Genome editing by CRISPR system in microalgae

I-Son NG*

*Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
E-mail: yswu@mail.ncku.edu.tw

Being the green gold of the future, microalgae have recently attracted considerable interest worldwide, due to their great commercial potential. They possess many applications and can be used to produce different kinds of metabolites, such as lipids, protein, pigments and bioactive compounds. In the last decade, the efforts attended to enhance high-value compounds in microalgae are motivated by genetic manipulation. Among them, the CRISPR/Cas9 system appears to be most efficient and novel technology. The system is constituted of a single guide RNA (gRNA) and a non-specific CRISPR-associated endonuclease protein (Cas9). In order to function, Cas9 will promote a double strand break (DSB) in the DNA loci targeted by gRNA. In this study, plasmid pCAMBIA1302 containing right border (RB), left border (LB) and a fragment of mGFP was transformed into microalgae *Chlorella* by electroporation under voltage 360 V at 25 μF and 200 ohm. Two of the transformants showed higher fluorescent value (28% and 67%, respectively) compared with wild type, proved plasmid with RB and LB is suitable for gene insertion in *Chlorella*. Aside from that, pHSE401 containing fragment of Cas9, RB and LB was used as vector, with sgRNA designed from fatty acid desaturase (*fad*3), which can affect the accumulation of lipid. Finally, Cas9-RNP (ribonucleoprotein complexes), a novel plasmid-free application of CRISPR system, is a potential technology to simplify genetic construction and feasible for seamless DNA editing. We applied the in vivo synthesis of Cas9-RNP by co-expression of Cas9 and single guide RNA (sgRNA) in two plasimds simultaneously in *E. coli* BL21(DE3) to reduce the cost and shorten the processing time. By adjusting the replication origin and regulator, as well as used one step purification process, we has obtained higher yeild of Cas9-RNP. Results showed that the *rbcL* was successfully been digested into two desired fragments. The Cas9-RNP remained the activity for 21 days at 4^{o}C. Meanwhile, the gene of *CrPEPC* and *ZEP* were also chosen for in vivo test which regulated the accumulation of fatty acid and zeaxanthin in the metabolism of CC-400, respectively. This is first-time to use CRISPR/Cas9 based technology for gene manipulation and regulation in *Chlorella*.

I-Son Ng

Associate Professor

Department of Chemical Engineering

National Cheng Kung University, Tainan (NCKU), Taiwan

Education:

PhD, 2002 – 2005 Department of Chemical Engineering, NCKU

MSc, 1996 – 1998 Department of Chemical Engineering, NCKU

BSc, 1992 – 1996 Department of Chemical Engineering, NCKU

Professional Career:

2017.08 ~ now Associate Professor, Department of Chemical Engineering, NCKU

2014.08 ~ 2017.07 Assistant Professor, Department of Chemical Engineering, NCKU

2010.05 ~ 2014.07 Associate Professor, Department of Biochemical and Chemical Engineering, Xiamen University

2006.08 ~ 2009.03 Postdoctoral, Academic Sinica, BEST center

Research Interests:

Biochemical Engineering

Bioremediation and carbon dioxide sequestration

Direct evolution of protein

Synthetic Engineering

Selected publications

1. I-Son Ng*, et al., (2019) Bioresource Technology 291, 121932
2. I-Son Ng*, et al., (2019) Bioresource Technology 289, 121625.
3. I-Son Ng*, et al., (2019) Scientific Reports, 9:7589
4. I-Son Ng, et al., (2019) Chemical Engineering Journal (in press)
5. I-Son Ng*, et al., (2019) Biotechnology Progress DOI: 10.1002/btpr.2834
6. I-Son Ng*, et al., (2018) Process Biochemistry, 73: 38-46.