CRISPR-EHCAS9 MOLECULAR BIOLOGY TOOL

A research group from CIBER, University of Alicante and CSIC has identified a new gene editing CRISPR-Cas9 tool that is functional *in vitro* for programmable DNA restriction and for genome editing in prokaryotic and eukaryotic cells.

The Need

CRISPR is a molecular biology tool used for *in vitro* and *in vivo* modification of target nucleic acids. It is remarkable the use of a protein from *Streptococcus pyogenes* (SpCas9) in this system, which requires a particularly short sequence for target recognizing, thus entailing a great advantage related to other Cas9 proteins that need longer sequences.

However, the big size of this Cas9 protein hampers *in vivo* assays in eukaryotic cells, making it necessary to identify alternative smaller Cas9 proteins.

The Solution

A CRISPR-EHCas9 tool has been developed from a new CRISPR-Cas system of a metagenome. This tool is functional *in vitro* in prokaryotic (*Escherichia coli,* Figure 1) and eukaryotic (N2a cells from mice, Figure 2) cells, and additionally the size of Cas9 protein is around 78% related to SpCas9.

Innovative Aspects

✓ The main innovative aspect is the design of a molecular biology tool derived from a new CRISPR-Cas system (CRISPR-EHCas9), that can be used in gene editing of bacteria and mammalian cells.

✓ It also has application as a sequence-specific antimicrobial and for cleavage of DNA molecules *in vitro*. The tool comprises the EHCas9 protein and a synthetic guide RNA, as well as the vectors constructed for the administration of theses elements to cells and their production.

Stage of Development:
Validated in prokaryotic (*Escherichia coli*) and eukaryotic (N2a cells from mice) cells.
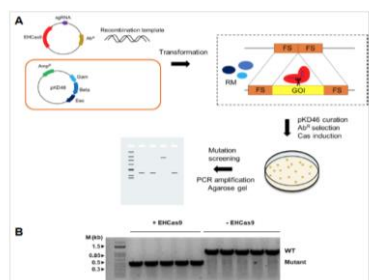
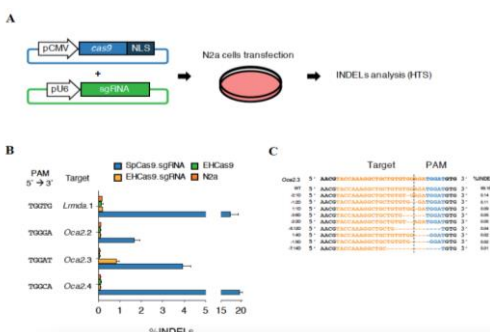

Figure 1. *E. coli* genome editing with EHCas9

Figure 2. EHCas9-mediated genome editing in N2a mouse cells showing the specific PAM sequence "TGGAT" required by EHCas9

Intellectual Property:

• Priority Spanish patent application filed in October 2022
• Suitable for international extension (PCT application)

Aims

Looking for a partner interested in a license and/or a collaboration agreement to develop and exploit this asset.

Contact details

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