

A forward genetic screen to identify hydrogenase-deficient mutants in the unicellular green alga *Chlamydomonas reinhardtii*

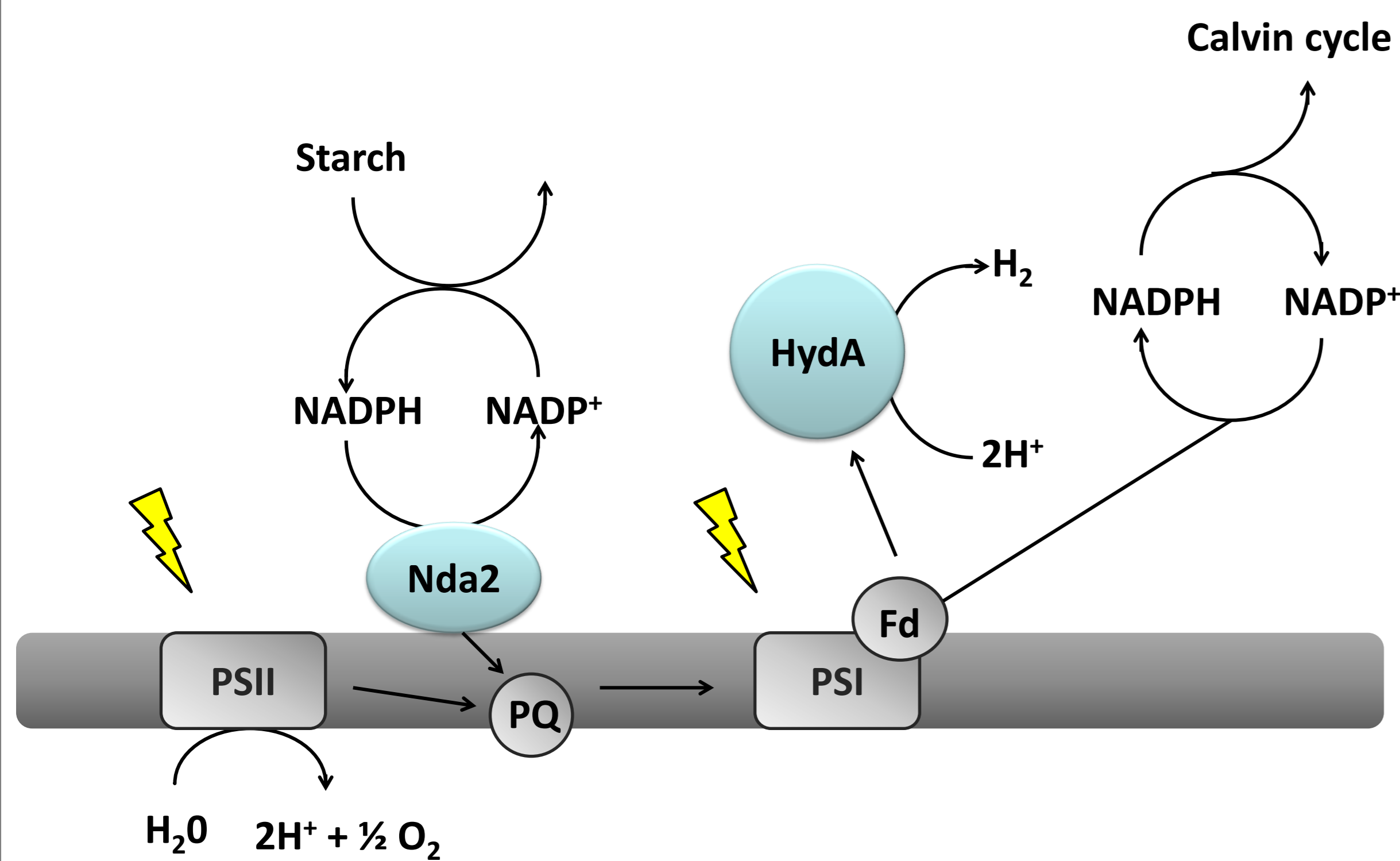
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1. Introduction



The ability of the unicellular green alga *Chlamydomonas reinhardtii* to evolve molecular hydrogen (H_2) is due to the presence in the chloroplast of oxygen-sensitive Fe-hydrogenases (HydA1/2), expressed in anoxic conditions that drive the photosynthetic electron flow to reduce protons into H_2 . Two different pathways can supply reduced ferredoxin for H_2 production in the light, PSII-dependent pathway and PSII-independent pathway (via Nda2) (Fig. 1).

