Name : Kher Xing Chan (Cindy)

Email address : kxc22@cam.ac.uk

Other team members:

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

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

Establishing a Procedure for Rapid Identification of Genetic parts for Use in Algal Biotechnology

Project Framework

This project aims to run a pilot experiment to investigate the feasibility of using DNase-SEQ to identify the regulatory elements in *Chlamydomonas reinhardtii*, with a view to add to the existing toolkit for the alga. As a test case, we will focus on identifying regulatory elements which controls the induction of the algal carbon concentrating mechanism (CCM). The project is divided into three parts (Figure 1).

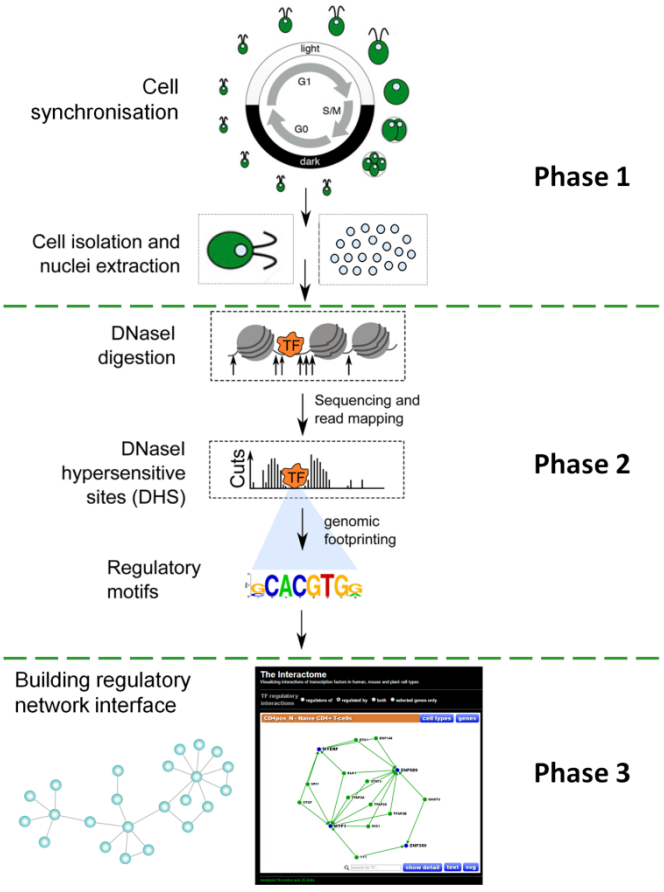

Figure 1: Overall framework of the project.

Progress Update

Phase I: Cell Synchronisation and Nuclei Isolation

Cell synchronisation was established using the method published by Mitchell et al. (2014)and cells can be synchronised and achieve the cell density required for nuclei isolation in a week.

Nuclei isolation was performed using protocol of Dr. Ivan Llorens but the quality and quantity of nuclei obtained was insufficient to continue with DNase I digestion. The nuclei were clumped together and degradation was observed (Figure 2). The outcome of this part of the project was presented as a poster during the OpenPlant Forum 2016 in Norwich.

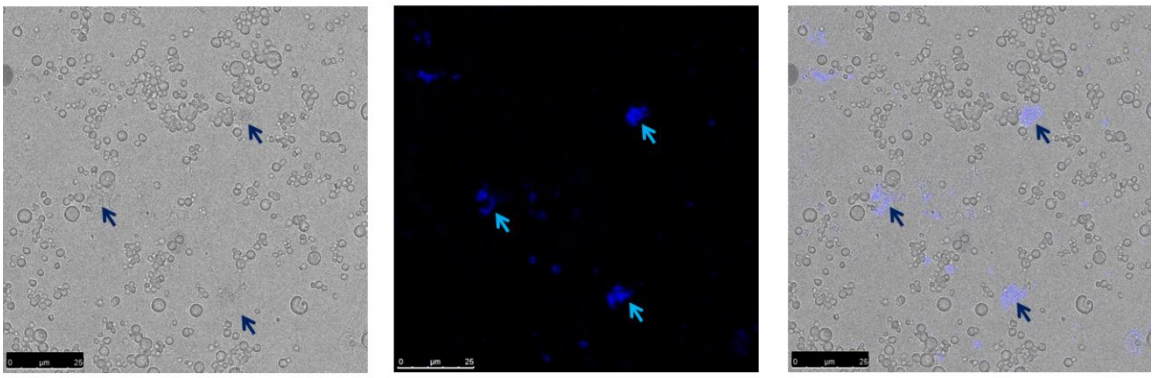

Figure 2: Bright field, DAPI-stained and DAPI-BF merged images of the nuclei isolated with the protocol of Dr. Ivan Llorens. The blue arrows point to DAPI-stained nuclei which were clumped together.

