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A company of the LifeScience Foundation

REFERENCE NUMBER TO 43-00013

# *CRISPR StAR*: sgRNA expression vectors for reliable and reproducible *in vivo* screenings

Keywords: CRISPR/Cas, sgRNA expression vector, stochastic activation, screening, essentialome, in vivo, organoids, reproducibility, reliability

## INVENTION NOVELTY

CRISPR/Cas-based negative selection screens are conducted by looking for the depletion of certain sgRNAs; i.e., a gene targeted by a depleted sgRNA is considered essential. Corresponding screens face various obstacles, and their outcome largely relies (among others) on the quality of the sgRNA library used, the efficiency of transduction, the regeneration of transduced cells, and the cellular heterogeneity. To obtain reliable results, it is of particular importance that a certain representation of the sgRNAs is maintained throughout gene editing (ideally: >300 cells/sgRNA), which is easy to achieve *in vitro*, but difficult (if not impossible) in organoids and *in vivo*. CRISPR StAR solves this problem by allowing actual gene editing to be postponed until all growth- and transduction-related bottlenecks have been passed, and sgRNA representation has had a chance to recover. By integrating an internal control, it further allows highly reproducible and reliable screens even under conditions of low sgRNA representation.

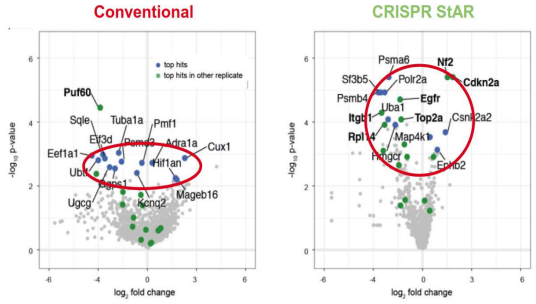
## VALUE PROPOSITION

Negative selection screens are used to identify essential genes, which in turn represent targets for disease-specific therapies. The approach is very promising and widely used, but also hampered by the fact that common methods do not provide reliable data *in vivo*. However, only in complex *in vivo* systems meaningful results can be obtained for a given disease model. Due to the limitations of conventional methods, true hits are hidden among many false-positive hits, making the identification and validation of novel disease targets, e.g., for cancer therapy, extremely difficult. CRISPR StAR overcomes this bottleneck by enabling the conduction of highly reliable and reproducible screens in complex *in vivo* systems and under conditions of low sgRNA representation.

## TECHNOLOGY DESCRIPTION

CRISPR StAR is based on CRISPR switch (see Partnering Opportunity for 44-00004), and takes advantage of sgRNA expression vectors, in which the sgRNA gene is interrupted by a disruption cassette flanked by a pair of recombinase recognition sites, which upon recombination facilitate the formation of an active sgRNA. However, the CRISPR StAR vector also carries a second pair of recombinase recognition sites which upon recombination facilitates the formation of an inactive sgRNA that serves as an internal control. Both recombination events are mutually exclusive and strictly stochastic, and accordingly yield a specific ratio of active and inactive sgRNA. In negative selection screens, the innovative sgRNA constructs are activated only after their representation has recovered. True essential genes are determined not only by depletion of the corresponding sgRNA (which is *per se* difficult to distinguish from a lack of transduction or recovery), but rather by a stochastic drift in the ratio of active and inactive sgRNA.

The figure shows the Volcano blots of a CRISPR screen in intestinal organoids using either state-of-the-art methodology (left) or CRISPR StAR (right). Each dot in these graphs represents a gene targeted by the sgRNA library employed. In an ideal world, the dots should center around the middle, and essential genes should be called with higher p-values (y-axis). As shown, only with CRISPR StAR, the dots are nicely centered, and top hits are identified with high confidence in the graph's upper part. Furthermore, and in contrast to the conventional method, the identification of essential genes is highly reproducible, as indicated by the colored dots. In summary, CRISPR StAR enables the detection of essential genes with high confidence even in heterogenous systems.




*Essentialome studies in intestinal organoids (Elling et al., 2021, unpublished).*

## DEVELOPMENT STATUS

CRISPR StAR has been successfully studied *in vitro* and in organoids and is currently further studied and used *in vivo*.

## COMMERCIAL OPPORTUNITY

CRISPR StAR is available for in-licensing.

## PATENT SITUATION

CRISPR StAR is protected by the international PCT application WO 2021/198233 A1.

## FURTHER READING

Elling, et al. (2021) Internal standard for CRISPR guide RNA. WO 2021/198233 A1.

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Last updated Sept. 2022