TransMIT
Gesellschaft für Technologietransfer mbH

Tool using BRET for determination of CRISPR/Cas9 restriction efficiency

Biosensor / CRISPR/Cas9 / restriction efficiency / BRET

DESCRIPTION OF TECHNOLOGY

The technology comprises a new method involving bioluminescence resonance energy transfer (BRET) ratio for determination of restriction efficiency of endonucleases or endonuclease systems comprising a guide-RNA that are used for induction of double-strand breaks at a DNA target sequence of interest for genome editing approaches.

The method is suitable for high throughput analyses of potential DNA target sites in combination with different endonuclease-guide-RNA complexes, e.g. CRISPR-Cas9-gRNA complexes.

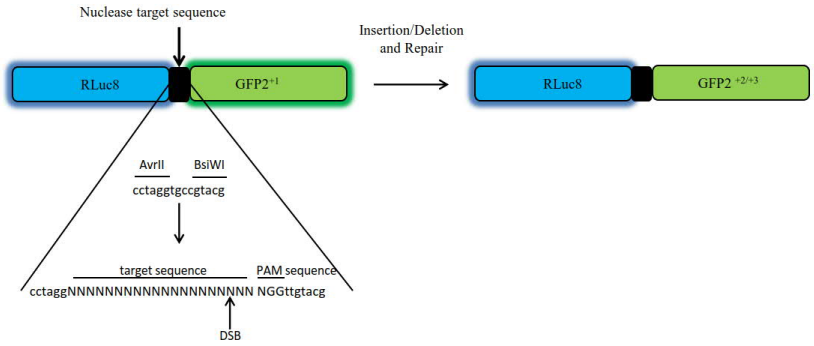

Fig. 1: Schematic representation of the biosensor. The DNA target sequence and a PAM sequence are cloned into the plasmid. During a double-stand break (DSB) in the DNA target sequence, by action of CRISPR/Cas9-gRNA, nucleotides are inserted or deleted. In 66% of the DSB events these insertions or deletions stay when the DSB is repaired by non-homologous end-joining (NHEJ), thereby leading to a frameshift in the gene encoding GFP2 and therefore expression of GFP2 gets lost. However, the expression of the luciferase (RLuc8) is not affected.

AT A GLANCE …

Application Fields

- Determination of CRISPR/Cas9 restriction efficiency in genetic applications of biotechnology or medicine

Business

- Pharma / Biotech / Medical Technology

USP

- Biosensor for CRISPR/Cas9 efficiency
- Less elaborate, cheap, highly sensitive
- Tool uses BRET
- Applicable for high throughput analyses

Development Status

- Tested in laboratory with selected targets
- Proof of concept and adaptation to high throughput application are next steps

Patent Status

- EP Patent granted.

REF. NO: TM 1006

APPLICATION FIELDS

Off-target mutations are an important caveat of endonucleases used for genome editing approaches, for example the CRISPR-Cas9 system, that need to be addressed. Even a low frequency of unintended mutations might have deleterious effects, so improving CRISPR-Cas9 specificity is essential for a reliable genome editing.

For the development of CRISPR-Cas9 systems comprising highly specific gRNAs that induce much less or even no off-target mutations, a reliable and cost-effective method for analysis of newly synthesized CRISPR-Cas9 systems and the DNA double-strand breaks induced by them is needed, which is furthermore suitable for high-throughput application.

The tool presented here provides a solution for this.

ADVANTAGES OVER THE PRIOR ART

The state of the art lacks methods that are less elaborate, cheap and sensitive enough. Furthermore, the known technologies are not suitable for high throughput analyses.

All of these disadvantages are overcome by the newly provided tool using the BRET system for determination of CRISPR/Cas9 restriction efficiency.

STATE OF THE PRODUCT DEVELOPMENT

The tool has been developed and tested with certain selected targets. Proof of concept and adaptation to high throughput application will be the next step.

MARKET POTENTIAL

A growth of the CRISPR/Cas systems market of up to US$ 25 billion is predicted by 2030. The market for genome editing, where CRISPR/Cas9 already accounts for more than half of the market, will expand strongly in the coming years. By 2021, the CRISPR/Cas9 market is expected to have annual sales of approximately $3.61 billion.

The market for screening libraries and gRNA design tools still has low market penetration. However, comprehensive end-to-end screening, design, and gRNA platforms are not yet available and therefore represent a key factor.

COOPERATION OPPORTUNITIES

On behalf of its shareholder Justus-Liebig-Universität Giessen TransMIT GmbH is looking for cooperation partners for further development or licensees.

A TECHNOLOGY OF

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

REF. NO: TM 1006

Contact

TransMIT Gesellschaft
für Technologietransfer mbH
Kerkrader Strasse 3
35394 Giessen
GERMANY
www.transmit.de

Contact Person

Dr. Michaela Kirndörfer
Tel: +49 (0) 641 9 43 64 - 16
Fax: +49 (0) 641 9 43 64 - 55
E-Mail:
michaela.kirndoerfer@transmit.de

TransMIT
Gesellschaft für
Technologietransfer mbH

SYSTEM PARTNER FOR INNOVATION