# CRISPR/Cas biotechnology

## Targeted and versatile gene editing in Petunia

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**Introduction**
At Hogeschool Inholland, we're currently working on a 4-year project on the application of the novel breeding tool 'CRISPR/Cas technology' for directed mutagenesis in multiple crop species. Via proof of concept experiments in *Petunia x hybrida*, we aim to show the applicability of this technique for crop breeding in *Solanaceae* species.

**Research Question**
*Is it possible to use this CRISPR/Cas technique to obtain a multeity of Petunia variants in shape, scent or colour, starting with a single plant?*

**Why?**
Breeders in our consortium are highly interested in this technique, since this would enable them to, relatively quickly, obtain plants with a mutation in a specific gene of interest. Enabling screening of crops with potential useful characteristics such as salt tolerance, altered nutrient values and altered crop yield.

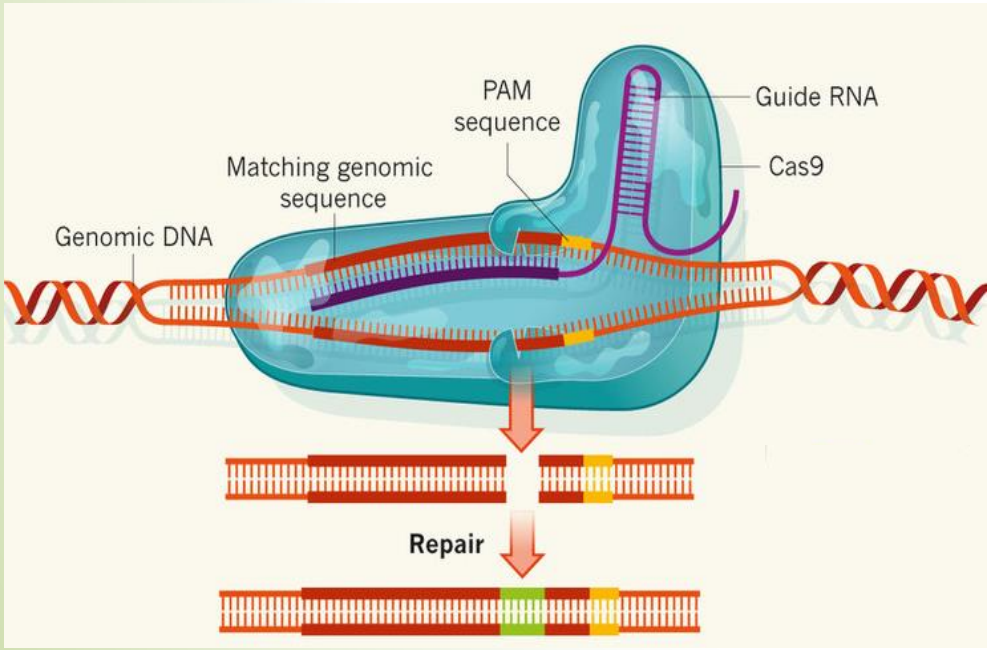


**Figure 1: Schematic representation of the CRISPR/Cas complex in action.** In this complex, the Cas 9 nuclease is directed to a specific site in the genome, by the guide RNA. At that site, a doublestrands break is induced. The cells' endogenous repair mechanism can erroneously miss out- or add some extra nucleotides, resulting in knockout mutants for specific genes. Altering the gRNA sequence results in a different target sites on the genomic DNA, making CRISPR/Cas a very versatile technique for creating multiple knockouts. Figure adapted from: Charpentier & Doudna (2013).

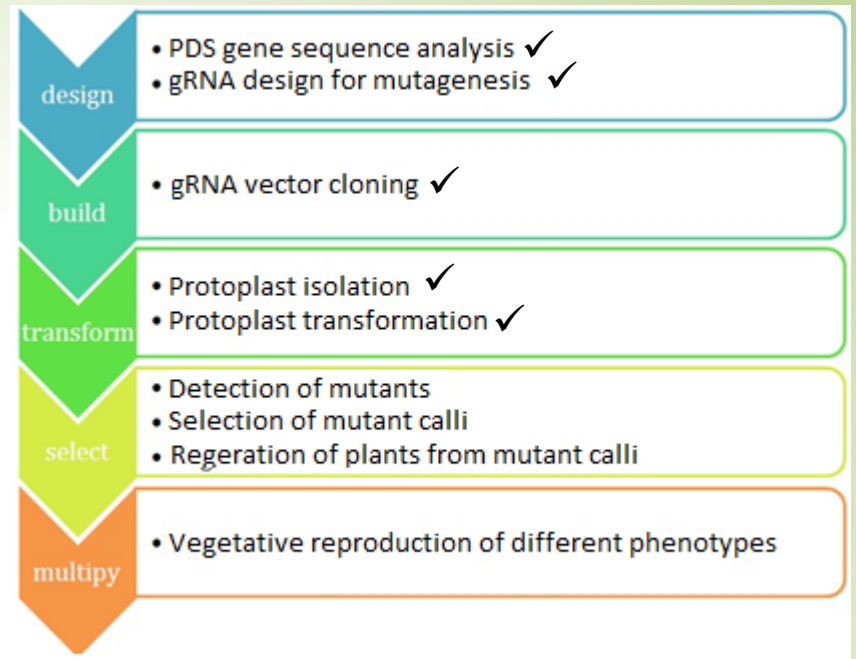


**Figure 2: Workflow and current progress of the project.**

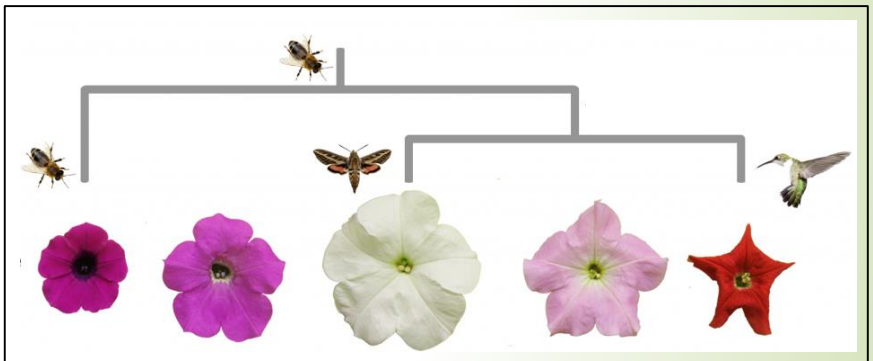

**Figure 3: Example of possible phenotypic mutants of Petunia.** Several different scent-, shape- and colour variants of Petunia flowers are shown in this figure. We investigate the possibility of obtaining such variants using the CRISPR/Cas technique. Figure adapted from Sheehan et al. (2015).

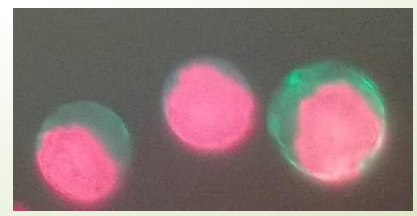

**Figure 4: Petunia protoplasts transformed with GFP plasmids.** Under the fluorescence microscope, GFP signal was observed in the most left and right protoplast in this figure, indicating our trial at Hogeschool Inholland for the transformation of Petunia protoplasts was successful.

**References**
Charpentier, Emmanuelle & Doudna, Jennifer A. 7 March 2013 *Nature* Biotechnology: Rewriting a genome, 495, 50–51
Sheehan et al. MYB-FL controls gain and loss of floral UV absorbance, a key trait affecting pollinator preference and reproductive isolation Nature Genetics 2015

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