Programmable Polycomb Repression: Engineering and Benchmarking a Dual-Module CRISPRi Platform Across Species

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Abstract

KRAB-based CRISPR interference (CRISPRi) is the most widely used programmable transcriptional repression system, but its efficacy depends on local chromatin context, and its key cofactors are poorly conserved outside vertebrates, limiting its use across diverse model organisms. To address these limitations, this thesis implements deeply conserved Polycomb group (PcG) complexes, which mediate stable developmental gene silencing for CRISPRi engineering. Using a hit-and-run recruitment screen in human cells to enrich for effectors and pathways associated with persistent transcriptional silencing, I identified Polycomb-linked components as strong engineering candidates. I then designed a programmable dual-module architecture (PcCRISPRi) that couples Polycomb Repressive Complex 1 (PRC1)-linked H2AK119ub1 activity to Polycomb Repressive Complex 2 (PRC2)-mediated H3K27 methylation, building on endogenous Polycomb self-reinforcement. Chemical perturbations and proteomics identified dependencies that govern system performance. Through pooled functional genomic screens at essential promoters and candidate distal enhancers, I benchmarked Polycomb and KRAB-based effectors, identified active chromatin environments associated with reduced Polycomb responsiveness, and tested combinatorial histone deacetylase (HDAC)-assisted CRISPRi designs that significantly improved repression. Finally, in Drosophila cells, host-compatible Polycomb modules demonstrated cross-species portability of acute CRISPRi repression.