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Title

Expression of Recombinant Proteins in Microalgae

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Our initial objectives in this project were three-fold. I. Express functional antibodies in the chloroplast of *C. reinhardtii* II. Express functional antibodies in the nucleocytoplasmic compartment of *C. reinhardtii* and III. Define optimal parameters for the large scale culture of antibody producing strains of *C. reinhardtii*.

Objectives I and III. Our initial work in this area focused on obtaining high levels of expression of reporter genes in the chloroplast of *C. reinhardtii*. Through a process of codon optimization, we were able to significantly enhance the levels of accumulation of recombinant protein. We first demonstrated this for green fluorescent protein (GFP, see attached GFP pdf) and have subsequently codon optimized and expressed eleven more genes for expression in the *C. reinhardtii* chloroplast with similar success. Codon optimization alone was shown to enhance recombinant protein accumulation at least 80 fold. This information then allowed for the efficient expression of a functional antibody in the chloroplast of *C. reinhardtii* (see attached antibody pdf). Since the publication of this result, we have gone on to express scfv antibodies as well as individual LC and HC constructs. We have just recently completed constructs which should express fully assembled LC/HC antibodies.

Aside from expressing a myriad of recombinant proteins in the chloroplast of *C. reinhardtii*, we have also examined the effect of over 16 different 5' and 3' UTR combinations on the accumulation of recombinant proteins. These combinations give us a range of expression levels spanning almost two orders of magnitude. Utilizing this information, we were able to select UTR combinations which resulted in high levels of protein accumulation. We then selected these UTRs to drive expression of several of our antibody constructs and have published results demonstrating that the specific utr combination drastically influences protein accumulation in a light dependent fashion (see antibody pdf). We currently have experiments underway to develop suitable husbandry practices for our non-transformed *C. reinhardtii* strain grown at 60,000 L scale, and will shortly apply that knowledge to growing transgenic strains for the production of antibodies.

Objective II. While we have been able to express several reporter genes from the nucleocytoplasmic compartment of *C. reinhardtii*, we have not as yet expressed an antibody. Most of this arises from the fact that our chloroplast based expression has worked so well, hence we have devoted a tremendous amount of time toward it. We are confident that given a similar effort, we will enjoy similar success with the expression of antibodies from the nucleocytoplasmic compartment as well.

Aside from the expression of antibodies, this work has allowed us to demonstrate that we can express a variety of recombinant proteins in the chloroplast of *C. reinhardtii*. We have been successful at expressing proteins ranging from antibodies to enzymes, allowing these organisms to be exploited to their fullest. We envision a day when a variety of protein products, ranging from industrial enzymes to human therapeutics are produced in this or similar systems for the benefit of all humankind.

Publications

Stephen P. Mayfield, Scott E. Franklin and Richard A. Lerner. 2003. Expression and assembly of a fully active antibody in algae. *Proc. Natl. Acad. Sci.* 100(2): 438-42.

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