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Michael McGill
MPMCGILL@CSBSJU.EDU

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Plant Genome Editing Using CRISPR/Cas9: Investigating the Role of TEN1 in the Maintenance and Protection of Telomeres in Arabidopsis thaliana

Michael McGill and Katherine Leehey, Ph.D.
College of St. Benedict and St. John’s University, Collegeville, MN

Background

- Telomeres are highly regulated, dynamic complexes that are located at the ends of linear chromosomes.
- Telomeres prevent unwanted DNA recombination and degradation, and inhibit activation of detrimental DNA damage response.
- Short telomeres have previously been associated with age-related and disease phenotypes.
- The TEN1 protein has previously been shown to be part of the CST complex at telomeres, but its function has yet to be fully characterized.

CRISPR/Cas9 is an adaptive immune system found in bacteria that has been exploited in genome engineering for its ability to create double stranded breaks at specific target sequences.

This study aims to establish a knockout line of Arabidopsis thaliana plants using CRISPR/Cas9 in order to investigate the role of TEN1 in telomere maintenance and protection.

How does CRISPR/Cas9 work?

CRISPR/Cas9 targets and cleaves specific sequences of the DNA, allowing for DNA insertions or deletions in the targeted region during DNA repair.

1. Identify TEN1 target sequences in A. thaliana genome
2. Design target for guide RNA and clone into vector
3. Transform A. tumefaciens, infect plants with bacteria

Methods & Materials

1) Identify TEN1 target sequences in A. thaliana genome
2) Design target for guide RNA and clone into vector
3) Transform A. tumefaciens, infect plants with bacteria

Introduction to plant cells via floral dip
Collect and select for transformed seedlings

Results

1) Protospacer (target) sequence
   P: 5′ – ATTGATCCTTCTTCTCTGTTTTAC – 3′
   R: 3′ – TAGGAGAAGAGAAGACAAATGCAA – 5’

2) Plasmid cloning

3) Gateway cloning of protospacer and guide RNA

Figure 1. Colony PCR results of ligation reaction into the entry vector. a) Gel electrophoresis of protospacer region PCR product. b) Sanger sequencing results of PCR product

Figure 2. Colony PCR results of gateway reaction into the destination vector. a) Gel electrophoresis of protospacer region PCR product. b) Sanger sequencing results of PCR product

Conclusion

- Successfully designed and cloned the CRISPR/Cas9 system targeting TEN1 in A. thaliana.
- Successful transformation of Agrobacterium tumefaciens and subsequent A. thaliana infection with CRISPR/Cas9 construct.

Current and Future Research

- Working to confirm a ten1 knockout plant line from the seedlings artificially selected from transformed plants

- After confirmation of a ten1 knockout line, future experiments will investigate the role of TEN1 in the protection and maintenance of telomeres.

References/Acknowledgments

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