CRISPR-mediated transcriptional activation with synthetic guide RNA

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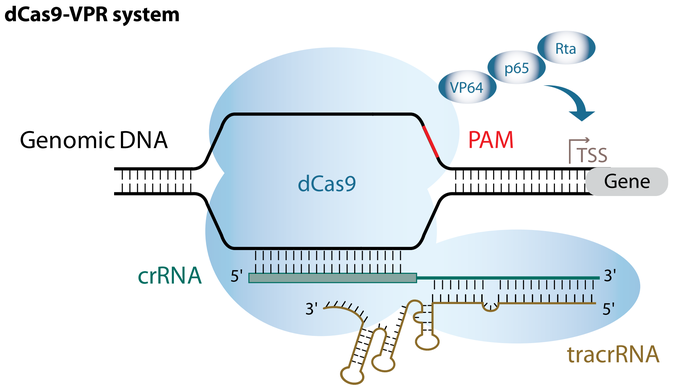
Abstract

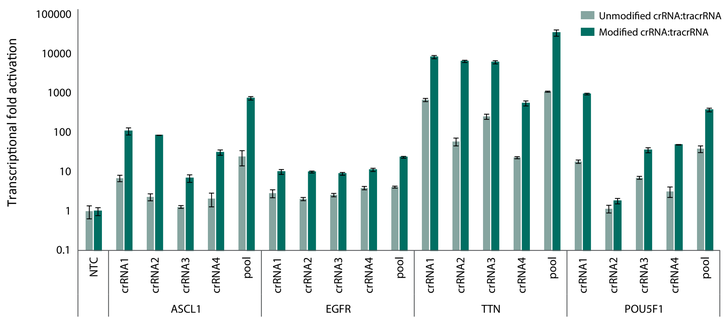
CRISPR activation (CRISPRa) platform has recently become a trusted tool to increase targeted gene expression *in vivo*, as well as in model systems. The technology utilizes gRNA directing deactivated Cas9 fused to transcriptional activation domains to promoter regions of a gene or genes of interest. The second-generation of CRISPRa, such as VPR, SAM, and SunTag, has emerged with the capability to recruit multiple components of the transcription machinery, which increases the potency of CRISPRa for targeted gene expression. Currently, all CRISPRa publications employ the use of gRNA-expressing vectors and lentiviral pools, which limits the technology from arrayed screening. Here, we show that synthetic crRNA:tracrRNA is as effective as expression gRNA vectors when using second-generation CRISPRa systems.

Synthetic guide RNA results in robust transcriptional activation using dCas9-VPR system

dCas9-VPR system

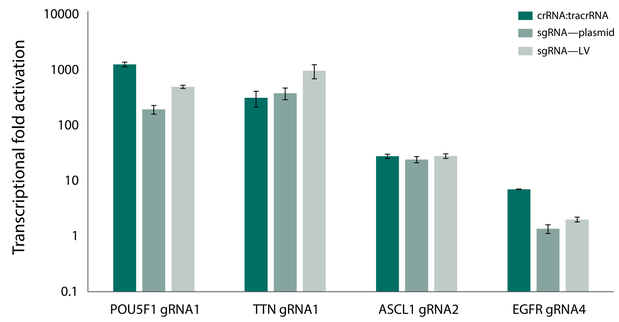


Chemical modifications and pooling of four different guides enhances transcriptional activation.



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.

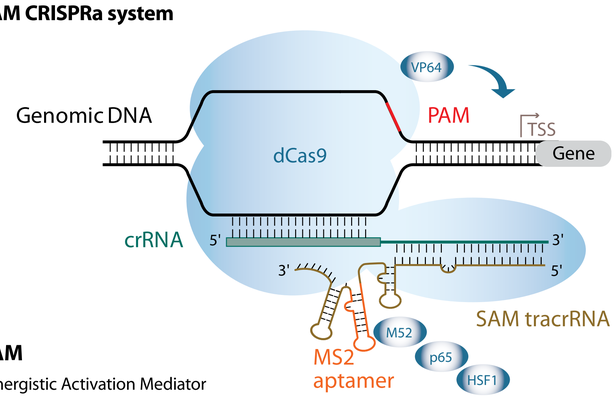
Synthetic crRNA:tracrRNA show comparable gene activation to expressed sgRNA



Comparison of gene activation from synthetic crRNA:tracrRNA transfected into U2OS-dCas9-VPR cells, sgRNA and dCas9-VPR plasmid co-transfection into U2OS cells and lentiviral transduction of sgRNA into dCas9-VPR-expressing U2OS cells. Transcriptional activation was measured at 72 h post-delivery for the synthetic gRNA and plasmid sgRNA, and at 4 days for the lentiviral sgRNA due to 48 h puromycin selection.

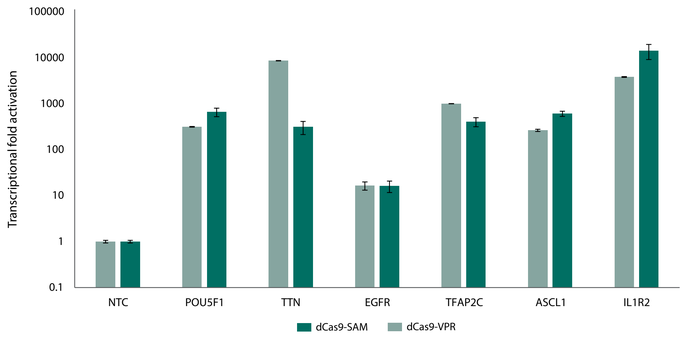
Synthetic guide RNA results in robust transcriptional activation with the SAM system

SAM CRISPRa system



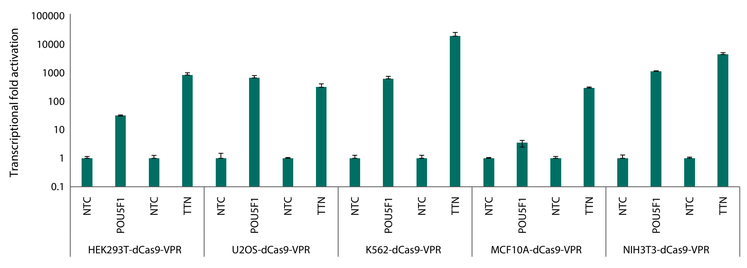
SAM
Synergistic Activation Mediator

Comparison of gene activation between SAM and VPR system across six genes.