The issue of clumped nuclei was addressed using the protocol with a nuclei isolation buffer of lower pH (Sikorskaite et al. 2013) (Figure 3). However, the optimal number of cells used for nuclei isolation needs to be determined in order to obtain sufficient amount of nuclei for DNase I digestion.

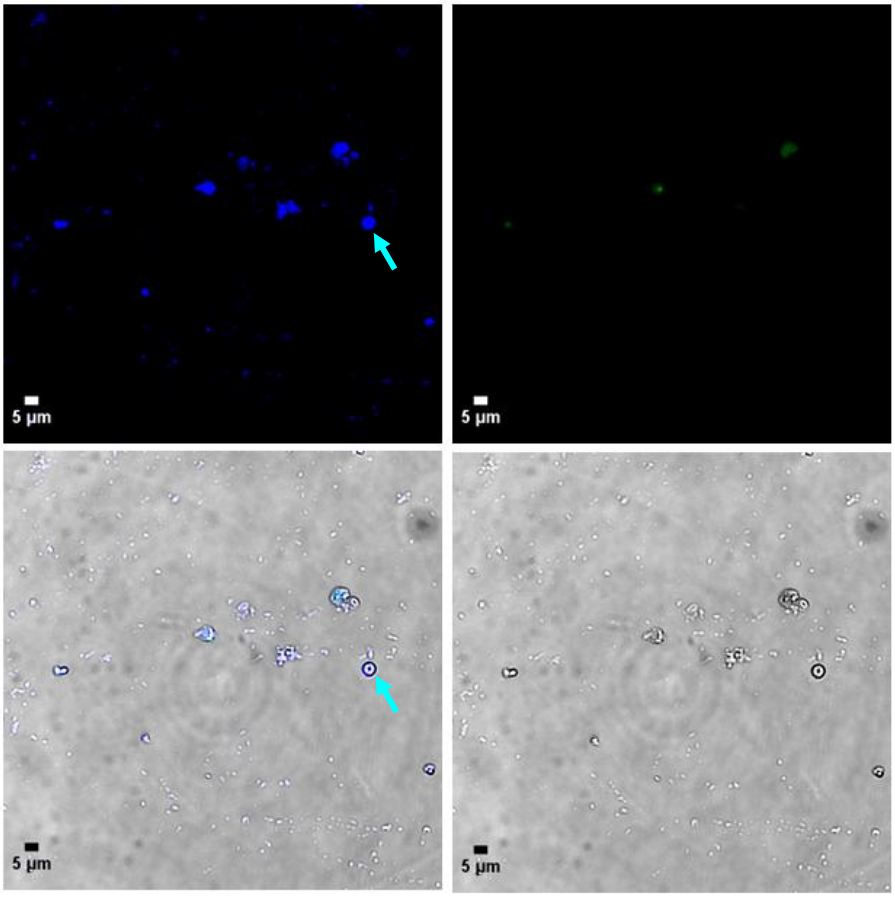

Figure 3: (A) DAPI-stained, (B) chlorophyll autofluorescence, and (C) DAPI-BF merged and (D) brightfield images of the nuclei isolated with the protocol from Sikorskaite et al. (2013) . The cyan arrows point to DAPI-stained nuclei.

Phase II: DNase I Digestion and DNase-SEQ

This phase will be conducted in the first quarter of 2017 upon achieving the optimal conditions for nuclei isolation process.

Phase III: Bioinformatics and Building Regulatory Network

Marielle Vigouroux of JIC uses a practice dataset from Dr. Steven Burgess to build the regulatory network while waiting for the data from our project.

Future Work

Due to commitment to other projects, the original schedule for this project is delayed but we would like to extend the current project to the first quarter of 2017. The final version of all protocols used for this project will be uploaded to protocols.io by the end of the project.