2024

Physcomitrium Patens CSLD Functional Analysis

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CSLD5/8 KO Genotype Analysis of *Physcomitrium patens*

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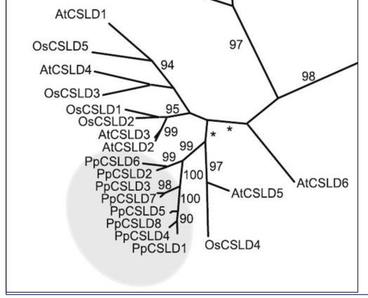
Abstract

Due to its small genome and dominant haploid life stage, Physcomitrium patens (formerly Physcomitrella patens) has become a model organism for studying plant genetics. The focus of this study was the Cellulose Synthase gene superfamily of P.patens, specifically the Cellulose Synthase-Like Ds (CSLD) family of genes. Previous studies into CSLD genes were preformed using RNA interference (RNAi) to preform a loss of function analysis on the entire PpCSLD gene family. The loss of function of the CSLD gene family resulted in inhibited protonemal tip growth indicating the CSLD gene family may regulate protonema formation. To further study the function of the CSLD genes in P.patens gene knockout (KO) of the CSLD5 and CSLD8 genes is preformed using CRISPR/Cas9 transformations. Over the summer analysis of the sg1 and sg2 cut sites was preformed on potential CSLD5/8 double knockouts from two different transformations, totaling 140 potential mutants being screened. Screening was preformed using competition based PCR (cbPCR). When preformed on wild type P.patens the cbPCR primers result in a greater ratio of forward inner primer product (around 564bps) to forward outer primer product (around 840bps), allowing for the screening of mutants by running the cbPCR products on agarose gels and determining the number of base pairs in the final PCR product via electrophoresis with a molecular weight ruler as reference. Of the 80 potential ppCSLD5/8 T2 KO samples screened 16/80 had the deletion genotype and were thus likely mutants while the other 64 either had the WT genotype or no amplification. Of the 60 T1 ppCSLD5/8 KO mutants screened 5/60 had the deletion genotype while 55/60 were either WT or failed to amplify.

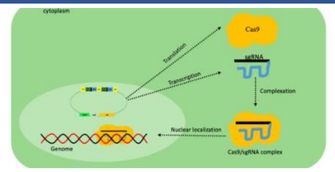
Physcomitrium patens CSLD family (Roberts, 2007)

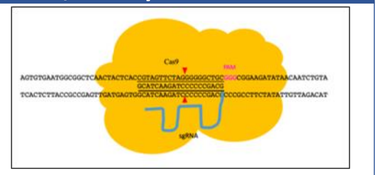


The P.patens CSLD gene family contains 8 genes which all share very high sequence similarity (Roberts et.al, 2007) and may share functional redundancies between them. (Dimos, 2010)

Previous studies preformed on the CSLD genes of Arabidopsis and rice have suggested potential roles in polarized tip growth and non-crystalline cellulose formation (Dimos, 2010)

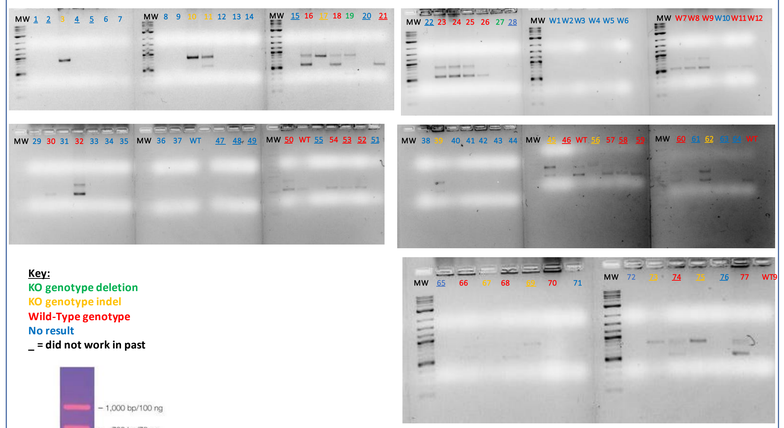
CRISPR Cas9 (Yuan, 2020)



