NU Tech
Technology Partnership of Nagoya University, Inc.

(B-32)

A Vector for Highly Efficient CRISPR/Cas9-Mediated Genome Engineering in Arabidopsis

Background:

The CRISPR/Cas9 system is a powerful tool for genome engineering. Only two components, Cas9 protein and single guide RNA (sgRNA) are needed to induce DNA double strand break (DSB) in a sequence-specific manner. Since the DSB results in an insertion or deletion of a few base pairs, it disrupts target gene function. Therefore, the CRISPR/Cas9 system enables us easily to produce knockout (KO) organisms.

Technology Overview:

Researchers at Nagoya University prepared three patterns of promoters to express Cas9 (Figure 1). Gene A and B are known to start their expression from a zygote and an egg cell, respectively. Transformants can be selected under a fluorescence microscope because OLE1p::OLE1:TagRFP on pFAST-R exhibits red fluorescence in seeds.

The gene B containing vector, pB-Cas9 was found to have a higher activity in inducing a target mutation. The vector had the activity to induce a whole albino phenotype in T1 plants. This phenotype suggests that the KO can be induced in the early stage of embryogenesis. pB-Cas9 was found to strongly induce transmittable mutations and can generate non-mosaic T1 plants.

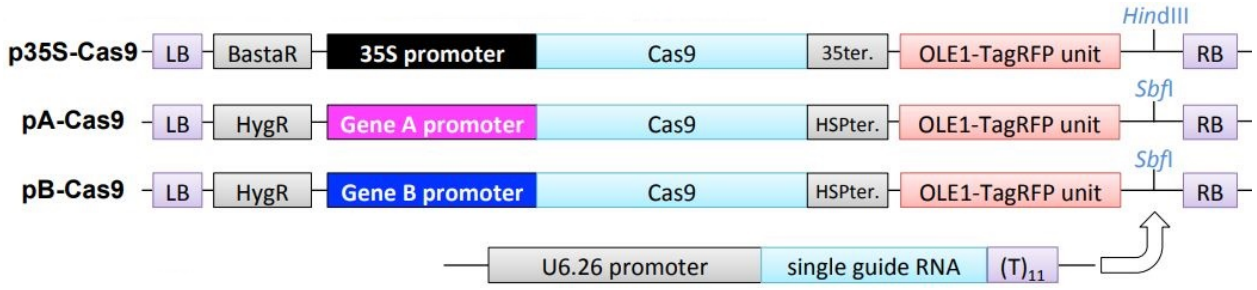

Figure 1: 35S/Gene A/Gene B promoter::Cas9 on pFAST-R

Benefits:

A highly efficient CRISPR/Cas9 vector
Strongly induced transmittable mutations
Cas9-free mutants can be isolated by fluorescence

Further Details:

Tsutsui H and Higashiyama T, pKAMA-ITACHI Vectors for Highly Efficient CRISPR/Cas9-Mediated Gene Knockout in Arabidopsis thaliana., Plant Cell Physiol. 2017 Jan

State of Development:

The researchers aim to stabilise Cas9 expression to further improve efficiency and enable mutant screening in the T1 generation.

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