Student driven CRISPR/Cas technology in petunia.

PW den Hollander, MA Plasmidhar; CM Kreits, 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.

Molecular cloning strategies:
Provided only with literature and a gene-of-interest our students have designed a project pipeline 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