PARTNERING OPPORTUNITY

CRISPR SWITCH: INDUCIBLE sgRNA EXPRESSION FOR TIGHTLY REGULATED, EFFICIENT & SAFER CRISPR/Cas9 GENOME EDITING

PARTNERING PROPOSAL

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. To address these challenges, IMBA and Vienna BioCenter Core Facilities GmbH (VBCF) scientists have developed ***CRISPR switch*** **(i.e. optimized sgRNA expression cassettes)** to facilitate (i) **tight OFF and ON 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.

IMBA is actively seeking for licensing partners with business interests in relevant areas of application, i.e. **screening, mutation analysis, safer genome editing, multiplexing and/or somatic gene therapy**.

BIOMEDICAL RELEVANCE

CRISPR switch **represents a recombinase-based system**, where induction of recombinase (i.e. CRE or FLP) expression leads to either activation or inactivation of sgRNA expression and eventually to genome editing (see Figure 1). The optimized sgRNA expression cassettes facilitate **tight temporal control, thereby dramatically improving the system's on-to-off target ratio**. In certain cassettes, introduction of a single **recombinase recognition site (i.e. loxP or FRT) actually stabilizes the sgRNA`s repeat:antirepeat stem loop, facilitating high, unimpaired editing activity and efficiency**. In contrast, ***CRISPR OFF switch*** **allows for tight time-controlled sgRNA inactivation, reducing off-target effects**, facilitating identification of the sgRNA targeting sequence, and permitting short-term editing for enriching mono-allelic editing in cases where heterozygotes are desired.

In conclusion, ***CRISPR switch*** **facilitates an efficient, absolutely tight, and safer CRISPR/Cas9-mediated genome editing**, which represents a prerequisite for practical (e.g. therapeutic) applications. In this context, most constructs **can be used along with already established Cas9-expressing cell lines or primary cells**.

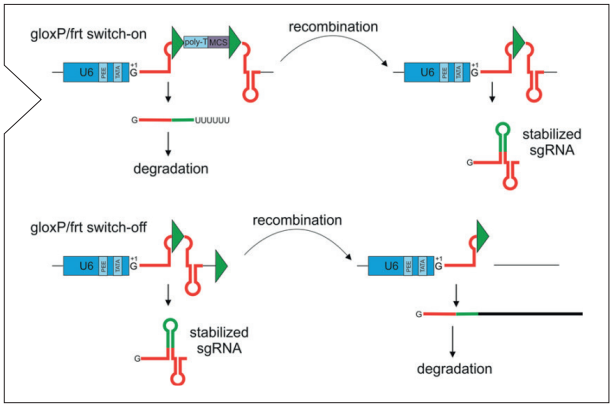

Figure 1. CRISPR switch expression cassettes. *Green triangles denote recombinase recognition sites. Upon recombination, sgRNA expression is switched ON (top) or OFF (bottom), respectively.*

SCIENTIFIC BACKGROUND

Homologous recombination represents a well-established tool for the inducible activation or deactivation of genes. Critical, in both cases, is the **presence of a loxP or FRT site within the gene of interest, which must not interfere with the gene's 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 expression, however, the corresponding placement of a recombinase recognition site is far from obvious, 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 regular promoter organization.

With the ***CRISPR switch*** system, IMBA and VBCF scientists have developed **optimized sgRNA expression cassettes that tolerate the presence of a loxP or FRT site, facilitating tightly regulated, inducible, recombinase mediated ON and OFF switches with reduced off-target activity and unimpaired efficiency**.

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BIOMEDICAL APPLICATIONS

Screening. Tight OFF switches allow for **control of the timing of genome editing and limit 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 (see Fig. 2), 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**.

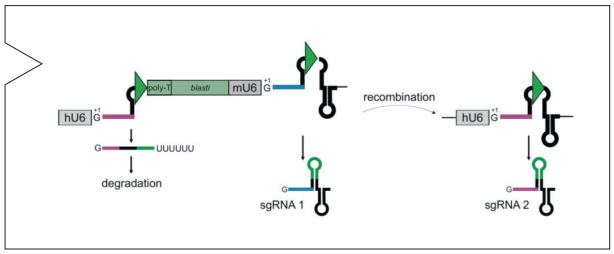

***Figure 2. Consecutive genome editing**. The expression cassette encodes two sgRNAs under control of their own promoters (hU6, human U6 RNA promoter; mU6, mouse U6 RNA promoter). In a non-induced state (left) active sgRNA 1 (blue) is transcribed and sgRNA 2 (purple) is prematurely terminated by a poly-T STOP cassette. Green triangles denote recombinase recognition sites. Upon recombination (right) STOP cassette the mU6 promoter and the sgRNA 1 guiding sequence are removed, allowing for expression of sgRNA 2*

STAGE OF DEVELOPMENT

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

IMBA and VBCF filed a European patent application followed by a PCT application (WO2017/158153). National phases have been entered in Europe and USA. The invention relates to expression cassettes for conditional expression of sgRNAs and uses thereof.

REFERENCES

Elling, Chylinski et al. (2017) Conditional CRISPR sgRNA expression. International patent application WO2017/158153.

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** Technology transfer consultant, supporting IMBA in commercializing this asset.*