DESIGN AND CONSTRUCTION OF A MODULAR IPTG-INDUCIBLE EXPRESSION SYSTEM IN *Chlorella vulgaris*

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Introduction. Microalgae are photosynthetic organisms that have the potential to satisfy several human needs, such as food supplements, biofuels and value-added compounds (1). A central issue for the production of high-value molecules, particularly recombinant proteins, is the low level of production achieved so far (1). Genetic engineering has been proved to be an alternative to overcome this problem (2, 3). However, development of genetic tools for the expression of exogenous genes in algae is crucial for the optimal use of these organisms at industrial scale.

The IPTG-inducible promoter has been proved to be a valuable tool in biotechnology, as it has been successfully implemented in several organisms for biotechnological purposes (4). Here, we propose a modular IPTG-Inducible System (IIS) in *C. vulgaris* for the production of a reporter protein, which could potentially be replaced for any gene of interest and could be implemented in other algae species.

Methods. *Biologic material. C. vulgaris 26* was purchased from "The Culture Collection of Algae" (University of Texas, USA) and used for the amplification of a 2-kbp region, comprising a portion of genes atpI-rps2. *Assembly of IIS. LacI* and RFP genes were amplified from plasmids distributed for the iGEM 2012 competition (iGEM Foundation, USA); the *psbA* promoter (P*psbA*) was amplified from the plastid genome of *C. reinhardti* strain cc125 mt+ (Chlamydomonas center, USA). All genes and plasmid manipulation were carried out following standard techniques in molecular biology.

Results. We have amplified the 2-kbp region of the genes atpI-rps2 from the chloroplast genome of *C. vulgaris*. This fragment has been inserted into vector pBlueScript KSII+ to generate the transformation vector pIA-1 (Fig 1a). For the assembly of the the IIS, the *LacI* and RFP genes have been amplified and inserted under the regulation of P*psbA* and P*lac*, respectively in a subcloning vector (Fig 1b). The fully assembled device of LacI and RFP was transferred to pIA-1 to obtain pIA-2 (Fig 1c).

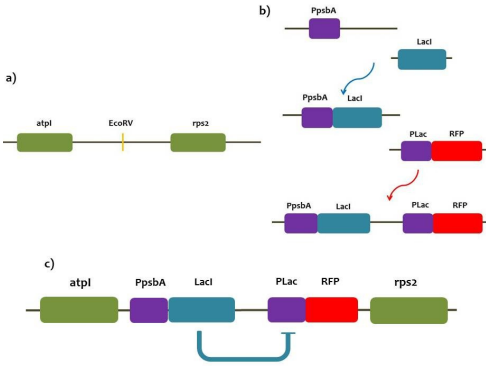

Fig.1 Schematic representation of transformation vectors pIA-1 and pIA-2. a) Homologous region in vector pIA-1; b) IIS assembly strategy; c) transformation vector pIA-2.

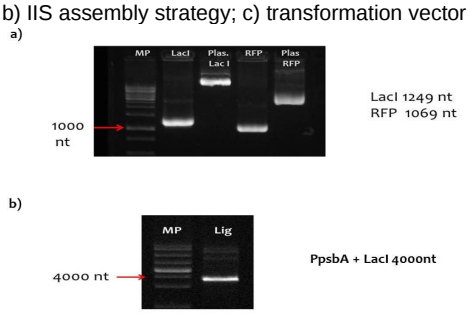

Fig.2 Gel electrophoresis of PCR amplification of *LacI* and RFP. a) Amplification of *LacI* and RFP genes. b) Restriction digestion to verify the ligation of P*psbA* with the coding region of *LacI*.

Conclusions. The modular design of the IIS here proposed will allow a systematic assembly of plastid transformation vectors for the regulated production of heterologous proteins in *C. vulgaris*. In addition, it is the first time IIS will be implemented in algae, to our knowledge. Once proven, the system could also be implemented in other algae species for the production of other proteins of interest, by replacing homologous recombination regions.

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