Cell-free transcription-coupled CRISPR/Cas12a assay for prototyping cyanobacterial promoters

최윤남, 신예림, 박종문, 이정욱†
포항공과대학교
(jeongwook@postech.ac.kr†)

Cyanobacteria are promising microbial hosts for the production of diverse biofuels and biochemicals. However, the relatively long time required by strain engineering—due to the variability of the genome copy number—makes cyanobacteria engineering challenging and time-consuming, thereby necessitating the development of a rapid and high-throughput prototyping tool for cyanobacteria. In this study, we developed a CRISPR/Cas12a-based assay coupled with cyanobacteria cell-free systems to rapidly prototype promoter characteristics. Using this newly developed assay, we demonstrated cyanobacteria cell-free transcription for the first time and confirmed a strong correlation between the in vitro and in vivo transcription performance. Furthermore, we generated a synthetic promoter library and, by using our assay, we efficiently evaluated the characteristics of its components in a high-throughput way. We believe that this study offers an easily applicable and rapid prototyping platform for characterizing promoters for cyanobacterial engineering.