CRISPR-mediated transcriptional activation and simultaneous gene knockout and activation with synthetic guide RNAs

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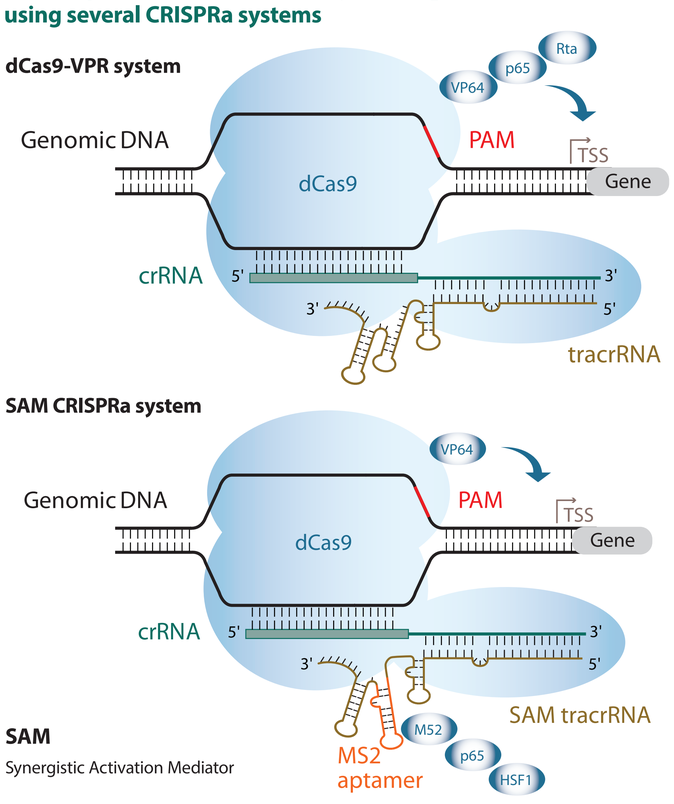
Abstract

The CRISPR-Cas9 system has been adapted for CRISPR modulation (CRISPRmod), including transcriptional activation (CRISPRa), where several second-generation CRISPRa systems (including VPR, SunTag, and SAM) have been developed to recruit multiple activators to a transcriptional start site. Here we show the effective use of synthetic guide RNA for gene activation with several of these second-generation CRISPRa systems. Methods of pooling multiple crRNA sequences targeting the same gene and chemically modifying the guide RNA can further enhance transcriptional activation levels, indicating the effectiveness of synthetic guide RNA for gain-of-function studies. Using combinations of full-length guide RNA and truncated guide RNA that are sufficient to localize to a genomic target site but not result in DNA cleavage, we show robust levels of simultaneous gene editing and activation of different gene targets with S. pyogenes VPR-Cas9 mRNA. Combinatorial genetic manipulations like this are an important method used to unravel complex gene regulatory networks in biological systems. Previously, these simultaneous gene knockout and activation experiments have been performed using two orthogonal Cas enzymes with unique guide RNA requirements: one to perform gene editing and the other for transcriptional activation. Using a single VPR-Cas9 effector with a multiplex guide approach for both gene editing and activation provides a useful tool for efficiently interrogating these complex regulatory pathways.

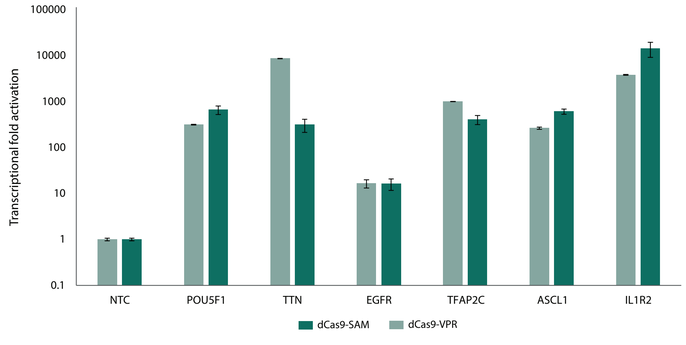
Transcriptional activation with synthetic guide RNA can be achieved using several CRISPRa systems



SAM

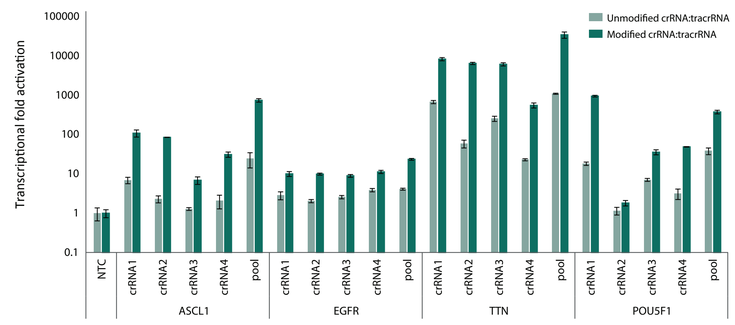
Synergistic Activation Mediator

Robust levels of activation with VPR and SAM systems across six gene targets with synthetic guide RNA



dCas9-SAM and dCas9-VPR expressing U2OS cells were transfected with pooled, synthetic crRNA:SAM tracrRNA and crRNA:tracrRNA, respectively and transcriptional activation was measured by RT-qPCR at 72 h post-transfection.

