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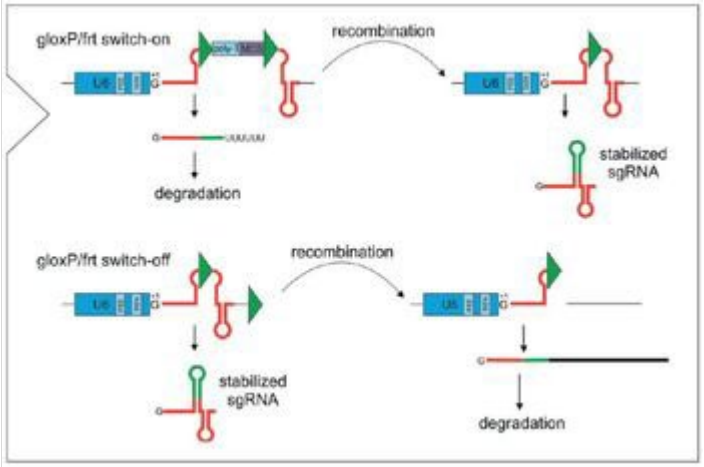
TO 43-00004

CRISPR SWITCH: INDUCIBLE SGRNA EXPRESSION FOR TIGHTLY REGULATED, EFFICIENT & SAFER CRISPR/CAS9 GENOME EDITING

CHALLENGE

CRISPR/Cas9 provides an easy, efficient and affordable tool for site-specific manipulation of genomes. Despite its promising characteristics, the technology needs further improvement, e.g. with regard to efficiency, regulation and reduction of off-target effects. A well-established strategy to accomplish a tightly regulated gene expression is the exploitation of recombinase-based systems, where induction of recombinase (i.e. CRE or FLP) expression leads to either activation (ON switch) or inactivation (OFF switch) of the gene of interest. Critical, in both cases, is the presence of recombinase recognition sites (i.e. loxP or FRT) within the gene of interest, which must not interfere with the gene's function and expression. For most eukaryotic genes this is easy to accomplish, given the presence of non-coding introns, which are post-transcriptionally removed. In case of sgRNA, however, placement of a recombinase recognition sites is far from trivial, given that sgRNAs do not possess introns, and are transcribed by RNA polymerase III, which has been optimized for highly efficient transcription of non-coding RNAs, but is extremely sensitive when it comes to disturbances of the native organization.



CRISPR switch expression cassettes; green triangles denote recombinase recognition sites. Upon recombination, sgRNA expression is switched ON (top) or OFF (bottom), respectively.

TECHNOLOGY DESCRIPTION

Scientist of IMBA and VBCF have developed CRISPR switch, i.e. optimized sgRNA expression cassettes that tolerate the presence of loxP or FRT sites, this way facilitating (i) tight ON and OFF switches of sgRNA expression, (ii) OFF switches without deletion of the guiding sequence for easy sgRNA target identification, (iii) optimized sgRNA scaffolds for efficient genome editing, and (iv) consecutive sgRNA expression for studying e.g. synthetic lethality, temporal order of lesions in tumor progression etc. Importantly, CRISPR switch is compatible with variations of CRISPR such as CRISPR-a, CRISPR-i and others.

- Biomedical applications of CRISPR switch technology include e.g.:
  Screening. Tight OFF switches allow for controlling the timing of genome editing, and limiting the risk of off-target effects. The sgRNA's guiding sequence is kept, facilitating subsequent target identification in screens. Tight ON switches, in turn, provide ideal sample control, especially in randomized or pooled library screens.
- Multiplexing. OFF switches of sgRNA expression in Cas9-expressing cells allows for multiple rounds of transformation with different sgRNA constructs without interfering off-targeting or titrating (Cas9) effects.
- In vivo genome editing. CRE/loxP recombination is well established in animal models. Hence, CRISPR ON switches allow for an inducible and/or tissue-specific expression of sgRNA, facilitating a spatial and temporal regulation of e.g. gene knock-outs and knock-ins. Using inducible promoters for expression of both recombinase and Cas9, further levels of specificity and control can be obtained.
- Synthetic lethality and tumor addiction studies. Consecutive genome editing allows the expression system to switch from one sgRNA to another. This can be used e.g. for studying synthetic lethality and tumor addiction.
- Therapeutic uses. CRISPR switch provides high activity and specificity, which represent prerequisites for therapeutic uses. Furthermore, the system allows for generation of no-escaping cell populations.

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COMMERCIAL OPPORTUNITY
CRISPR switch technology is available for in-licensing and co-development.

DEVELOPMENT STATUS
Practicability of all aspects of the CRISPR switch system has been broadly validated in vitro. In all studies conducted, the system showed tight control, minimal leakiness, rapid induction and high editing activity.
Performance of CRISPR OFF switches was tested e.g. on GFP and was shown to be both rapid and efficient (high level of editing after 24 h, saturation after 48 h). The system allowed for titrating of sgRNA activity, which is crucial for avoiding/minimizing off-target effects.
Performance of CRISPR ON switches, in turn, has been tested e.g. for the deletion of 16 essential genes. For all genes and most sgRNAs, 90% homozygous deletion efficiency could be observed.
Practicability of consecutive genome editing was challenged in mouse embryonic stem cells and shown to allow for time-controlled sequential deletion of two genes. In alternative setups, the system can be also exploited for e.g. controlled gene activation / repression, knock-in / mutagenesis, DNA modifications, as well as combinations thereof.

PATENT SITUATION
CRISPR switch technology is protected by national/regional patent applications in the US and EP (based on WO 2017/158154 A1).

FURTHER READING
Chylinski et al. (2019) CRISPR-Switch regulates sgRNA activity by Cre recombination for sequential editing of two loci. Nature Commun. 10: 5454.

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