Redirecting metabolic flux via combinatorial multiplex CRISPRi-mediated repression for isopentenol production in *E. coli*

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Project Goals: We aim to establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts at JBEI. Re-directing carbon flux to the targeted pathway is an important approach to improve production titer, rate, and yield. CRISPR interference (CRISPRi) is a novel approach that can be used to knock down endogenous genes in competing pathways. In this study, we constructed a CRISPRi-mediated multiplex repression system to silence transcription of several endogenous genes in order to increase precursor availability in a heterologous isopentenol biosynthesis pathway. The result shows that multiplex combinatorial knockdown of competing genes using CRISPRi can increase production of target metabolite, while the repression level needs to be adjusted to balance the metabolic network and to achieve the maximum titer improvement.

CRISPR interference (CRISPRi) via target guide RNA (gRNA) arrays and a deactivated Cas9 (dCas9) protein has been shown to simultaneously repress expression of multiple genomic DNA loci. By knocking down endogenous genes in competing pathways, CRISPRi technology can be utilized to re-direct metabolic flux toward target metabolite [1]. In this study, we constructed a CRISPRi-mediated multiplex repression system to silence transcription of several endogenous genes in order to increase precursor availability in a heterologous biosynthesis pathway for isopentenol which is a drop-in biofuel and a precursor for commodity chemicals [2]. To identify genomic knockdown targets in competing pathways, we first designed a single-gRNA library with 15 individual targets, where 3 gRNA cassettes targeting gene *asnA*, *prpE*, *gldA* increased isopentenol titer by 18-24%. We then combined the 3 single-gRNA cassettes into two- or three-gRNA array and observed up to 98% enhancement in production by fine-tuning the repression level through titrating dCas9 expression [1]. Our strategy shows that multiplex combinatorial knockdown of competing genes using CRISPRi can increase production of target metabolite. In this approach, we also showed that the repression level needs to be adjusted to balance the metabolic network and achieve the maximum titer improvement.

References

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