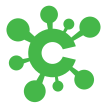


CRISPRa Tools for Gain-of-Function Genetic Screens

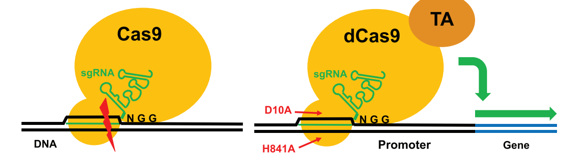
Nadya Isachenko[1], Donato Tedesco[1], Debbie Deng[1] and Alex Chenchik[1]
[1]Cellecta, Inc., Mountain View, CA

ABSTRACT

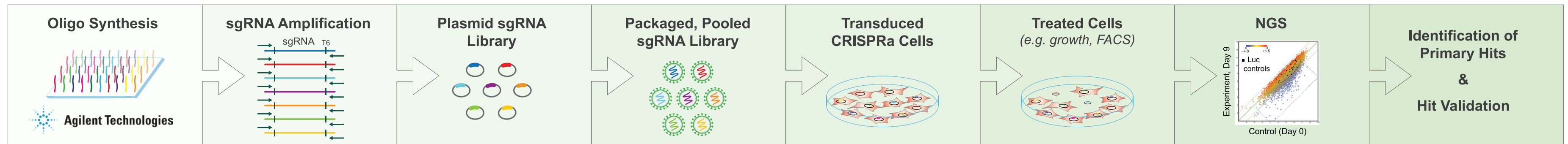
Catalytically dead SpCas9 (dCas9) can be fused to Transcriptional Activators (TA) and used to activate transcription from endogenous promoters, in an approach called CRISPR activation (CRISPRa). Initial CRISPRa systems resulted in only moderate gene activation and were not applicable for genome-wide screens. A significant improvement has been achieved by TA multiplexing, by either direct fusion to dCas9 or recruitment to a modified tracrRNA. Further improvement can be achieved by promoter sgRNA multiplexing. Unfortunately, sgRNA multiplexing with the dCas9 system is limited. Other class 2 endonucleases, like Cpf1, can be engineered for activation of transcription. Thanks to its innate crRNA processing activity, sgRNA multiplexing is much easier with Cpf1, making it an attractive alternative to SpCas9.

So far, no consensus has been reached on which system works best in high-throughput genetic screens. In this work, we compare the potency of gene activation of the most popular CRISPRa systems (dCas9-VPR, SAM system) and of dCpf1 fused with different combinations of activators. Elucidating which CRISPRa system best achieves a strong activation of a broad range of genes is central in the development of a new generation of genetic screens based on gene activation.