H_2 photoproduction is only transient since O_2 is generated by PSII and inhibits hydrogenase activity which is physiologically relevant during transition from dark to light in wild type strain.

The aim of this project is to conduct forward genetic screen in order to identify new players involved in H_2 photoproduction in *Chlamydomonas*.

Fig. 1 : Simplified scheme of photosynthetic electron transport chain during H_2 photoproduction. PSII: photosystem II ; PQ: plastoquinone pool ; PSI: photosystem I ; Fd: ferredoxin ; Nda2: type II NAD(P)H dehydrogenase; HydA: hydrogenase.

2. Experimental and procedures

An insertion mutant library was generated using cassettes conferring resistance to an antibiotic (hygromycin or paromomycin) and analyzed by an *in vivo* fluorescence imaging screen based on the different kinetics of photosynthesis induction between wild type (WT) (Fig. 2A) and hydrogenase-deficient mutants (Hyd⁻) (Fig. 2B) [1].

Fluorescence recorded by the camera is emitted by the chlorophyll a from PSII and varies in function of the PQ's redox state. The more PQ is reduced (noted PQH₂), the more fluorescence is high and vice versa. In dark anoxic conditions, the cellular redox poise is high and the photosynthetic electron transport chain is fully reduced in both WT and Hyd⁻ strains.