Chemical modifications and pooling of four different guides enhances transcriptional activation

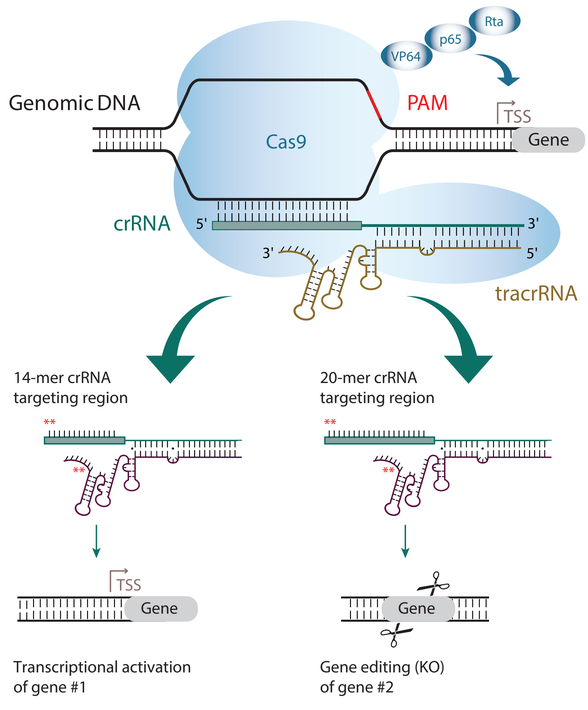


Guide RNAs were modified with two MS modifications at the 5' end of the crRNA and at the 3' end of the tracrRNA. Guide RNAs were delivered in dCas9-VPR expressing U2OS cells and transcriptional activation was measured by RT-qPCR at 72 h post-transfection.

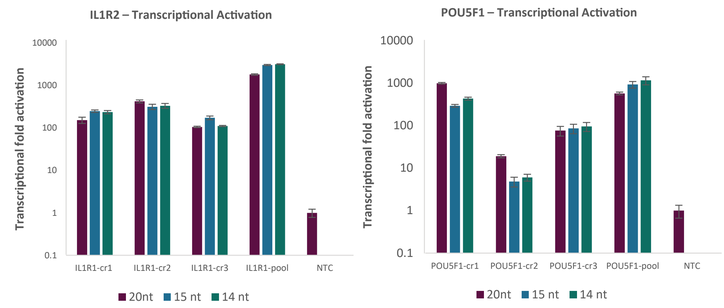
Synthetic crRNA enables simultaneous activation of multiple genes

dCas9-VPR expressing U2OS cells were transfected with synthetic crRNA:tracrRNA targeting IL1R2, POU5F1 and TFAP2C individually, two or three genes simultaneously (25 nM total RNA concentration per well). Transcriptional activation was measured at 72 h using RT-qPCR.

Simultaneous activation and gene editing with wild-type Cas9-VPR

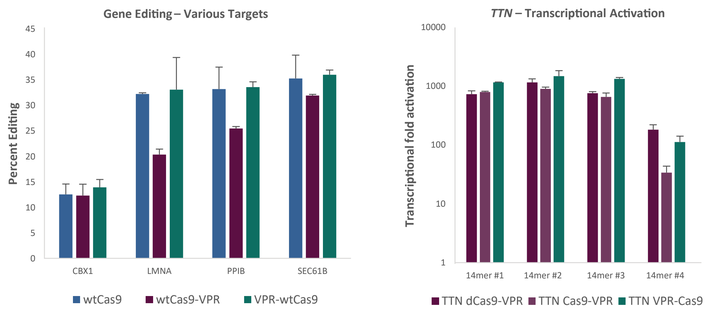


Synthetic truncated guide RNAs are capable of transcriptional activation



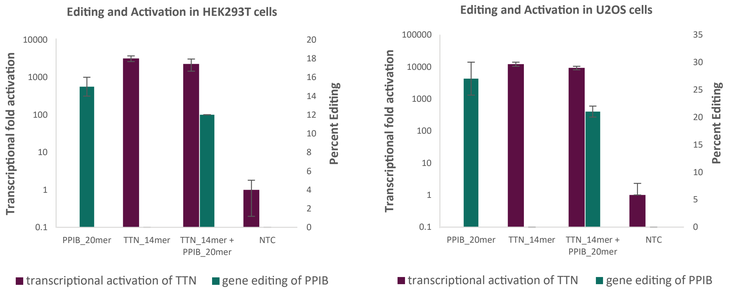
dCas9-VPR expressing U2OS cells were transfected with synthetic guide RNAs with target sequences of variable lengths. Transcriptional activation was measured by RT-qPCR at 72 h post-transfection.

Positioning of VPR at the Cas9 N-terminus improves gene editing while maintaining robust transcriptional activation



U2OS cells were co-transfected with synthetic guide RNAs containing 20nt target sequences along with mRNA with VPR at the N- or C-terminus of wtCas9 for assessment of gene editing at 72 h by T7EI mismatch detection assay. Transcriptional activation was measured by RT-qPCR from transfections with 14nt guide RNAs targeting TTN.

Simultaneous activation and editing with truncated and full-length synthetic guides targeting different sites



HEK293T or U2OS cells were co-transfected with synthetic guide RNAs with target sequences of variable lengths along with VPR-wtCas9 mRNA. At 72 h, gene editing was assessed at by T7E1 mismatch detection assay and transcriptional activation was measured by RT-qPCR

Conclusions

- Synthetic guide RNA, chemically modified with 2MS at both the 5′ end of the crRNA and 3′ end of the tracrRNA, can successfully be used with second generation CRISPRa systems (VPR, SAM) for transcriptional activation
- Multiple crRNAs targeting the same gene can be pooled to achieve higher transcriptional activation of a single gene
- Multiple crRNAs targeting different genes can be used for simultaneous activation
- Truncated 14nt targeting crRNA can be used with full-length 20nt targeting crRNA for simultaneous transcriptional activation and gene editing with VPR-Cas9 mRNA