Analysis of gene function by CRISPR/Cas9 deletions and transcriptomics

Análisis de la función génica mediante delecciones por CRISPR / Cas9 y transcriptómicas

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Abstract

CRISPR-Cas9, a revolutionary technique currently used to modify the genome in a precise way, is based on DNA repair mechanisms activated locally by DNA breakage. CRISPR is associated with an endonuclease called Cas9. This complex is directed by a gRNA (guide) conferring specificity of action to a given DNA sequence. The aim of the present work is to use the CRISPR / Cas9 technique to develop a series of mutagenetic plants using *in vitro* transformation.

Keywords: CRISPR/Cas9; genetic engineering; developmental genetics.

Resumen

CRISPR-Cas9, una técnica revolucionaria actualmente utilizada para modificar el genoma de una manera precisa, se basa en los mecanismos de reparación del ADN activados localmente por la rotura del ADN. CRISPR se asocia con una endonucleasa llamado Cas9. Este complejo está dirigido por un gRNA (guía) que confiere especificidad de acción para una secuencia de ADN dada. El objetivo del presente trabajo es utilizar la técnica CRISPR / Cas9 para desarrollar una serie de plantas mutagenéticos utilizando la transformación *in vitro*.

Palabras clave: CRISPR/Cas9; ingeniería genética; genética del desarrollo.

1. INTRODUCTION

Genome editing is a technique that allows controlled modification of genetic information, using homologous recombination. Adding and modifying bases in DNA sequences has become progressively easier. The genome revolution is in progress after the discovery of CRISPER technique. The CRISPR / Cas9 system includes the non-specific endonuclease (Cas9) and gRNA that is responsible for transporting Cas9 to the targeted DNA site (figure 1).

The Cas9 enzyme generates cleavages in the double-stranded DNA using its two catalytic centers to cleave the target DNA next to the PAM sequence which corresponds to a 20 nucleotide sequence of the guide RNA (sgRNA) [1].

The use of the CRISPR / Cas 9 system for eukaryotic genomes allows a modification of several traits simultaneously and precisely. Moreover, this technique does not require any protein engineering process [2].

Several research studies have shown that the CRISPR / Cas9 technology has been successfully used in *Petunia*. The objective of the thesis proposal is to use the CRISPR / Cas9 technique to develop a series of mutagenetic plants using *in vitro* transformation [3].

The aim of the present PhD proposal is to use the CRISPR / Cas9 technique to develop a series of mutagenetic plants using *in vitro* transformation

2. MATERIALS & METHODS

2.1. Plant material

The plants chosen for this investigation, *Petunia hybrida* and *Antirrhinum majus*, will be grown in in a growth chamber under controlled conditions. For transformation of the CRISPR / Cas9 constructs, seeds of the *Antirrhinum majus* line 165E and of *Petunia hybrida* variety Mitchell will be sterilized on the surface, and grown on Murashige and Skoog medium in a growth chamber under a photoperiod of 12/12 h light / dark and a temperature of 25ºC / 18ºC. Transformation will be performed on hypocotyls (*A.m.*) or leaves *(P.h.)*.

2.2. Vector construction

The constructions were made following conventional construction cloning procedures. Primers of gRNA for PCR will be designed in order to confirm certain genes within the construct (Figure 2).

2.3. Transformation by *Agrobacterium*

The plant transformation chosen in this study will be carried out using *Agrobacterium tumefaciens* via a vector appropriate to the specific context.

3. RESULTS AND PROSPECT

To develop a series of CRISPR/Cas9 alleles in several plants in order to obtain loss of function alleles in several genes.

To develop transgenic plants via *in vitro* transformation, help to genotype the outcoming T0, and obtain T1 segregating plants for further phenotypic analysis.

To establish homozygous lines for transcriptomic and bioinformatic analysis in order to identify target genes that can be modified by the loss of function alleles in the target tissues.

4. CONCLUSIONS

New genomic editing techniques have become easier and faster. The CRISPR/Cas9 system is a valuable tool for genome editing. The system has allowed researchers to perform accurate genetic modifications, easier to use and cheaper compared the previous gene editing techniques such as Transcription activators-like effective nucleases (TALENS).

An additional advantageous lies in the fact that this technology has the ability to modify several genes simultaneously.

