Engineered CRISPR-Cas12a for higher-order combinatorial chromatin perturbations

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Background:

Multiplexed genetic perturbations are critical for testing functional interactions among coding or non-coding genetic elements. Combinatorial genetic perturbations can greatly facilitate the development of combination therapies for prostate cancer. Compared to double-stranded DNA cutting, repressive chromatin formation using CRISPR interference (CRISPRi) avoids genotoxicity and is more effective for perturbing non-coding regulatory elements in pooled assays. However, current CRISPRi pooled screening approaches are limited to targeting one to three genomic sites per cell.

Methods:

Inspired by prior biophysics literature on Cas12a, we engineer an Acidaminococcus Cas12a (AsCas12a) variant, multiplexed transcriptional interference AsCas12a (multiAsCas12a), that incorporates R1226A, a mutation that stabilizes the ribonucleoprotein–DNA complex via DNA nicking.

Results:

The multiAsCas12a-KRAB fusion improves CRISPRi activity over DNase-dead AsCas12a-KRAB fusions, often rescuing the activities of lentivirally delivered CRISPR RNAs (crRNA) that are inactive when used with the latter. multiAsCas12a-KRAB supports CRISPRi using 6-plex crRNA arrays in high-throughput pooled screens. Using multiAsCas12a-KRAB, we discover enhancer elements and dissect the combinatorial function of cis-regulatory elements in human cells. These results instantiate a group testing framework for efficiently surveying numerous

combinations of chromatin perturbations for biological discovery and engineering. We showed that the multiAsCas12a platform supports multiplexed transcriptional targeting in prostate cancer cell models.

Conclusions:
We developed and benchmarked multiAsCas12a CRISPRi as a new combinatorial functional genomics platform that paves the way for future studies of combinatorial genetic dependencies in prostate cancer.

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Conflicts of Interest Disclosure
C.C.H., C.M.W., R.D. and L.A.G. have filed patent applications related to multiAsCas12a. J.S. is a scientific consultant for Treeline Biosciences. L.A.G. has filed patents on CRISPRof/on and CRISPR functional genomics and is a co-founder of Chroma Medicine. The other authors declare no competing interests.