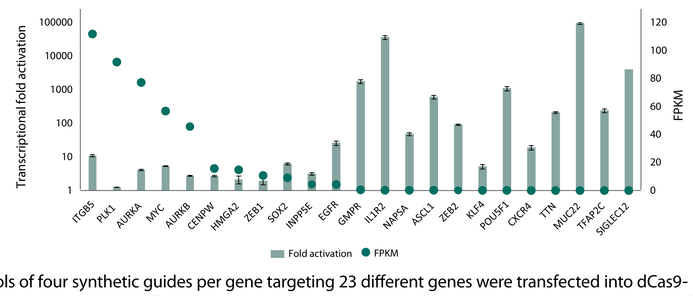
dCas9-SAM and dCas9-VPR expressing U2OS cells were transfected with pooled, synthetic crRNA:SAM tracrRNA and crRNA:tracrRNA, respectively and transcriptional activation was measured by RT-qPCR at 72 h post-transfection.

Synthetic guide RNA successfully activates gene expression in different cell lines



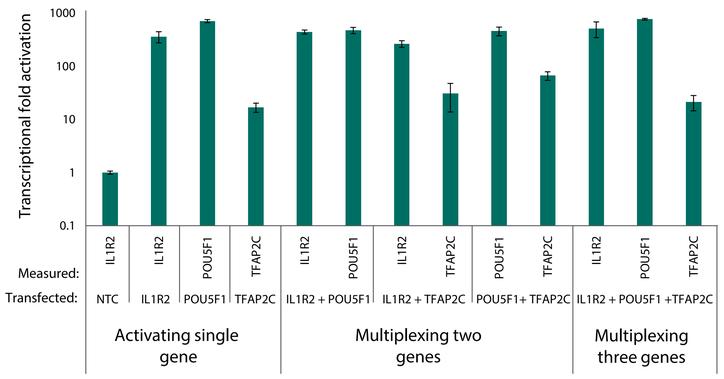
crRNA:tracrRNA targeting POU5F1 and TTN or negative controls (NTC) were transfected into five different cell lines expressing dCas9-VPR and transcriptional activation was measured at 72 h.

Low level of endogenous gene expression is typically activated at a higher level



Pools of four synthetic guides per gene targeting 23 different genes were transfected into dCas9-VPR expressing U2OS cells. Transcriptional activation at 72 h (RT-qPCR, gray bars) is shown vs basal expression level of each target gene (RNA-Seq FPKM (Fragments Per Kilobase Million), green circles).

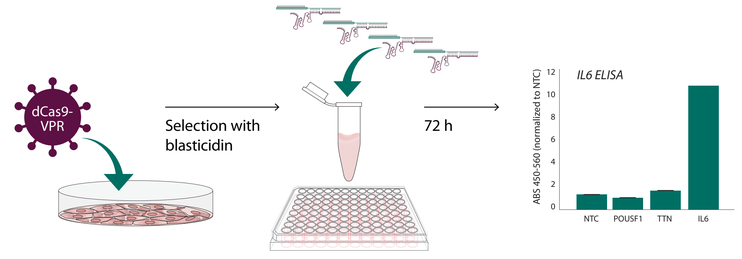
Synthetic crRNA enables simultaneous activation of multiple genes

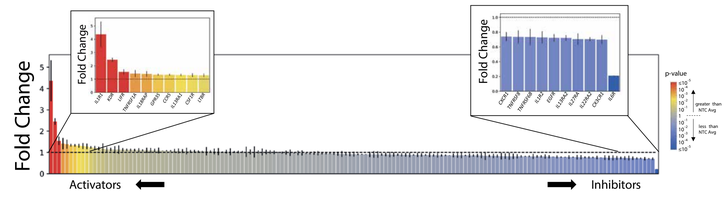


dCas9-VPR expressing U2OS cells were transfected with synthetic crRNA:tracrRNA targeting IL1R2, POU5F1 and TFAP2C individually, two or three genes simultaneously (25 nM total RNA concentration per well). Transcriptional activation was measured at 72 h using RT-qPCR.

Synthetic guide RNA enables arrayed CRISPRa screening

Generate dCas9-VPR stable cells | **Transfect with crRNA:tracrRNA library** | **Measure secreted IL6**





dCas9-VPR expressing U2OS cells were transfected with the CRISPRa library (153 cytokine receptor targets) in an arrayed fashion. Each gene was targeted with a pool of four predesigned synthetic crRNAs. IL-6 secretion was monitored by an ELISA, which helped us identify positive (red bars) and negative regulators of IL-6 (blue bars).

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).
- Transcriptional activation with synthetic crRNA:tracrRNA is comparable to activation achieved by expression vectors.
- Multiple crRNAs targeting the same gene can be pooled to achieve a better transcriptional activation of a single gene.
- The use of synthetic crRNA is ideal for simultaneous activation of multiple genes or use with dCas9-VPR mRNA.
- Synthetic CRISPRa guide RNA can be used for high throughput, arrayed screening applications.