CRISPRa Tools for Gain-of-Function Genetic Screens

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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.

SCREENING

Oligo Synthesis > sgRNA Amplication > Plasmid sgRNA Library > Packaged, Pooled sgRNA Library > Transduced CRISPRa Cells > Treated Cells (e.g. growth, FACS) > NGS > Identification of Primary Hits & Hit Validation

CRISPR-KO vs. CRISPRa

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.

Crispr Optimation

Multiplexed sgRNA Targeting Promoter

Multiplexing sgRNA for dCas9 System

Synergistic Improvement of Transactivation

MDA-MB-231 cells expressing dCas9-VPR 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.

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

U2OS/MG6-A231/C26WT

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

U2OS/MG6-A231/C26WT

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