inholland hogeschool

Green Biotechnology

Site-directed mutagenesis in Petunia using CRISP/CAS9 technology.

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The Green Biotechnology research group focusses on the application of molecular breeding/biotechnological tools and also on the development/analysis of new tools, for the breeding of enhanced vegetable crops and ornamental plants. The research group is positioned within Inholland University of Applied Sciences, Life Sciences & Chemistry and serves as a link between the breeding companies and our education of the skilled technicians of tomorrow.

We are working on the development of a method for targeted mutagenesis of plant genomes using the bacterial CRISPR-Cas system. This method greatly enhances the effectiveness and speed by which new crops and plants can be developed.

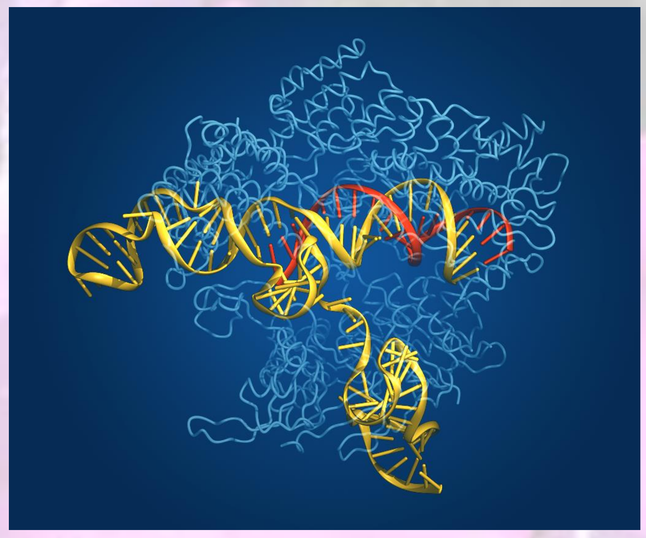

Figure 1: Crystal structure of the Cas9 gene-editing enzyme (light blue) in complex with an RNA guide (red) and its target DNA (yellow).
From: http://directorsblog.nih.gov/tag/cas9/

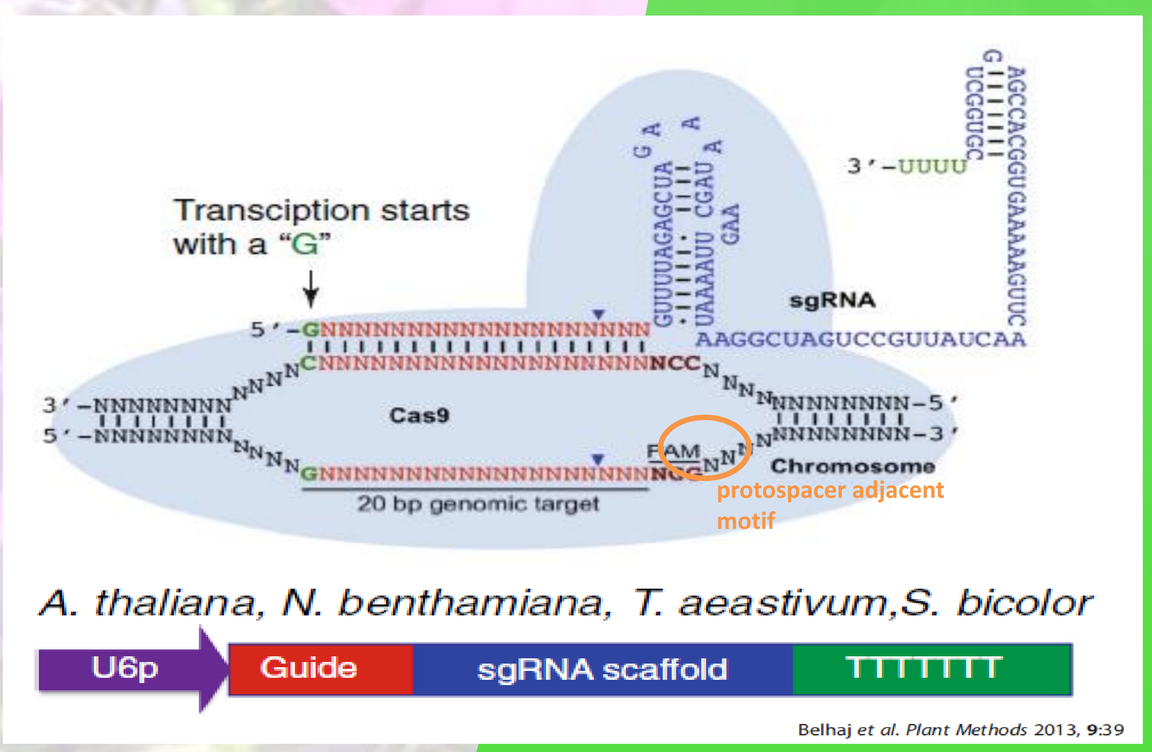

Figure 2: schematic representation of the CAS9 nuclease (light blue) with bound guideRNA and template DNA molecules. In lower panel the gene cassette required for plant transformation is depicted. Adapted from Belhaj *et al.* Plant Methods 2013, 9:39

The CRISPR-CAS system originates from bacteria where it serves to detect and inhibit bacteriophage infection. The two main components of this endonuclease system are the Cas9 endonuclease and the single guide RNA molecule (sgRNA) (Figure 1). The sgRNA contains a specific targetsequence, and together with the Cas9 protein introduces a double strand break at the targeted complementary sequence.

A fraction of the introduced double strand breaks are repaired by the error prone non-homologous end joining mechanism of the endogenous DNA repair system, causing the introduction of mutations at the pre-defined sites. Modifications of the CRISPR/CAS system are required for its use in plants, the 19-22 nucleotide guide sequence is required to contain a protospacer adjacent motive (PAM) at its 3'end for proper Cas9 endonuclease activity (see Figure 2).

Figure 3: *Petunia x hybrida*

We are currently developing an experimental pipeline for the introduction of site-directed mutations in the Solanaceae model species Petunia x hybrida (Figure 3). Petunia is a close relative of many agricultural crop species and in cooperation with various partners we aim to expand our method to other crop species and ornamental plants in the near future

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