

# NEW METHOD TO DETECT SPACER ACQUISITION IN CRISPR STRUCTURES

**P** PATENTED TECHNOLOGY



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## ABSTRACT

CRISPR structures are a component of a recently discovered prokaryotic immune system. CRISPR structures are arranged in clusters and interspaced by unique spacer sequences, which serve as a guide for the recognition and restriction of infectious nucleic acids. The research group of Molecular Microbiology of the University of Alicante has developed a novel method that enables detection of spacer integration in artificial CRISPR structures, called insertion modules. The main advantage of this technology is that it makes possible the positive selection of cells that have acquired a new spacer without relying on the immunity these spacers may confer. A spacer insertion in these artificial modules brings about a switch in the reading frame of an out-of-frame reporter gene, rendering a functional protein. Ensuing protein activity identifies adapted cells where an insertion has taken place.

The method at hand can be used in industrial sectors related to genetics and biotechnology as well as research in microbiology and molecular biology. The research group is looking for companies acquiring this invention for licensing agreement or technical cooperation.

## TECHNOLOGY ADVANTAGES AND INNOVATIVE ASPECTS

Insertion of new CRISPR spacer units is very infrequent in most species. Detection of these events usually requires large screenings of CRISPR clusters of a high number of clones. In order to decrease the number of clones to be tested, it is possible to select adapted cells when such acquisition changes the immunity pattern (i.e. enables for the degradation of target molecules). This causes a bias against detection of insertions of other sequences and cannot be executed in cells with silenced CRISPR immunity.

In this sense, the availability of a selectable tool for readily detecting spacer insertion independently of its consequences over the degradation of target genetic elements is highly advantageous compared to the methods currently in use.

## MARKET APPLICATIONS

The present invention makes reference to a new method for acquiring spacers by an interference-independent selection based on artificial CRISPR structures.

CRISPR Cas systems can be used for the following applications:

- Comparative studies among isolates from the same species.
- Studies related to Microbial Ecology and Metagenomics.
- To engineer bacteria of biotechnological interest, providing them with immunity against phages or plasmids conferring antibiotic resistance.
- Development of Molecular Biology and Genetic Engineering tools. CRISPR-Cas systems are being optimized for genetic expression

regulation and genome editing of prokaryotic and eukaryotic organisms (including the human species). It allows in vivo silencing or replacement of genes. Some of the applications are gene therapy or plant improvement for agri-food.

According to these considerations, artificial structures described in the present invention are suitable for the acquisition of new spacers that can act as a guide ("antibodies") for the system.

Main market applications:

- GENETIC ENGINEERING/MOLECULAR BIOLOGY:

Recombinant DNA

- MEDICAL/HEALTH RELATED:

Therapeutic

- CONSUMER RELATED:

Food and Beverages

- INDUSTRIAL PRODUCTS:

Other Industrial Products

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**COLLABORATION SOUGHT**

The research group is looking for companies acquiring this technology for commercial exploitation through:

- Patent licensing agreement.
  - Financial opportunities to develop new applications, adaptation to company needs, etc.
  - Knowledge transference agreement.
  - Technical cooperation.
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