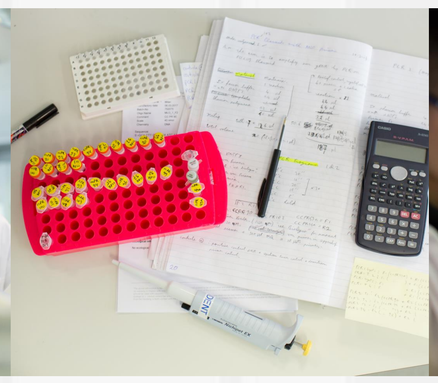
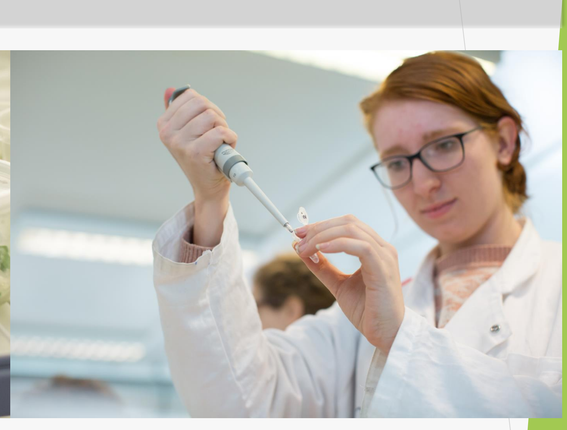
Student driven CRISPR/Cas technology in petunia.

PW den Hollander, MM Plasmeijer, CM Kreike,
University of applied sciences Inholland, Amsterdam, The Netherlands

Introduction:

The biotechnological breakthrough of this millennium might well be the discovery that CRISPR-associated RNA-guided endonuclease Cas9 is able to cleave non-prokaryotic DNA *in vivo*. At **InHolland University of Applied Sciences** we train undergraduate students in technical skills and knowledge on current topics in biotechnological sciences. Recently we therefore implemented the CRISPR/CAS9 technique in several theoretical and practical courses of our bachelor program. Student-driven learning has encouraged students to Study, Design, Execute and Optimize CRISPR/CAS9 site-directed mutagenesis in *Petunia x hybrida*. These students have shown that the CRISPR/CAS9 site-directed mutagenesis can be applied in Petunia protoplasts and illustrated that this technique is a captivating and challenging educational toolkit for the training of bachelor students in biotechnological sciences.

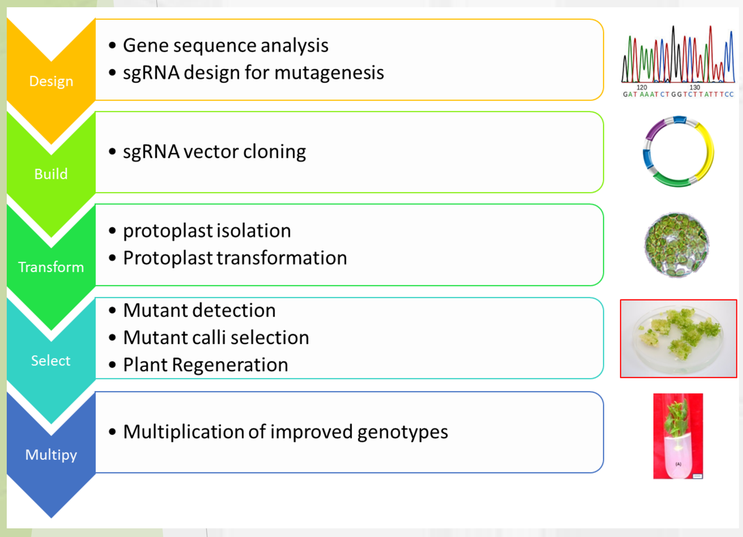


Figure 1: Experimental pipeline for directed mutagenesis using the CRISPR-CAS9 system.

Figure 2: Depiction of part of a students' cloning strategy.

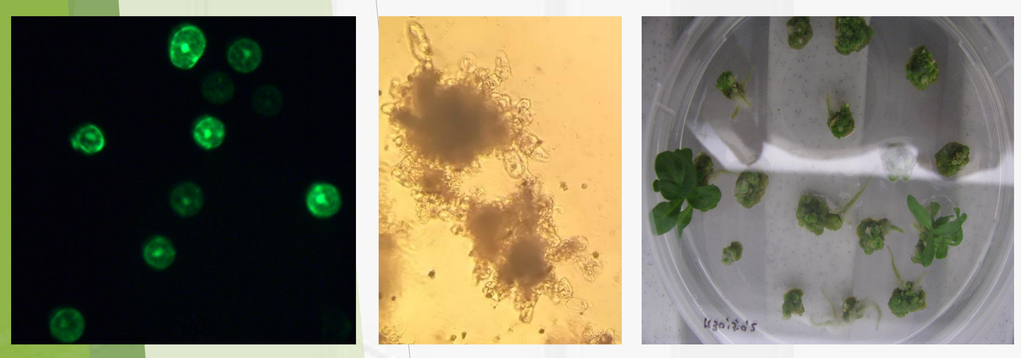


Figure 3: Transformation and regeneration results. Showing (L to R) transformed, dividing and regenerated petunia protoplasts.

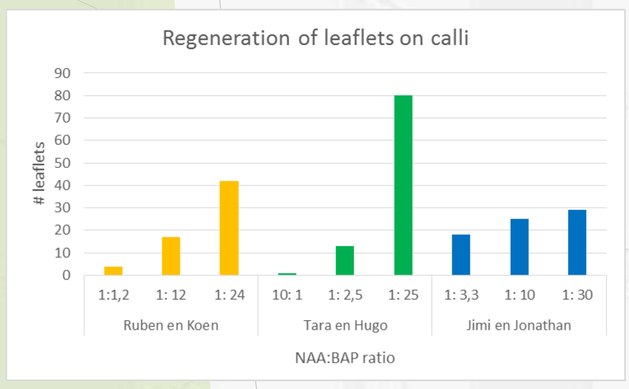


Figure 4: Quantification of leaflet formation on calli.

Molecular cloning strategies:

Provided only with literature and a gene-of-interest our students have designed a project pipe-line for CRISPR/CAS9 site-directed mutagenesis in *Petunia x hybrida* (figure 1).

Students learned to design and execute the procedure for introduction of new sgRNA-sequences in a donor vectors.

The do-it-yourself approach induces good insights in experimental design (figure 2). By peer-review the students are able to fine tune the various steps and controls that need to be taken into account.

Transformation Experiments:

Polyethylene-glycol (PEG) mediated transformation of protoplasts was a bottle-neck in this process. Several student couples have tackled different experimental parameters that could be of influence on transformation efficiency.
Some of the variables tested were;

- Polyethylene-glycol size
- duration of membrane disruption
- recovery media used.

Current transformation rates are 50-70% (figure 3),
a vast improvement in comparison to our old procedure.

Regeneration experiments:

Students choose their own media for regeneration of protoplasts. To induce shoot regeneration different media-compositions were tested (figure 4), including various hormone concentrations and coconut-milk additions.

Conclusion:

Undergraduate students are highly motivated and well able to design and execute modern molecular techniques. Their combined efforts yielded various reports that describe the CRISPR-CAS9 system adequately. Additionally, their efforts in the lab have helped improve the efficiency of several steps in the directed mutagenesis pipeline.

More information?
Paulus.denhollander@inholland.nl

inholland hogeschool