# Our first-hand experience with CRISPR interference approach for gene silencing in mycobacteria

By

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**ABSTRACT**

Tuberculosis (TB) is a dreadful disease killing people at an alarming rate of 1.5 million annually and infecting another 9 million worldwide. Identification and characterization of new drug targets and vaccine candidates in the causative agent of TB, *Mycobacterium tuberculosis* (Mtb) is urgently required for combating the menace of this deadly infectious disease. Convergence of complete genome sequence and advanced genetic tools provides a roadmap to understand the complex biology of TB-causing pathogen, which further contributes in helping the scientific community achieving the milestones of ongoing anti-TB drug and vaccine programmes. Although, various available mycobacterial genetic tools such as allelic exchange, insertional mutagenesis and regulatory expression system have proven useful in decoding some of the unknown genes' functions, these approaches have largely failed in deducing the function of complete mycobacterial genome due to their poor efficiency, particularly for genes of essential cellular functions.

In order to generate a rapid, cost-effective and efficient tool for targeted gene inactivation, we have systematically optimized and employed a novel approach namely *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats-*i*nterference (CRISPRi) for silencing the expression of a variety of genes in several mycobacterial species including Mtb. CRISPRi opens a new avenue for rapidly characterizing the function of unknown genes in Mtb to better understand their place in the cascade of essential cellular and molecular events.