Abstract title:

Neutralization of competition in CRISPRi circuits via dCas9 feedback regulator

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Abstract content (300 words w/ references)

CRISPR interference (CRISPRi) has emerged as a powerful tool for modulating complex endogenous transcriptional programs and for building larger scale synthetic genetic circuits [1,2].

However, highly specific sgRNA-promoter binding is, alone, insufficient to achieve independent transcriptional regulation of multiple targets. Indeed, due to competition for dCas9, the repression ability of one sgRNA reduces significantly when another sgRNA becomes expressed [3].

To solve this problem, decoupling sgRNA-mediated regulatory paths, we created a dCas9 concentration regulator that implements a negative feedback on dCas9 level [4]. This allows any sgRNA to maintain an approximately constant dose-response curve, independent of other sgRNAs.

We demonstrate the regulator performance on both single-stage and layered CRISPRi-based genetic circuits, zeroing competition effects of up to 15-fold changes in circuit I/O response encountered without the dCas9 regulator.

This recovery was confirmed even varying DNA copy numbers, strains and signaling molecules activating the systems;

Our dCas9 generator is implemented in a dedicated plasmid, to be easily transported across compatible bacterial strains, thus enabling true scalability of CRISPRi-based transcriptional programs.

We establish an important new tool of broad interest for CRISPRi users to either control endogenous transcriptional programs or to create new genetic circuits for any application.

[1] Gao Y, Xiong X, Wong S, et al. Complex transcriptional modulation with orthogonal and inducible dCas9 regulators. Nat Methods 13, 12 (2016).

[2] Kiani S, Beal J, Ebrahimkhani MR, et al. CRISPR transcriptional repression devices and layered circuits in mammalian cells. Nat Methods 11, 7 (2014).

[3] Zhang S, Voigt CA. Engineered dCas9 with reduced toxicity in bacteria: implications for genetic circuit design. NAR 46, 20 (2018).

[4] Huang H-H, Bellato M, Qian Y, et al. dCas9 regulator to neutralize competition in CRISPRi circuits. Nat Commun 12, 1692 (2021).

Title: **NEUTRALIZATION OF COMPETITION EFFECTS IN CRISPRI-BASED GENETIC CIRCUITS VIA A DCAS9 FEEDBACK REGULATOR.**

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Content (up to 2200 characters w/o spaces)

CRISPR interference (CRISPRi) has emerged as a powerful tool for modulating complex endogenous transcriptional programs [1] and for building larger scale synthetic genetic circuits [2].
While CRISPRi-mediated gene regulation allows simultaneous control of many genes, its highly specific sgRNA-promoter binding is, alone, insufficient to achieve independent transcriptional regulation of multiple targets. Indeed, due to competition for dCas9, the repression ability of one sgRNA reduces significantly when another sgRNA becomes expressed [3]. As a consequence, theoretically independent regulatory paths become coupled through dCas9 sharing. Thus, simultaneous and independent control of multiple genes remain a challenge. This problem cannot be addressed by simply increasing dCas9 expression since this protein is toxic in many bacteria when expressed in high amounts. Hence, alternative solutions are required.
In this work [4], we created a dCas9 expression system in which the level of apo-dCas9 (dCas9 devoid of sgRNA) is regulated to match sgRNAs demand. The regulator ensures that apo-dCas9 concentration is kept at an approximately constant, non-toxic level, regardless of loads by sgRNAs. This is accomplished by engineering a negative feedback loop composed of a constitutively transcribed sgRNA that specifically targets a strong promoter expressing dCas9.
When one sgRNA becomes transcribed and loads dCas9, the level of apo-dCas9 initially drops. This, in turn, reduces the amount of sgRNA-dCas9 complex targeting dCas9 promoter, which becomes de-repressed, thereby increasing dCas9 expression, compensating for the initial decrease in its level. As a result, any sgRNA in the system always has about the same amount of available apo-dCas9 and hence keeps a constant repression strength, independent of the amount of transcribed sgRNAs.
We tested our dCas9 regulator on both single-stage and layered CRISPRi circuits, varying DNA copy numbers, strains and signaling molecules activating the systems.

In all conditions, the regulator is able to neutralize interference between the desired sgRNAs and a competitor sgRNA, demonstrating the wide applicability of the approach.
Our dCas9 generator is implemented in a dedicated plasmid and can be easily transported across compatible bacterial strains, enabling true scalability of CRISPRi-based transcriptional programs.
We establish an important new tool of broad interest for CRISPRi users to either control endogenous transcriptional programs or to create new genetic circuits for any application.

[1] Gao Y, Xiong X, Wong S, Charles EJ, Lim WA, Qi LS. Complex transcriptional modulation with orthogonal and inducible dCas9 regulators. Nat Methods 13, 12 (2016). doi:10.1038/nmeth.4042
[2] Kiani S, Beal J, Ebrahimkhani MR, et al. CRISPR transcriptional repression devices and layered circuits in mammalian cells. Nat Methods 11, 7 (2014). doi:10.1038/nmeth.2969
[3] Zhang S, Voigt CA. Engineered dCas9 with reduced toxicity in bacteria: implications for genetic circuit design. NAR 46, 20 (2018). https://doi.org/10.1093/nar/gky884
[4] Huang H-H, Bellato M, Qian Y, Cárdenas P, Pasotti L, Magni P, Del Vecchio D. dCas9 regulator to neutralize competition in CRISPRi circuits. Nat Commun 12, 1692 (2021). https://doi.org/10.1038/s41467-021-21772-6