大连理工大学生物工程学院
SCHOOL OF BIOENGINEERING,DALIAN UNIVERSITY OF TECHNOLOGY

GreenGate cloning technology introduces enormous advantages for studying pathogenicity factors of plant diseases

Nan Wu*, Wanqing Jiang, Yu Cheng, Xuan Zhou, Xinran Zhao, Mahinur S. Akkaya*
wu_nan@mail.dlut.edu.cn and msa@dlut.edu.cn

School of Bioengineering, Dalian University of Technology, No.2 Linggong Road, Dalian, Liaoning, 116023, China.

ABSTRACT

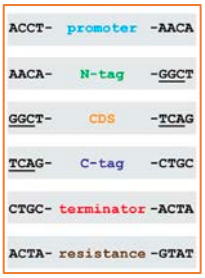
One of the yield limiting factors in agriculture is pathogens, which are the causal agents of destructive diseases. Knowing the mechanistic details how pathogen effectors manipulate plant immunity and how the plant molecules sense the activity of pathogen effectors are important topic in the study of molecular plant pathology. In pathogen effector studies, gene of effector cloning into binary vectors for plant transformation to validate its function is an essential first step. Traditionally, gene cloning has relied on restriction enzyme digestion and ligation. In recent years, however, a recent technology has developed a reliable alternative cloning; GoldenGate methodology. GreenGate system is designed by Lampropoulos et al. (2013) uses this methodology to rapidly assemble essential constructs for plant transformation which contains six types of insert modules (plant promoter, N-terminal tag, coding sequence, C-terminal tag, plant terminator and plant resistance cassette). Based on six modules with different genes for each type of module, together with the empty modules, it is possible to generate many different clones for various purposes. The characteristics of GreenGate cloning technology is sufficient to simultaneously construct a variety of effector expression vectors. All these make GreenGate versatile and applicable cloning system for the field. The availability of GreenGate technology will accelerate the high-throughput study of pathogen effectors in plant immunity.

PRINCIPLE OF GREENGATE CLONING (Lampropoulos et al.,

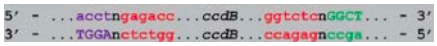
Type IIS restriction endonucleases cut at a precisely defined distance regardless of the local sequence.

*Bsa*I

GreenGate system has various destination and entry vectors containing the 6 modules to be assembled forming a plant transformation vector for expression of gene of interests.



Destination vector with engineered *Bsa*I cleavage sites having a different antibiotic resistance then that of modules, including *ccdB*.



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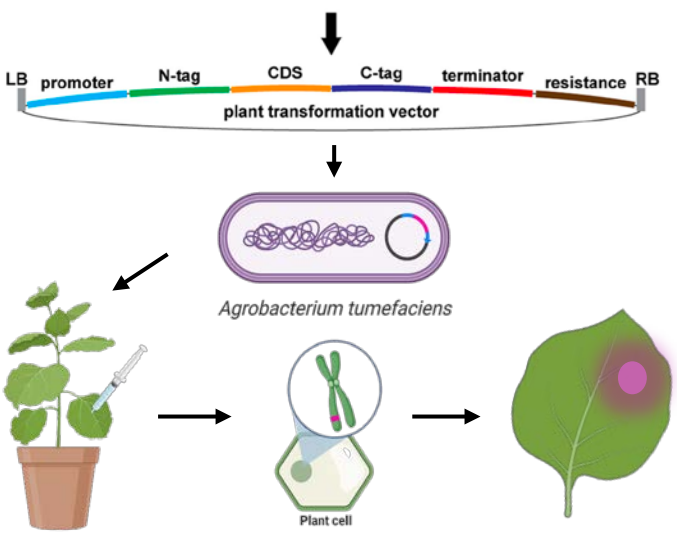
Module vectors with engineered cleavage sites and antibiotic resistance genes.



PROCEDURE

If gene of interest (GOI) has a *Bsa*I site it should be removed with a silent mutation.

GOI can be either cloned into CDS-module or as a PCR product with inverted *Bsa*I sites can be included into a single reaction tube having other 5 types of pre-cloned modules and a destination vector from GreenGate Cloning kit.

GreenGate reaction contains 150 ng of each module plasmids, 1.5 µL of CutSmart Buffer (NEB), 1.5 µL of ATP (10 mM), 1 µL of 30U/µL T4 ligase and 1 µL of 20 U/µL BsaI-HF in a 15 µL reaction volume by 50 cycles of 5 min at 37℃ and 5 min at 16℃ steps for continual digestion and ligation, followed by 5 min at 50℃ and 5 min at 80℃ enzyme inactivation steps.

The only circular ligation product would be plant transformation vector which is resistant to further *Bsa*I cleavage, since all the other molecules; the original destination vector and the modules retain the *Bsa*I cleavage sites, although they will spontaneously relegate but they will be re-digested again and again. Thus other then the final plant transformation vector construct, all the other molecules will disappear from the reaction over time.

Escherichia coli is transformed with the GreenGate reaction product and clones are confirmed by sequencing.

The verified plant transformation construct is used for transforming agrobacterium either transient or stable transformation of plant for expression of the GOI for follow up experiments.

REFERENCE

Lampropoulos A, Sutikovic Z, Wenzl C, Maegele I, Lohmann JU, Forner J. GreenGate---a novel, versatile, and efficient cloning system for plant transgenesis. *PLoS One*. 2013, 8(12):e83043. doi: 10.1371/journal.pone.0083043.