CRISPR interference (CRISPRi) for Dynamic Regulation of Gene Expression in Diatoms

Tessema Kassaw[1*](tessema.kassaw@colostate.edu), Graham Peers[1], **Andrew Allen**[2]

[1]Colorado State University, Fort Collins, CO; [2] J. Craig Venter Institute, San Diego, CA.

Project Goals*: Reprogram metabolic networks using in vivo synthetic modules to increase the flux of energy and carbon into biofuel precursors. Goal 1) Profiling the transcriptome, proteome and metabolome to investigate cell responses to physiologically relevant conditions. Goal 2) Identify and manipulate key factors involved in the control of inorganic C assimilation, photosynthetic efficiency and regulation of lipid accumulation. Goal 3) Development of Phaeodactylum genome reconstruction /modeling and our development of novel synthetic genomic tools to achieve our overall goal of increasing productivity.*

CRISPRi-based gene inactivation is an attractive tool to probe genome-wide gene functions and to reprogram metabolic networks due to its high target specificity, reversibility, and multiplexing capacity. However, the current state of the art for gene expression knockdown in most algae is RNAi. While RNAi can occasionally be successful in producing strains with lowered cellular enzyme activities, it is less efficient, and the underlined mechanism is still unknown in diatoms. We recently developed two episome-based small molecule responsive transcriptional control systems in the model diatom alga, *Phaeodactylum tricornutum*. These systems are activated by the addition of exogenous β-estradiol and digoxin. We demonstrated that our transcriptional control systems are tunable and reversible maintaining a dynamic range of up to ~180-fold. We employed our inducible system to regulate the expression of *P. tricornutum* codon optimized dCas9 to establish a CRISPRi system for at will control of target protein production from an episome. We first assessed the feasibility of our CRISPRi approach using eYFP reporter system. We found that up to 80% repression can be achieved by targeting the reporter protein close to its transcription start site using quantitative fluorescence assay. We then selected and designed sgRNA for multiple endogenous loci (nitrate reductase, urease) to demonstrate the β-estradiol inducible CRISPRi application. Based on our preliminary result, we observed ~20% and ~70% growth reduction by targeting nitrate reductase and urease genes respectively. To our knowledge this is the first report on exploiting inducible CRISPRi system for targeted gene inactivation in any algal system. The procedure described here will expand the synthetic biology toolkits in diatoms and can be adapted for high-throughput interrogation of genome-wide gene functions and redirection of metabolic fluxes towards target chemical production.

Reference:

Kassaw, T.K.; Paton, A.J.; Peers G. Episome-Based Gene Expression Modulation Platform in the Model Diatom Phaeodactylum tricornutum. ACS Synthetic Biology, 2022. DOI 10.1021/acssynbio.1c00367.

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