PARTNERING OPPORTUNITY

SCON – A CONDITIONAL INTRON DEVOID OF HYPOMORPHIC TARGET GENE EFFECTS - FOR ONE-STEP GENERATION OF CONDITIONAL ANIMAL MODELS

BIOMEDICAL RELEVANCE

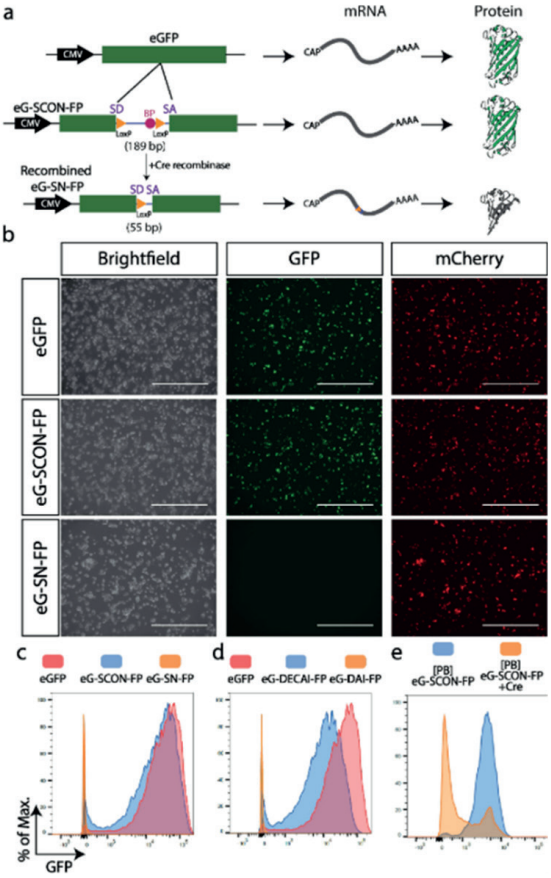
Gene knockouts (KO) in cells or animals have made substantial contributions to understanding the molecular functions of genes. For essential or developmentally active genes, a conditional knockout (cKO) strategy is often required to elucidate their functions. To prevent potential developmental or other unwanted physiological effects, the expression level of such targeted genes needs to be unaffected. This remains a major, unresolved challenge even in the CRISPR era.

PARTNERING PROPOSAL

"SCONs" (Short COnditional INtrons) were developed to overcome these limitations by analyzing the minimum requirements for a functional, artificial, conditional intron. The SCON methodology is based on genomic integration of the conditional intron within an exon sequence. It was demonstrated that an integrated SCON does not interfere with natural gene function and expression level; a hypomorphic effect, which has been observed for currently known "artificial intron" strategies, such as DECAI (DEgradation based on Cre-regulated- Artificial Intron), was not observed.

Upon transcription of the targeted gene, an additional splice event takes place which removes the inserted SCON, reconstituting the original mRNA sequence of the targeted gene. The conditional gene knock out is induced by a recombinase-based genomic event which partially removes the artificial intron, preventing splicing.

IMBA is actively seeking licensing partners with business interests in the field of animal model generation or novel CRISPR applications, to further exploit applications for the SCON technology.



SCON functionality test with an eGFP overexpression construct
a) Schematic diagram of SCON functionality test in an eGFP overexpression construct including intact eGFP, eG-SCON-FP and recombined eG-SN-FP. SD, splice donor; BP, branch point; SA, splice acceptor. b) Images of transfected HEK293T cells on day 1, with intact eGFP, eG-SCON-FP and recombined eG-SN-FP. All constructs were co-transfected with an mCherry-overexpression plasmid.
c), d), Histograms of the flow cytometry analysis of transfected HEK293T cells showing comparisons between eGFP (red) and eG-SCON-FP (blue) (c), between eGFP (red) and eG-DECAI-FP (blue) (d), and the respective recombined forms eG-SC-FP and eG-DAI-FP (yellow) (c,d). e) Flow cytometry analysis of mouse ES cells with integrated piggyBac-eG-SCON-FP transfected with Cre-expressing plasmid (yellow) or empty vector (blue).

SCIENTIFIC BACKGROUND

Conditional animal models are currently only available by methodologies that make use of the insertion of large fragments. However, efficacies are comparably low for introduction of such fragments by conventional homologous recombination or via CRISPR-Cas gene editing. In contrast, the SCON approach offers a simple, more efficient, and rapid approach for the generation of conditional animal models that are otherwise hard to create, and opens up a new field of applications for the use of artificial introns in different species.

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AREAS OF APPLICATION

GENERATION OF CONDITIONAL GENETIC MODELS

- The SCON technology has already proven its applicability for an easy and straightforward generation of conditional gene knockout models in cell lines of different species and in mice.
- The technology is especially suitable for the generation of animal models that address essential or developmentally active genes which require an unaltered gene expression throughout development.
- Due to the simple procedure of integrating an approximately 300 bp single-stranded nucleotide-strand, the technology offers an easy way to generate conditional models which could be broadly explored across a variety of species.
- SCONs can be also used in combination with other gene editing technologies such as, ZFNs and TALENs, as well as being integrated via classical homologous recombination.
- Development of targeted SCONs is based on a rationale for selection of optimized integration sites.
- SCONs are compatible with both LoxP and FRT sites, allowing the use of different recombinases for knockout generation.
- SCON design enables uncritical regions between the two recombination sites to be varied in length, offering adaptation to tailored sequences.

STAGE OF DEVELOPMENT

The SCON technology has been validated in HEK293T cells, zebrafish and *Xenopus laevis* embryos, mouse ES cells and mouse intestinal organoids. Proof of concept experiments for generation of mouse models by targeted integration of SCON elements were performed for developmentally essential genes, such as Ctnnb1 (beta-catenin) and Sox2, via direct injection into one or two cell mouse embryos.
Commercially synthesized single-stranded deoxynucleotides consisting of the SCON sequence and short homology arms for correct integration were used for injection. About 8% of the offspring carried the precise heterozygous SCON integration. Mice developed normally, were bred to homozygosity, and further analyzed for conditional gene inactivation.

To demonstrate broad applicability across species the scientists are seeking to validate the approach in a variety of animal models (mice, rats, zebrafish, medaka) in collaborative approaches.

REFERENCE

Wu et al., (2021) bioRxiv preprint [doi: https://doi.org/10.1101/2021.05.09.443220]

PATENT SITUATION

IMBA filed a European patent application in May 2021 (EP21172761). The application claims the architecture of SCONs, the methodology to generate cells and animals comprising a SCON element, such modified cells or animals, the methodology of investigating the function of a gene by inactivating its expression via a SCON element, and a kit for the generation of alleles comprising a SCON. The preferred methodology claims CRISPR/Cas for targeted integration of a SCON into a gene of interest.

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