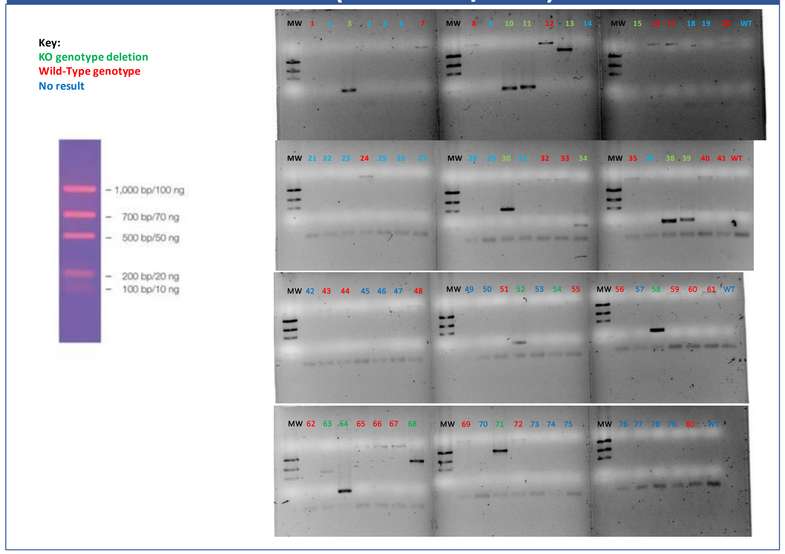


Gene editing by the CRISPR/Cas9 system. A CRISPR/Cas9 plasmid vector is used for PEG-mediated protoplast transformation. Inside the protoplast, the vector drives expression of the Cas9 protein and sgRNA, and the Cas9/sgRNA complex targets the moss genome for editing.

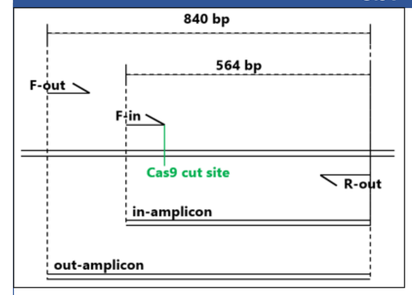
Results (T1 CSLD 5/8 KO)



Results (T2 CSLD 5/8 KO)



cbPCR



The inner primers anneal to the same genomic region as the cas9 cut site, thus indels at this site will lead to a greater ratio of F-out product (840bps) to F-in product (564bps) in mutant cells. For WT cells this ratio is reversed, leading to a greater ratio of F-in product to F-out product

Discussion

- Of the T2 ppcsld5/8 KO mutants screened; 16/80 had a D5/8 deletion genotype, 30/80 had a WT genotype, and 34/80 had no amplification
- Of the 16 T2 mutants with a deletion genotype; 1/16 had an sg1 indel genotype, 5/16 had an sg2 indel genotype, with the remaining samples being WT or failing to amplify
- The 6 samples with confirmed indel genotypes were sent for sequencing

- Of the T1 ppcsld5/8 KO mutants screened; 5/60 had a D5 deletion genotype, 1/60 had a D8 deletion genotype, 45/60 were WT, and 10/60 had no amplification
- Of the T1 samples screened with the D5 cb sg1 Fout/Rout/Fin primers; 1/40 had an indel genotype, 32/40 were WT, and 7/40 failed to amplify

References / Acknowledgments

1. Yuan, K. (2020). Functional Analysis of the CSLD Genes in *Physcomitrella Patens* by CRISPR/Cas9 Gene Knockout (Doctoral dissertation, University of Rhode Island).
2. Dimos, C. S. (2010). *Functional analysis of the cellulose synthase-like D (CSLD) gene family in Physcomitrella patens*. University of Rhode Island.
3. Roberts, A. W. and J. T. Bushoven (2007). "The cellulose synthase (CESA) gene superfamily of the moss Physcomitrella patens." Plant molecular biology 63(2): 207-219

This project is supported by National Science Foundation Grant NSF 21-506 Division of Integrative Organismal Systems Core Programs.
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