PPTIM1 targeted gene knockout by homologous recombination in *Physcomitrella patens*

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Abstract: *Physcomitrella patens* has become a model organism for biological studies in lower plants for about 20 years. However, the function of all MADS box genes, which play important roles in plant development remains elusive. Recent transciptomics study in all developmental stages of *P. patens* was shown that expression of *PPTIM1* which is type I MADS box gene was increased during transition from early sporophyteto middle sporophyte stage which is the stage for sporogenesis. Thus, *PPTIM1* might play a role in spore development, and the functional study is needed in order to elucidate the function of *PPTIM1*. One approach to investigate the gene function is to create the knocked-out mutant and characterize to phenotype of the mutant line. In this research, *PPTIM1* knocked-out line was generated using gene targeting via homologous recombination method and the *PPTIM1*gene would be substituted by *sGPF* open reading frame and zeocin resistant gene. With this construct, the expression of GFP is expected to regulate with *PPTIM1* promoter, and it can be used to follow the expression of *PPTIM1*gene. The vector for *PPTIM1* was successfully generated and the recombination region sequence was confirmed by DNA sequencing. Two transformation methods, heat shock protoplast transformation and particle gun bombardment transformation, were used to create the transgenic lines. Heat shock protoplast transformation could not generate positive survival clones, while the *P. patens* colonies transformed by particle gun bombardment could germinate on zeocin plates. The validation of inserted fragments via PCR will be done in the next step prior to phenotype characterization.

Keywords: *Physcomitrella patens, PPTIM1, Homologous recombination, MADS box genes*

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