TYPE III CRISPR-CAS SYSTEM FOR ROBUST RNA KNOCKDOWN AND IMAGING IN EUKARYOTES

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PATENT STATUS

Patent Pending

BRIEF DESCRIPTION

Type III CRISPR-Cas systems recognize and degrade RNA molecules using an RNA-guided mechanism that occurs widely in microbes for adaptive immunity against viruses. The inventors have demonstrated that this multi-protein system can be leveraged for programmable RNA knockdown of both nuclear and cytoplasmic transcripts in mammalian cells.

Using single-vector delivery of the S. thermophilus Csm complex, RNA knockdown was achieved with high efficiency (90-99%) and minimal off-targets, outperforming existing technologies of shRNA- and Cas13-mediated knockdown. Furthermore, unlike Cas13, Csm is devoid of trans-cleavage activity and thus does not induce non-specific transcriptome-wide degradation and cytotoxicity. Catalytically inactivated Csm can also be used for programmable RNA-binding, which the inventors exploit for live-cell RNA imaging. This work demonstrates the feasibility and efficacy of multi-subunit CRISPR-Cas effector complexes as RNA-targeting tools in eukaryotes.

SUGGESTED USES

This invention has the potential to completely supplant RNAi and Cas13-based RNA knockdown technologies.

Suggested uses include:

» research purposes requiring gene perturbation at the transcript (RNA) but not genome (DNA) level.
» efficiently knocking down both cytoplasmic and nuclear RNAs.
» RNA imaging in live cells.
» RNA detection for diagnostic purposes.
» utilizing the enzyme's ssDNase and/or cyclic oligoadenylate synthesis activities.

ADVANTAGES

The advantages include:

» yields robust, highly efficient RNA knockdown with minimal off-targets and no cell toxicity
» cheap, easy to use, and easy to deliver as an all-in-one vector
» allows for gene perturbation at the transcript (RNA) level
» efficiently knocks down both cytoplasmic and nuclear RNAs

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- COMPOSITIONS AND METHODS FOR IDENTIFYING HOST CELL TARGET PROTEINS FOR TREATING RNA VIRUS INFECTIONS
- Lentivirus-like Particle Delivery of CRISPR-Cas9 & Guide RNA for Gene Editing
- Cas13-mediated DNA Detection Reporter Molecules
- Improved guide RNA and Protein Design for CasX-based Gene Editing Platform
- Cas13a/C2c2 - A Dual Function Programmable RNA Endoribonuclease
- CasX Nickase Designs, Tans Cleavage Designs & Structure
- A Dual-RNA Guided CasZ Gene Editing Technology
- CRISPR-CAS EFFECTOR POLYPEPTIDES AND METHODS OF USE THEREOF ("Cas-VariPhi")