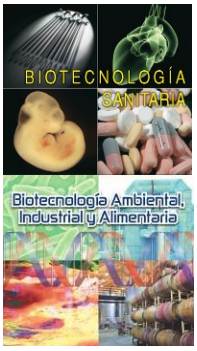
Poster



Analysis and optimization of the efficiency of CRISPR/Cas systems in gene editing and expression depletion in the fission yeast S. Pombe.

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ABSTRACT

The CRISPR/Cas system is a gene editing tool that was discovered as a protection mechanism in bacteria. This system has the ability to perform alterations in DNA and RNA sequences in a precise way, through the action of an RNA molecule that acts as a guide and a Cas protein that interacts with the guide and cuts the target strand [1]. In this project, we aim for a double goal. First, we implement a previously published CRISPR/Cas9 editing system in our biological model to enable a versatile tool for genome editing in our lab. We have used a replicative plasmid for Cas9 expression together with corresponding gRNAs to perform and analysing the tagging efficiency of mex67 gene (nuclear protein involved in mRNA transport) with a fluorescent protein; in comparison to the traditional method. Second, we aim to develope a novel, highly efficient, method to deplete gene expression by targeting the Cas13d RNA nuclease to specific RNAm molecules. We are developing a fusion of Cas13d with Mex67 to maximize the physical interaction between target RNA molecules and the RNA nuclease in obtaining RNA knockdowns [1,2,3]. Fluorescence microscopy in living cells results show that it is possible to perform fluorescent labeling of mex67 by the CRISPR/Cas9 system, although not at the reported efficiency in the literature for other genes. Nevertheless, up to our knowledge, this is the first Mex67 fluorescently tagged version under its own promoter in the field. We show that the intracellular distribution of this protein is nuclear; cells are viable and they proliferate as wild type controls, which make this protein a good candidate to acomplish our second goal.

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