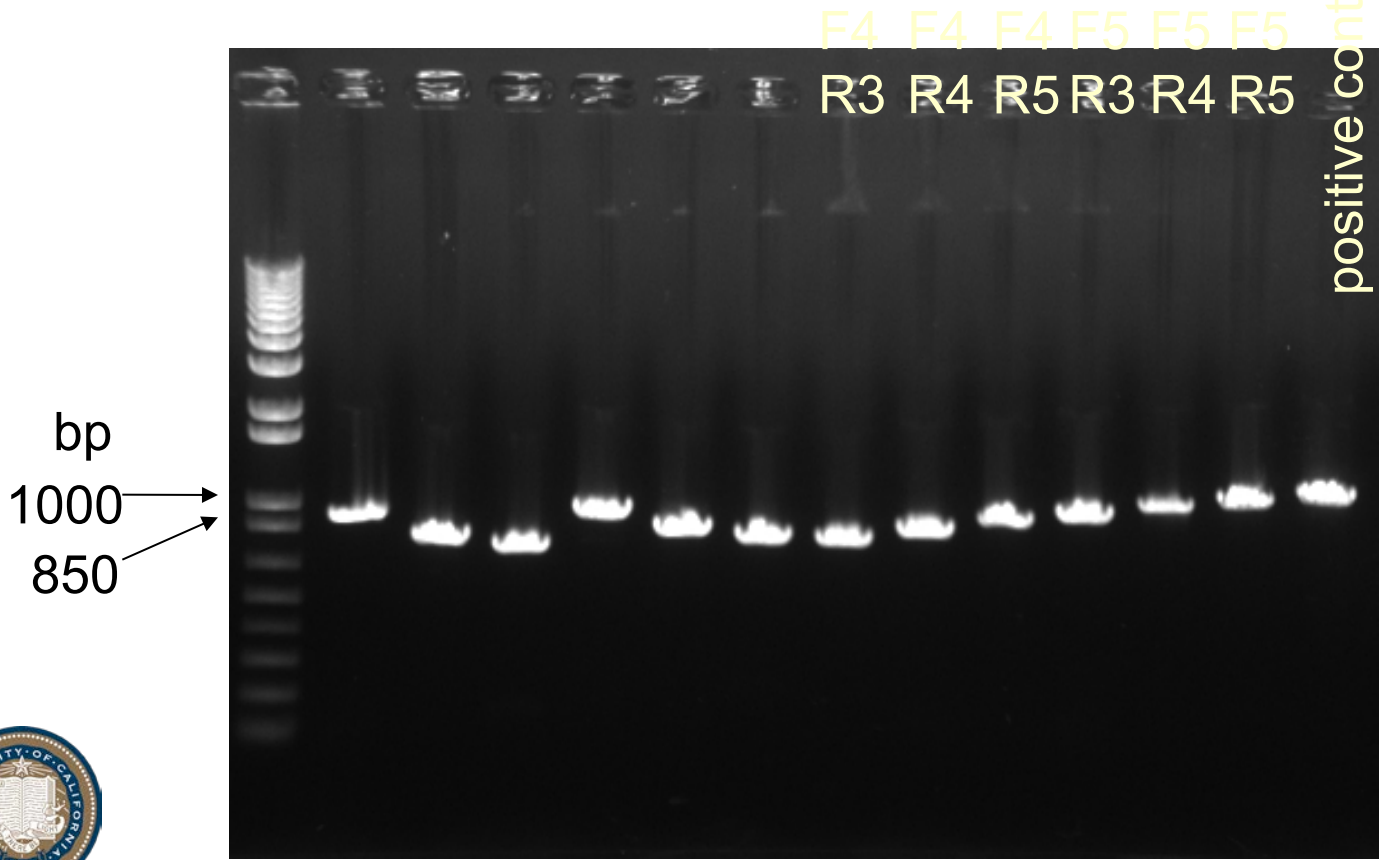
Expected product size

Primer set	Length [bp]
F1R1	935
F2R1	819
F3R1	769
F1R2	1003
F2R2	887
F3R2	837









Expected product size

Primer set	Length [bp]
F4R3	825
F4R4	894
F4R5	949
F5R3	1008
F5R4	1077
F5R5	1132

Chl Antenna Size vs Light Utilization Efficiency
Utilization Efficiency of Absorbed Light Energy

- **Wild type antenna size = 470 Chl molecules (100%)**
  (PSII=230; PSI=240)
  Photon use efficiency of WT photosynthesis = ~6-10%
  Utilization Efficiency of Absorbed Light Energy by WT: ~3-5%

- ***tla1* antenna size = 275 Chl molecules (59% of control)**
  (PSII=115; PSI=160)
  Photon use efficiency of *tla1* photosynthesis = ~20%
  Utilization Efficiency of Absorbed Light Energy by *tla1*: ~10%

- ***tla2* antenna size = 195 Chl molecules (42% of control)**
  (PSII=80; PSI=115)
  Photon use efficiency of *tla2* photosynthesis = ~30%
  Utilization Efficiency of Absorbed Light Energy by *tla2*: ~15%

- **Long-term goal: 132 Chl molecules (28% of control)**
  (PSII=37; PSI=95)
  Photon use efficiency of photosynthesis *goal* = ~60%
  Utilization Efficiency of Absorbed Light Energy *goal*: ~30%

Phycobilisome-Chlorophyll antenna size In Cyanobacteria