

ENABLING GENETIC ANALYSIS OF DIVERSE BACTERIA WITH MOBILE-CRISPRi

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INVENTION NOVELTY

Researchers at UCSF, funded in part by the Chan Zuckerberg Biohub, have generated a modular and effective CRISPRi system for the genetic dissection of non-model bacteria.

VALUE PROPOSITION

- ▶ A suite of CRISPRi systems that combines modularity, stable genomic integration and ease of transfer to diverse bacteria by conjugation
- ▶ Inducible and tunable conditional knockdown, allowing for analysis of essential and non-essential genes
- ▶ Stable genetic integration without antibiotic selection, enabling studies of gene and antibiotic function, in which maintaining selection is problematic or impossible

TECHNOLOGY DESCRIPTION

The vast majority of bacteria, including human pathogens and microbiome species, lack genetic tools needed to associate genes with phenotypes. A method of gene disruption, clustered regularly interspaced short palindromic repeats interference (CRISPRi), enables the robust silencing of target genes by interfering with gene transcription. CRISPRi has demonstrated utility in species ranging from bacteria to humans. However, the difficulty of establishing robust genetic tools, such as CRISPRi, in non-model bacteria has profoundly limited our understanding of the contributions of genes and gene networks to bacterial physiology and pathogenicity.

APPLICATION

- ▶ Genetic analysis of pathogenic bacteria lacking genetic tools
- ▶ Analyses of microbiome function, antibiotic resistances and sensitivities of pathogenic bacteria
- ▶ Comprehensive screens for host-microorganism interactions
- ▶ Mechanism of action studies in relevant human pathogens

STAGE OF DEVELOPMENT

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OTHER INFORMATION

KEYWORDS

CRISPRi, genome
 integration, genetic
 dissection, bacterial
 pathogenesis, non-model
 bacteria

CATEGORIZED AS

- ▶ **Research Tools**
- ▶ Animal Models
- ▶ Nucleic Acids/DNA/RNA

RELATED CASES

2019-228-0

The inventors have developed Mobile-CRISPRi, a suite of modular CRISPRi systems for use in non-model bacterial systems.

Mobile-CRISPRi can be stably integrated into diverse bacterial species and used in combination with organism-specific genetic libraries and tools. Mobile-CRISPRi modules achieve transfer and genomic integration by distinct mechanisms, while ensuring a specified orientation and without gene disruption. Importantly, genomic integration is stable for more than 50 generation, even in the absence of selection. The inventors demonstrate the efficacy and applicability of Mobile-CRISPRi in drug-gene synergy studies in several pathogenic bacteria.

RELATED MATERIALS

► [Enabling genetic analysis of diverse bacteria with Mobile-CRISPRi - 01/07/2019](#)

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	12,018,258	06/25/2024	2019-228

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