Bacterial Genome Editing Strategy for Control of Transcription and Protein Stability

In molecular biology and cell factory engineering, tools that enable control of protein production and stability are highly important. Here, we describe protocols for tagging genes in Escherichia coli allowing for inducible degradation and transcriptional control of any soluble protein of interest. The underlying molecular biology is based on the two cross-kingdom tools CRISPRi and the N-end rule for protein degradation. Genome editing is performed with the CRMAGE technology and randomization of the translational initiation region minimizes the polar effects of tag insertion. The approach has previously been applied for targeting proteins originating from essential operon-located genes and has potential to serve as a universal synthetic biology tool.