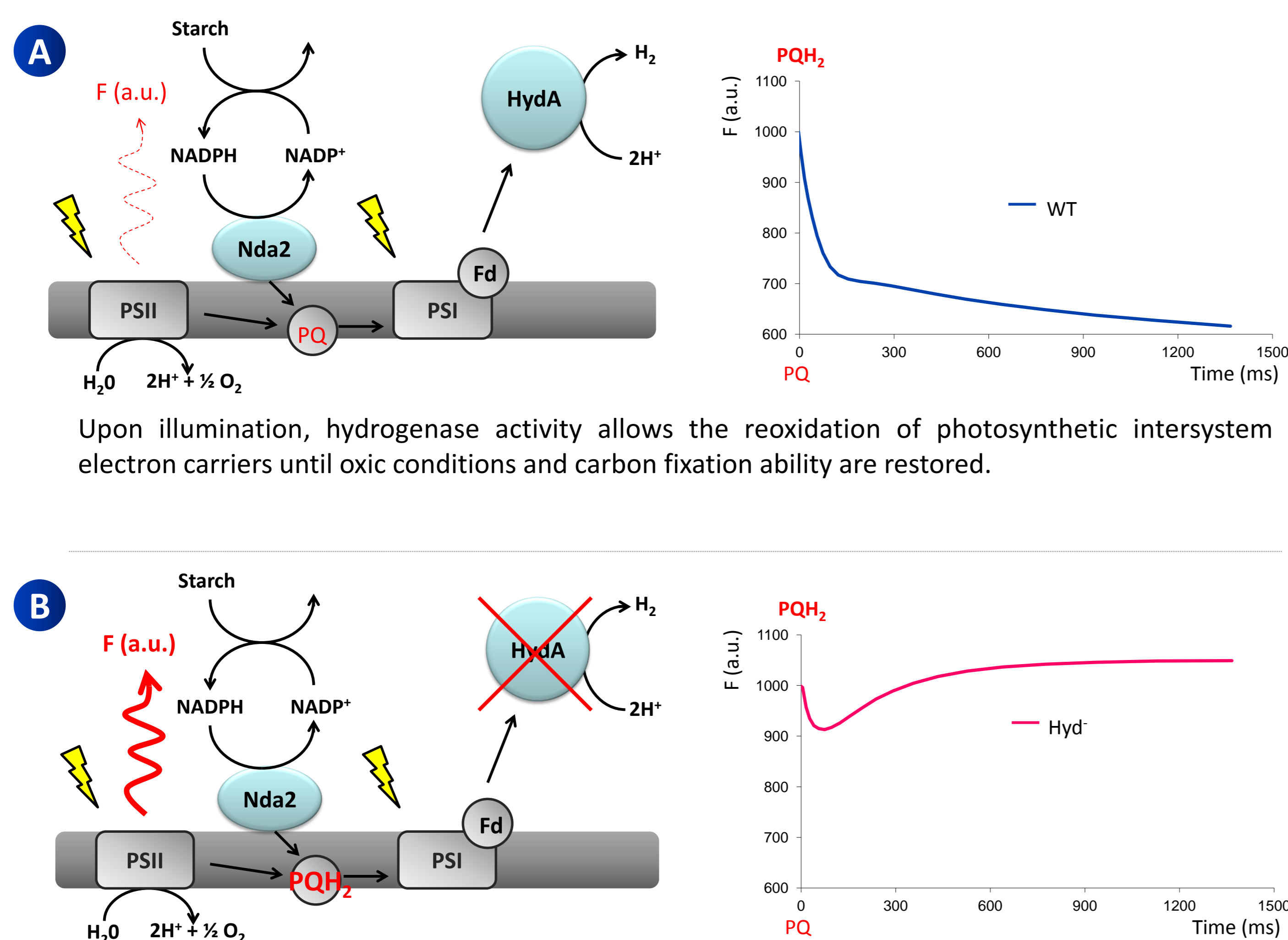


Fig. 2 : Fluorescence imaging screen.

3. Results and discussion

At this stage, twelve putative hydrogenase-deficient mutants have been identified on 16,000 transformants. Molecular characterization of these mutants is ongoing (Fig. 3).

A A cross between hydrogenase mutant strain and WT strain is conducted and meiotic progeny is selected on non selective medium before to be replicated in presence of antibiotic. 50% of meiotic resistant progeny (R⁺) means that only one resistance cassette is inserted in the genome of the mutant.

Analysis of the linkage between the antibiotic resistance and the fluorescence phenotype :

B Untagged mutant : R⁺ meiotic products show both kinetics, Hyd⁻ (●) and WT (●).
→ Insertion of the resistance cassette is not responsible for the fluorescence phenotype. Such mutants are not analyzed subsequently.

C Tagged mutant : R⁺ meiotic products show only Hyd⁻ kinetic (●).
→ Insertion of the resistance cassette is responsible for the fluorescence phenotype.

D Identification of the insertion site of the resistance cassette into the genome of tagged mutants by TAIL-PCR. This technique allows the amplification of DNA regions flanking the insertion site of the antibiotic cassette using primers specific to the cassette (blue arrows: primers 1, 2, 3 on Fig. 3D) and degenerated primers designed for the nuclear *Chlamydomonas* genome (black arrow on Fig. 3D). PCR products are sequenced and similar nucleotide sequences are searched into *Chlamydomonas* genome sequence database using Blast tools.

