



# Impact of CRISPR interference on strain development in biotechnology

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## Abstract

Genetic perturbation systems are of great interest to redirect metabolic fluxes for value-added production, as well as genetic screening for the development of new drugs, or to identify new targets for biotechnological applications. Here, we review CRISPR interference (CRISPRi), a method for gene expression using a catalytically inactive version of the CRISPR-associated protein 9 (dCas9) of the widely applied CRISPR-Cas9 genome editing system. In combination with the appropriate sgRNA, dCas9 binds to specific DNA sequences without causing double-stranded DNA breakage but interfering with transcription initiation or elongation. Besides manifold uses to interrogate the physiology of a bacterial cell,

CRISPRi is used in applications for metabolic engineering and strain development in industrial biotechnology. Albeit in its infancy, CRISPRi has already delivered the first success stories; however, we also analyze limitations of the CRISPRi system and give future perspectives. © 2020 The Authors. *Biotechnology and Applied Biochemistry* published by Wiley Periodicals, Inc. on behalf of International Union of Biochemistry and Molecular Biology Volume 67, Number 1, Pages 7–21, 2020

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**Keywords:** CRISPRi, genetic screening, metabolic engineering

## 1. Introduction

Metabolic engineering of microorganisms increases the host cells' production of a broad range of compounds, including fine-chemicals, drugs, and biofuels. Since the complex metabolic networks are used for the survival of microorganisms, changes in the network structure can have a dramatic impact on the viability of the production host. Hence, strict regulatory mechanisms for the fine-tuned balancing of competing pathways are needed. These can rely on, for example, transcriptional regulators that bind to their specific DNA operator sequences to repress or activate transcription of target genes or operons. Most transcription factors respond to intra- and extracellular signals. Therefore, a number of transcriptional regulation tools

have been developed for application in metabolic engineering. Thus, by RNA interference (RNAi) gene expression of one or more genes can be inhibited [1]. Other RNAi approaches in eukaryotes include *i.a.* morpholinos, siRNA, shRNA, and S-DNA [2,3]. However, these approaches typically suffer eventual off-target effects and therefore their use in metabolic engineering is limited.

Recently, the acquired resistance system against phages and plasmids named Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated protein 9 (Cas9) of *Streptococcus pyogenes* have been repurposed for gene repression. Sequence-specific gene repression by this method has been named CRISPR interference (CRISPRi) [4–6]. This genetic tool only requires a dCas9 protein and a chimeric single-guide RNA (sgRNA) to target a DNA sequence of interest [5]. The dCas9 protein consists of the well-known Cas9 protein, lacking its endonucleolytic activity by two amino acid exchanges (D10A and H841A) in the RuvC and HNH endonuclease domains of the original Cas9 protein [5]. Because the complex of dCas9 enzyme and gRNA is still able to bind target DNA, this RNA-guided transcription repression system operates without altering the target DNA sequence (Fig. 1). Furthermore, CRISPRi-mediated gene repression by up to 99% has been observed, which leads to consistent phenotypical results [7]. Application of this easy and fast-to-modify method [6]

**Abbreviations:** CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; CRISPRa, CRISPR activation; dCas9, catalytically inactive version of the CRISPR associated protein 9; RNAi, RNA interference; sgRNA, single-guide RNA.

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