CRISPR-KO vs. CRISPRa

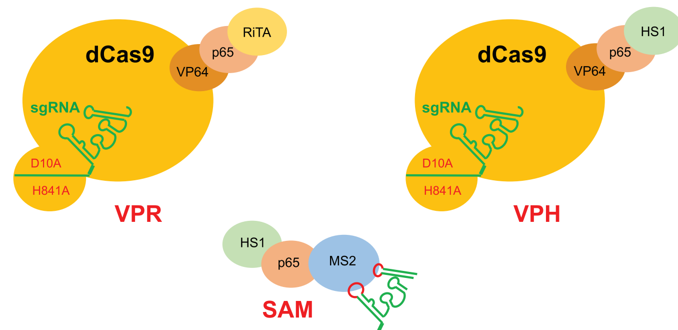


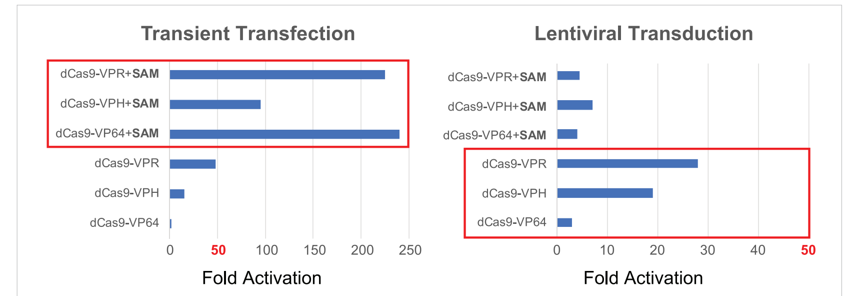
SCREENING



CRISPRa OPTIMIZATION

Choice of Transcriptional Activators

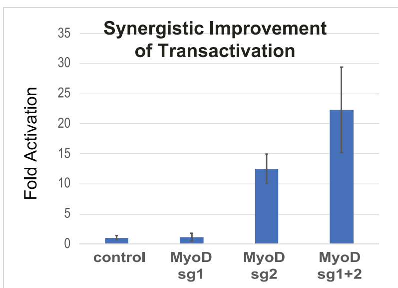




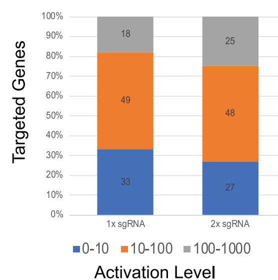
293T cells were transfected and MDA-MB-231 transduced with a combination of different dCas9 transactivators and sgRNAs targeting IL1R2 promoter. IL1R2 transactivation was measured by qRT-PCR.

Multiplexed sgRNA Targeting Promoter

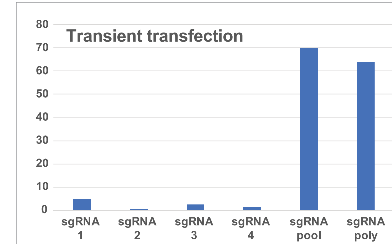
Multiplexing sgRNA for dCas9 System



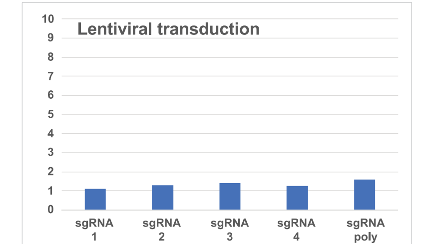
MDA-MB-231 cells expressing **dCas9-VPH** transactivator were transduced with sgRNAs targeting MyoD promoter. MyoD transactivation was measured by qRT-PCR.



Multiplexing sgRNA for dCpf1 System



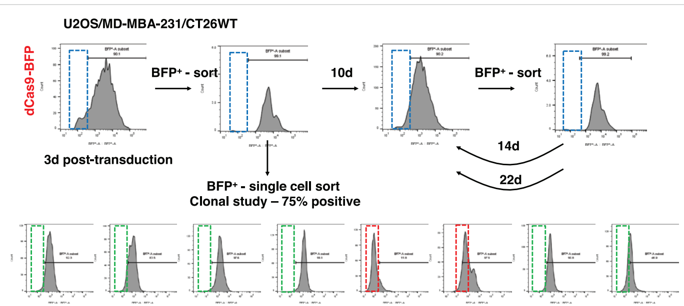
293T cells were transfected with plasmids expressing **dCpf1-VPR** transactivator with sgRNAs targeting IL1R promoter. IL1R transactivation were measured by qRT-PCR.



U20S cells expressing **dCpf1-VPR** transactivator were transduced with sgRNAs targeting IL1R promoter. IL1R transactivation were measured by qRT-PCR.

Endonuclease Expression

Expression of dCas9 is Stable, Allowing Screens with Pooled Cell Lines



U2OS cells were transduced with **dCas9-BFP** (left) or **dCpf1-BFP** (right). After 3 days, cells were FACS sorted. Expression of dCas9-BFP was stable, but expression of dCpf1-BFP was not. Clonal selection was used for further investigation.

Clonal Selection of Cells Expressing dCpf1 Seems Necessary For Screens