

# A NEW GENOME EDITING TOOL FOR HEALTH AND AGRI-FOOD APPLICATIONS

 TECNOLOGÍA PATENTADA

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## RESUMEN

The Molecular Microbiology group has developed a CRISPR tool, based on a new Cas9 protein, which overcomes some limitations of commonly used CRISPR-Cas9 tools.

The technology has application in genetic engineering and production of antibacterial agents, in the biomedical, agri-food and biotechnological sectors.

Entities interested in acquiring the technology for commercial exploitation or for the development of new applications or tools are sought.

## VENTAJAS Y ASPECTOS INNOVADORES

### MAIN ADVANTAGES OF THE TECHNOLOGY

CRISPR-Cas technology has advantages over conventional genetic modification technologies used in sectors such as agri-food (i.e., mutagenesis with chemical substances and ionizing radiation), among which the following stand out:

- Targeting specific genes
- Absence or reduction of unwanted modifications

Regarding other targeted gene editing methodologies, such as those based on transcription activator-like effector nucleases (TALEN), zinc-finger nucleases (ZFN), or oligonucleotide-directed mutagenesis (ODM), the tool has the following advantages:

- Easy to use
- Lower cost

In addition, the tool of this invention has the following advantages over other CRISPR-Cas9 systems:

- Smaller size of the EHCas9 protein (about 78% of the size of SpCas9).
- Greater ease of administration to both bacteria and mammalian cells.
- It allows the incorporation, in a single vector molecule, of sequences of accessory genetic elements, such as regulatory sequences or templates for gene editing.
- Facilitates the administration of inactive derivatives of nuclease fused with peptides with different DNA-related activities.
- Exceptionally narrow operating temperature range, which allows easy control of its activity by modulating incubation conditions.
- It requires the presence of a very short and frequent PAM motif, which facilitates the selection of target sequences.
- Lower tolerance to variations in PAM results in higher specificity when compared to SpCas9.
- It can be used as a very effective system for the positive selection of mutant bacteria, without the need to introduce selection markers.

### INNOVATIVE ASPECTS

The CRISPR-EHCas9 system has been identified by researchers from the Molecular Microbiology group of the University of Alicante in a

metagenome generated from a water sample collected in El Hondo Natural Park (Spain), a previously unexplored natural environment.

Comparison of the amino acid sequence of EHCas9 protein with those of the Cas9 proteins available in databases shows a sequence identity of less than 68%. When the comparison is made against native Cas9 proteins used in genome editing in mammalian cells, a sequence identity of less than 29% is observed. Furthermore, the sequence of interaction with PAM differs considerably.

Unlike other Cas9 proteins currently available, the system of this invention allows sequences of accessory genetic elements, such as regulatory sequences or templates for gene editing, to be incorporated into a single vector molecule.

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#### APLICACIONES DE LA OFERTA

The present invention falls within the field of genetic engineering. More specifically, the object of the invention refers to a new EHCas9 endonuclease protein and a CRISPR-EHCas system comprising said protein for gene editing in cells and production of antibacterial agents.

CRISPR-Cas systems enable gene silencing or deletion, mutagenesis, and corrections of specific cell genome sequences in an easy, fast, and highly accurate manner. Its numerous applications include the diagnosis and treatment of diseases, as well as the production of sequence-specific antimicrobials.

The European Union is considering excluding plants produced using new genomic techniques from GMO legislation. If so, the use of CRISPR-Cas systems in the agri-food sector could be boosted in the European market.

Therefore, the tool of this invention would have application in **agri-food, biotechnology, environmental, biochemical and molecular biology companies, and the health sector.**

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#### COLABORACIÓN BUSCADA

We are looking for companies interested in acquiring this technology for **commercial exploitation** through:

- Patent license agreements.
- Technical cooperation agreements, through the development of joint R&D projects or personalized technical assistance, to adapt the technology to the needs of the company or develop new applications or tools.
- Subcontracting agreements for technical assistance, training, etc.

**Company profile sought:**

- Companies in the biotechnology sector
  - Companies in the biomedical sector
  - Companies in the pharmaceutical sector
  - Companies in the agri-food sector
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