Developing multilevel CRISPR-based kill-switches for biocontainment of genetically engineered microbes and microbiota

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Abstract

Microbial biocontainment is a critical goal for engineering safe living therapeutics, pollutant-degrading microbes, and nitrogen-fixing bacteria [1,2]. However, the genetic stability of biocontainment circuits, including kill switches, is a huge challenge that must be addressed. Kill switches are among the most difficult circuits to maintain due to the strong selection pressure they impart, leading to high potential for evolution of escape mutant populations. We developed two CRISPR-based kill switches in the probiotic *Escherichia coli* Nissle 1917: a single-input chemical-responsive switch and a 2-input chemical- and temperature-responsive switch [3]. We employed parallel strategies to address kill switch stability, including functional redundancy within the circuit, modulation of the SOS response, antibiotic-independent plasmid maintenance, and provision of intra-niche competition by a closely related strain. We demonstrate that strains harboring either kill switch can be selectively and efficiently killed inside the murine gut, while strains harboring the 2-input switch are additionally killed upon excretion into the environment. Leveraging redundant or layered strategies, we demonstrate robust biocontainment of our kill switch strains and provide a generalizable platform for future kill switch development, facilitating safe applications of genetically engineered microbes and microbiota in agricultural, environmental, medical, and manufacturing sectors [4].

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