Establish a Procedure for Rapid Identification of Genetic Parts for Use in Algal Biotechnology

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The Idea

We propose to run a pilot experiment to investigate the feasibility of using DNase-SEQ to identify of regulatory elements in Chlamydomonas reinhardtii; with the view to producing a genetic toolkit for this alga. DNase-SEQ is a powerful approach to identify transcription factor (TF) binding sites (He et al. 2014) which can then be utilised as genetic parts. To date there have been no reports of DNase-SEQ being applied to C. reinhardtii so the first stage of the project will be to establish the procedure.
As a test case we will focus on identifying regulatory elements that control the induction of the algal carbon concentrating mechanism (CCM). The reason for this is that the Griffiths lab is interested to understand the CCM, and a set of genetic parts that induce expression upon CCM induction could serve as useful tools for future analyses, such as high throughput screening of carbon concentrating components which could be engineered into higher plants for crop yield improvement.
Additionally, exploring DNase-SEQ data can be difficult for those without bioinformatics experience; we therefore aim to develop an open access, online tool to facilitate this process.

Who We Are

Cindy Chan (kxc22@cam.ac.uk)

- PhD student in Physiological Ecology group, Department of Plant Sciences, University of Cambridge under the supervision of Professor Howard Griffiths.
- Current research focus is the carbon concentration mechanism (CCM) of Chlamydomonas reinhardtii with a focus on the pyrenoid biogenesis.
- Role: In charge of cell culture and harvesting. Nuclei extraction and DNAse I digestion.

Dr. Steven Burgess (sjb287@cam.ac.uk)

- Post-doctoral research associate in Molecular Physiology group, Department of Plant Sciences, University of Cambridge
- Current research areas include identification of regulatory elements using DNase-SEQ
- Role: Provide technical assistance in performing DNase-SEQ and a pipeline for DNase-SEQ analysis.

Marielle Vigouroux (marielle.vigouroux@jic.ac.uk)

- Support specialist in computational and systems biology in John Innes Centre, Norwich
- Current research includes the development of codon optimization tools in Chlamydomonas reinhardtii and analysis of RNA-Seq data.
- Role: Generation of an online tool for exploration of DNase-SEQ data.
- Implementation

Implementation

Step 1: (3 months)

1. Synchronous culture of wild type reinhardtii cells at ambient air condition – Cindy
2. Cell harvest at five time points: -2D, -1D, 1L, 3L, 6L (D: dark, L: light) – Cindy
3. Nuclei harvest – Cindy & Steven
4. DNase I digestion – Cindy & Steven
5. Library preparation – sequencing service (Dept. of Pathology, Cambridge)

Step 2: (2 months)

1. Sequencing – sequencing service (Dept. of Pathology, Cambridge)

2. DNase-SEQ pipeline refinement – Steven
3. Data analysis – Cindy

Step 3: (6 months)

1. Development of webpage – Marielle

Note: We have some preliminary data which can be used to establish the online tool so that the webpage development will be run in parallel to the experimental part of the project.

Benefits and outcomes

1. A set of candidate light responsive sequences for use as genetic parts in C. reinhardtii.
2. A protocol for performing DNase-SEQ analysis in C. reinhardtii.
3. An open-source pipeline for DNase-SEQ analysis.
4. A biologist-friendly website of the regulatory network of carbon concentrating mechanism of Chlamydomonas reinhardtii which would be open to public access.

Budget

NextSeq 500 Mid Output: £1450.00
Library Preparation: £2500.00
Webpage Development: £50.00
Total: £4000.00