A Fused Deactive Cas9 Complex

Technology #19138

Applications

The inventors have developed a novel, fused Cas9 complex for engineering synthetic gene circuits. Potential applications for this invention include basic synthetic regulatory network engineering, therapeutics, metabolic engineering, and biological research.

Problem Addressed

Gene regulatory networks are circuits in which molecular regulators, such as DNA-binding transcription factors, interact to control the expression of genes in a cell. Modulating gene regulatory networks, particularly through engineering of synthetic gene circuits, is essential for practical applications in biological engineering as well as for understanding the fundamentals of biological evolution. However, implementing synthetic biological circuits on the scale of hundreds of genes is difficult. As a result, CRISPR-based systems have emerged as useful tools for modulating regulatory circuits. Previous approaches utilize a catalytically inactive form of Cas9 (dCas9) that acts as a repressor after being directed to specific genes. However, when dCas9 is expressed at the high levels, as is common when building complex circuits, this protein becomes extremely toxic in bacterial cells due to non-specific binding. Therefore, there remains a need to engineer CRISPR/dCas9-based systems for regulatory network use in prokaryotes in which dCas9 can be expressed at high concentrations while maintaining specificity and avoiding toxicity.

Technology

The inventors have developed a novel, non-toxic dCas9 complex that enables construction of complex biological circuits in bacterial cells. To decrease non-specific binding, and thus reduce toxicity, the inventors have engineered a mutant variant of dCas9 that can no longer recognize the PAM sequence, yet still retains DNA binding capabilities. This Cas9 variant is fused to a transcriptional repression factor, Ph1F, using a linker peptide. Thus, when Ph1F-binding DNA sequences are placed in front of cognate promoters, the fused dCas9 complex can effectively and specifically repress the expression of target genes. Approximately 104 copies of fused dCas9 protein can be expressed in a cell without compromising cellular health. Furthermore, fusion of dCas9 to Ph1F improved cooperativity compared to the existing dCas9 system, which is critical for building intricate gene circuits.

Advantages

- Novel, Cas9 fusion complex with low toxicity for CRISPR-based engineering of synthetic biological circuits in prokaryotes
- Fused Cas9 protein can be expressed at high levels without damaging cellular health, enabling construction of complex gene circuits

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Categories For This Invention:

Life Sciences
Research Tools
Expression Systems
Vector & Plasmid
Synthetic Biology
Bacterial
Therapeutics
Gene Silencing

Intellectual Property:

Engineered dCas9 with reduced toxicity and its use in genetic circuits
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Engineered dCas9 with reduced toxicity and its use in genetic circuits
PCT

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