5. ACKNOWLEDGMENTS

The experiments will be conducted at the Institute of Plant Biotechnology (IBV), Cartagena, Polytechnical University of Cartagena (UPCT)

6. REFERENCES

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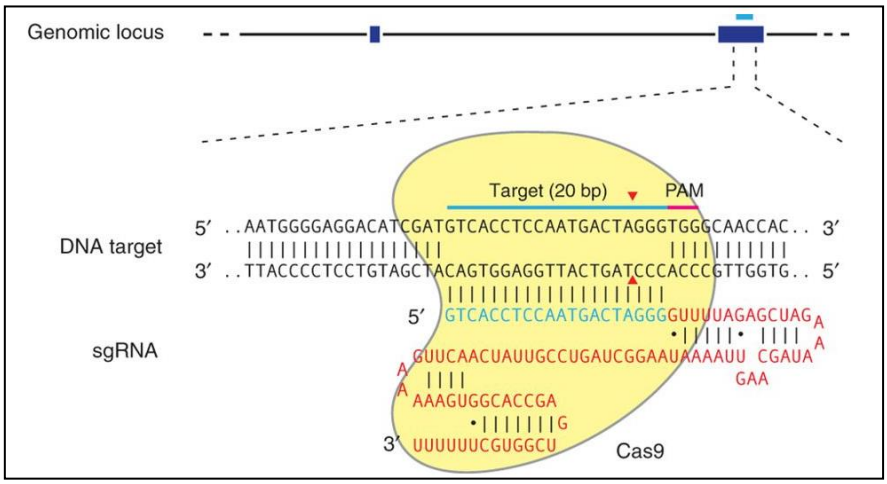

Figure 1. Schematic of the RNA-guided Cas9 nuclease [1].

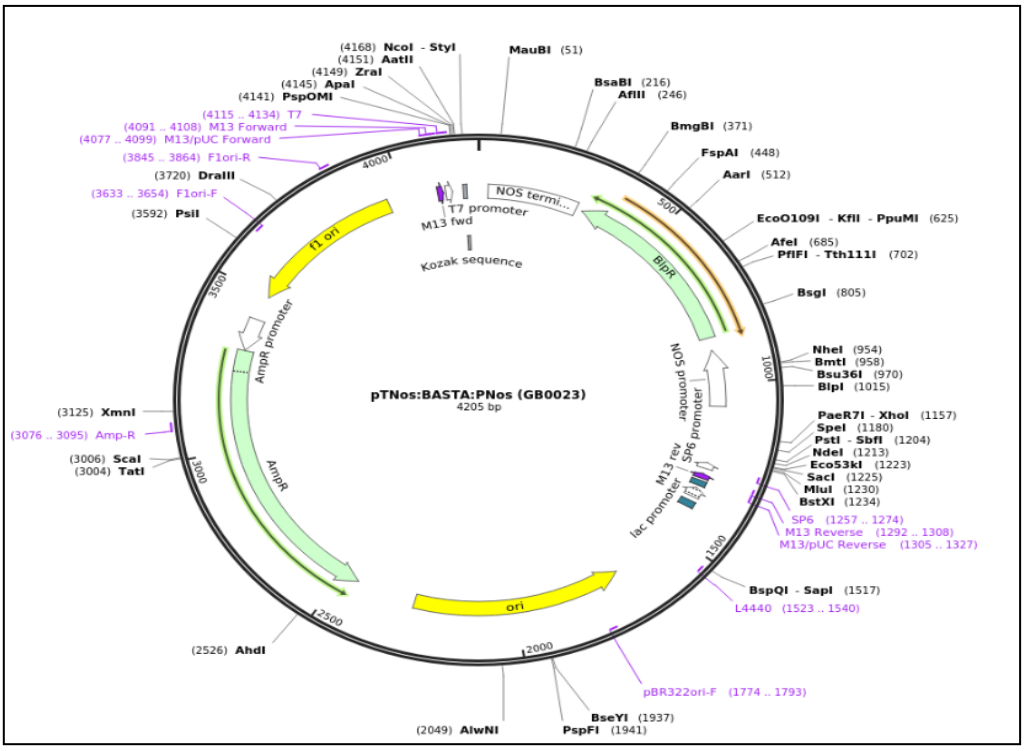

Figure 2. pTNos:BASTA:Pnos Vector system will be used in this experimental setup.