Max-Planck-Innovation

Technology Offer

# RNA-CASing: means and methods for selective isolation and stabilization of RNA molecules

Ref.-No.: 0706-5433-MSG

**RNACasing: A novel and selective way of isolating and stabilizing RNA molecules, which enables processing, storing and shipping of RNA at room temperature.**

## Background

CRISPR-Cas systems are adaptive immune systems in archaea and bacteria. Thereby, there are different types of operating systems. Type I systems employ multi-subunit Cascade (CRISPR-associated complex for antiviral defence) complex to facilitate duplex formation between a CRISPR RNA spacer and the complementary DNA-strand protospacer. In contrast, Type I-Fv Cascade of *Shewanella putrefaciens* consists of Cas5fv, the crRNA endonuclease Cas6 and the backbone protein Cas7fv. Since 2015, it has been known that Cas7fv can form extended filaments with unspecific RNA molecules and formation of Cas5fv/Cas7fv dimers was observed. A conserved network of amino acids of Cas5fv provides specific recognition of the 5'-terminal 8nt tag of crRNA (5'-CTTAGAAA-3'), thus Cas5fv shows high specificity for RNA target selection. This feature is unique for Type I-Fv Cascade assemblies, as other CRISPR-Cas systems harbor additional large subunits with contacts to the repeat tag. It is moreover known that Cas5fv interacts with Cas7fv and initiates the formation of a six subunit Cas7fv backbone that spans the entire length of the CRISPR RNA spacer sequence.

## Technology

Our scientists have found a process, which provides RNA segments from chemical and enzymatic ribolysis, thereby enabling the storing and shipping of RNA at room temperature. Briefly, this technology is based on the discovery that the 5' RNA tag can be fused with any RNA molecule, resulting in specific recognition by Cas5fv and filament protection by Cas7fv. Our scientist termed these filaments "RNA-casing", as they provide protection from chemical and enzymatic ribolysis.
The technology thereby relies on two plasmids that have been designed for transformation into *e.coli.* Plasmid 1 enables the recombinant production of Cas5fv, Cas7fv and Cas6. Plasmid 2 enables the fusion of desired RNA sequences downstream of the repeat tag sequence. This repeat tag sequence can be processed by a provided Cas6 endonuclease. The designed sequences can be transferred onto plasmids that provide compatibility with other organisms.

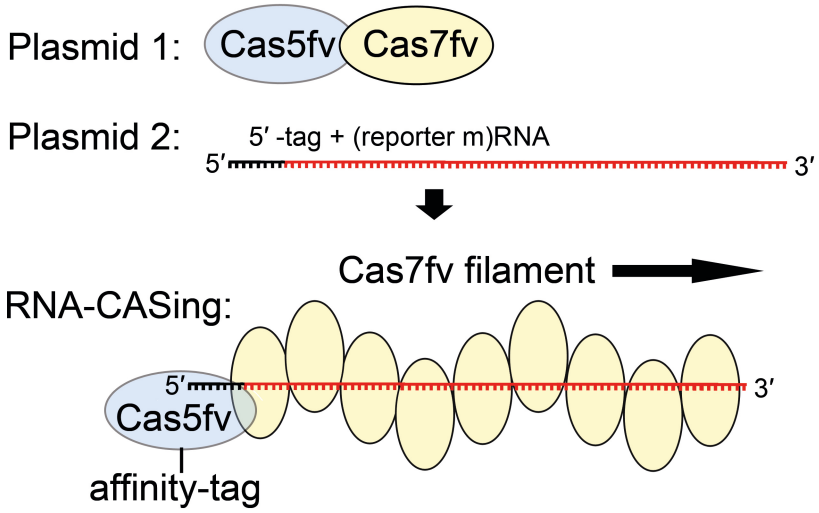
Additional envisioned applications contain the possibility to

- produce toxic RNAs which can be generated *in vivo* and shielded from contacts with other proteins
- fuse Cas5fv with fluorescent proteins and localize the tagged RNAs in the cell
- fuse Cas7fv to proteins in order to compartmentalize the proteins required to interact in metabolic pathways
- maintain regulatory RNA functions which rely on base paring, as the stabilized RNA is not fully encased, and five-nt segments are available for interactions with RNA.



Max-Planck-Innovation GmbH
Amalienstr. 33
80799 München
www.max-planck-innovation.de

**Filamentation of mRNA molecules**




Recombinant production of (i) the Cas5fv/Cas7fv dimer and (ii) the desired RNA (red) fused to a 5'-tag (black) in *e.coli* results in stable filament formation, termed RNA-CASing. Reporter mRNA can be silenced and affinity purification with tagged Cas5fv allows for the isolation of stable ribonucleoprotein complexes.

We are now looking for either a licensing partner, or a collaboration partner to further develop this exciting project.

## Patent Information

The PCT application EP18/070739 was filed on 31.07.2018.

## Contact

**Dr Matthias Stein-Gerlach**

Senior Patent- & License Manager
Biologist

Phone: +49 (0)89 / 29 09 19 - 18
eMail:
stein-gerlach@max-planck-innovation.de



Max-Planck-Innovation GmbH
Amalienstr. 33
80799 München
www.max-planck-innovation.de