Methods and Compositions for RNA-guided Genetic Circuits

Technology #18596

Applications

This technology allows rational forward engineering of complex cellular behaviors using RNA-guided genetic circuits. It has potential applications in a wide variety of biotechnology fields including therapeutics and agriculture.

Problem Addressed

Genetic engineering of cells to respond to particular stimuli and perform complex tasks has unlocked a new horizon in biotechnology. However, there are many challenges in designing molecular circuits in living cells, and there is a compelling need to develop molecular tools to increase the robustness of circuit function. These inventors describe a technology with five major improvements that help to surmount some of the issues associated with designing and utilizing RNA-guided genetic circuitry.

Technology

This technology uses catalytically inactive CRISPR-dCas9 that is guided by gene specific sgRNAs to design genetic circuits. The technology includes five significant improvements to RNA-guided circuitry. The first improvement is the generation of point mutants of dCas9 that reduce cellular toxicity, which increases cellular growth and reduces dCas9 silencing. The next improvement includes strategies to introduce non-linearity to signal responses by using “decoy” DNA sites or sRNAs to degrade RNAs. This increases the robustness of the system by reducing noise. The third improvement is a map of nucleotide substitutions that can be made in sgRNAs without reducing function. This diversity of sgRNAs reduces the rate of homologous recombination when many sgRNAs are expressed. The fourth improvement is the use of a heterologous T7 RNA polymerase to drive circuit expression. The use of a heterologous polymerase means that the system can be rapidly used across many different organisms without significant adaptation. Finally, the inventors developed a software that automates the design of RNA-guided genetic circuits. The software uses simple user-defined inputs and outputs a wiring diagram, sgRNA sequences, and predictions of circuit performance.

Advantages

- Reduced dCas9 cellular toxicity
- Non-linear responses to improve circuit robustness and reduce noise
- Diverse sgRNAs to reduce homologous recombination
- Heterologous T7 expression for simple adaptation across organisms
- Circuit design software to simplify circuit construction

Categories For This Invention:

Agriculture

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External Links:

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