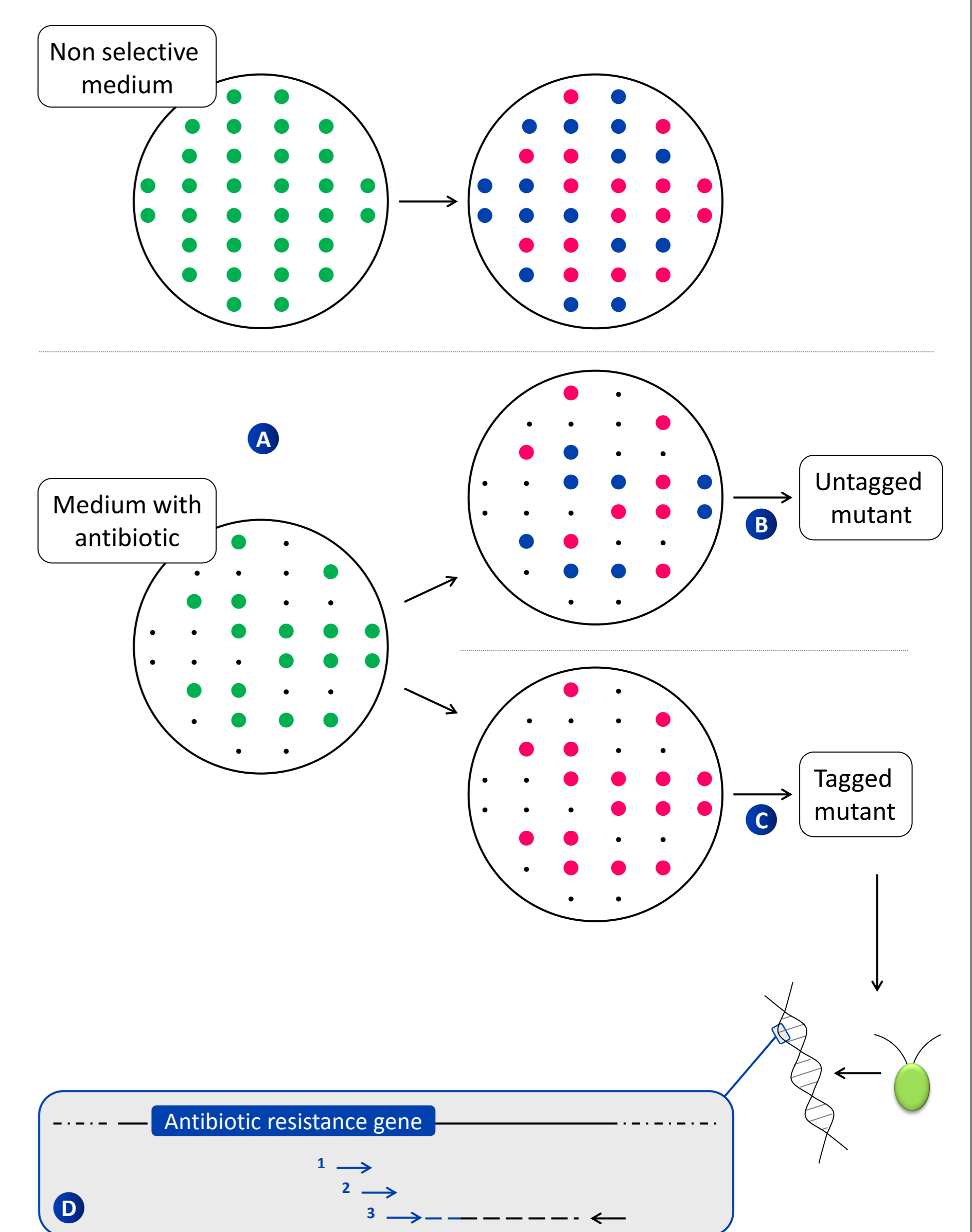


Fig. 3 : Scheme of molecular characterization of putative hydrogenase-deficient mutants.

The first results showed that two mutants were untagged with the resistance while three tagged mutants were deficient for the HydG assembly factor, necessary for active hydrogenase.

4. Conclusion

This screening procedure should allow to identify new players in hydrogenase transcription, translation, maturation and regulation processes. Compared to other screening methods previously developed [2] to identify mutants defective in H_2 photoproduction or hydrogenase activity/expression, the present screen based on distinctive fluorescence kinetics of hydrogenase-deficient mutants has two main advantages :

- (i) This is a non invasive procedure since *Chlamydomonas* cells can recover from exposure to dark anoxic conditions.
- (ii) The protocol is quite fast (a few thousands transformants analyzed a day) by using a camera with a high sensitivity which enables the direct screen of colonies.

[1] D. Godaux, B. Emonds-Alt, et al., "A novel screening method for hydrogenase-deficient mutants in *Chlamydomonas reinhardtii* based on *in vivo* chlorophyll fluorescence and photosystem II quantum yield", International Journal of Hydrogen Energy, Vol.38, 1826-36, (2012).
[2] M. Seibert, D.K. Benson, T. Flynn, "Method and apparatus for rapid biohydrogen phenotypic screening of microorganisms using a chemochromic sensor", in, vol. US 6277589 B1. Kansas City, MO (US): Midwest Research Institute, (2001).