CRISPR/Cas9-based genome editing for simultaneous interference with gene expression and protein stability - DTU Orbit (09/12/2018)

CRISPR/Cas9-based genome editing for simultaneous interference with gene expression and protein stability

Interference with genes is the foundation of reverse genetics and is key to manipulation of living cells for biomedical and biotechnological applications. However, classical genetic knockout and transcriptional knockdown technologies have different drawbacks and offer no control over existing protein levels. Here, we describe an efficient genome editing approach that affects specific protein abundances by changing the rates of both RNA synthesis and protein degradation, based on the two cross-kingdom control mechanisms CRISPRi and the N-end rule for protein stability. In addition, our approach demonstrates that CRISPRi efficiency is dependent on endogenous gene expression levels. The method has broad applications in e.g. study of essential genes and antibiotics discovery.