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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

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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*

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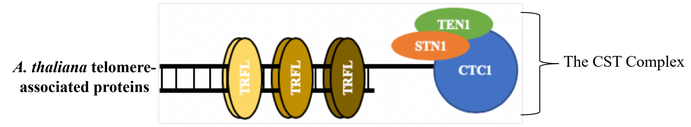
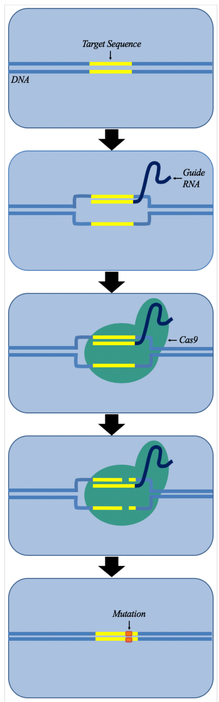
## Background

- 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 it's 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 *ten1* 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.**

The **protospacer** (yellow) portion of guide RNA is complimentary to the target sequence and guides the Cas9 to the target.

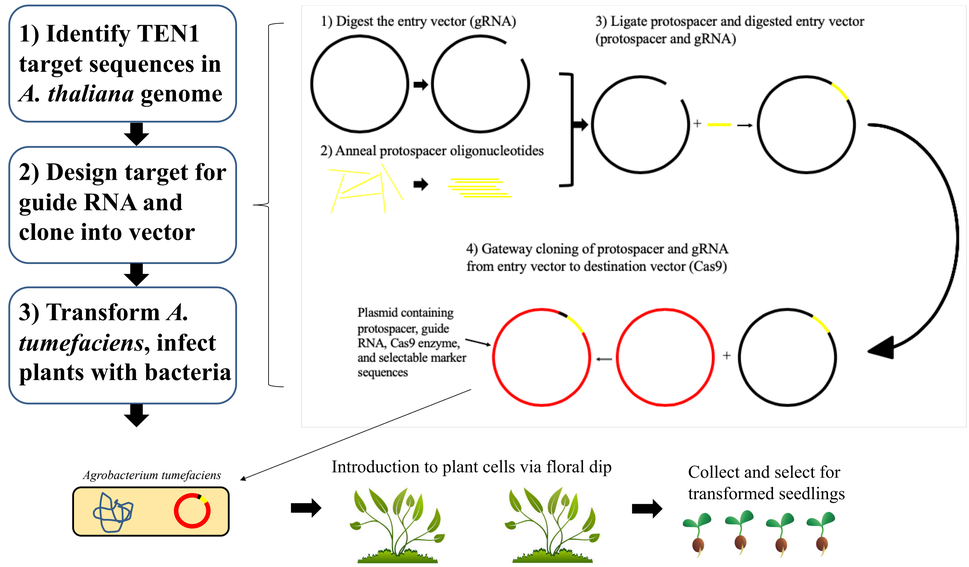
The **scaffold** (black) portion of guide RNA binds to the Cas9.

The Cas9 enzyme binds to the guide RNA at the target sequence.

The Cas9 enzyme cuts both strands of DNA at the target sequence.

The DNA break is repaired, sometimes introducing a mutation.

## Methods & Materials




## Results

**1) Protospacer (target) sequence**

```
F: 5′ –  ATTGATCCTTCTTCTCTGTTTTAC  – 3′
R: 3′ –      TAGGAAGAAGAGACAAAATGCAAA  – 5′
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**2) Plasmid cloning**

**a) 0.7% TAE gel electrophoresis**

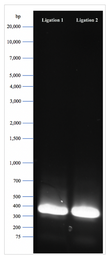
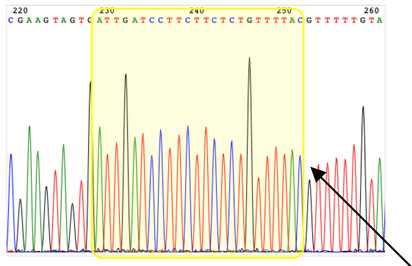


**Figure 1. Colony PCR results of ligation reaction into the entry vector.** a) Gel electrophoresis of protospacer region PCR product. b) Sanger sequencing results of PCR product.

**b) Sanger sequencing (GENEWIZ)**



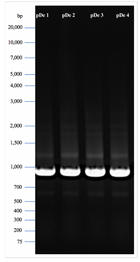
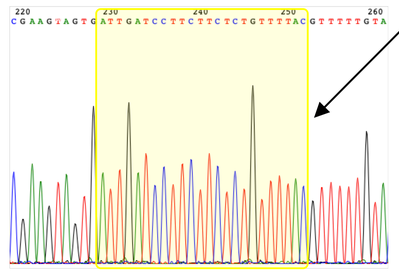



**Figure 2. Colony PCR results of gateway reaction into the destination vector.** a) Gel electrophoresis of protospacer region PCR product. b) Sanger sequencing results of PCR product.



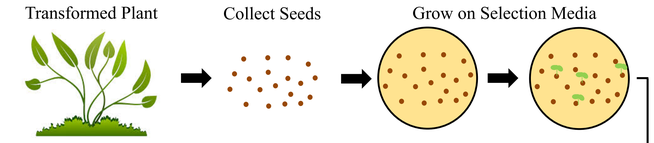

Protospacer Sequence

## 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



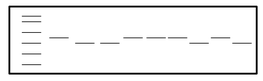

Identifying a *ten1* knockout line:

1. Collect plant tissue
2. Extract genomic DNA via phenol-chloroform extraction
3. Amplify TEN1 gene from each plant

a. Evaluate TEN1 gene for DNA insertions or deletions ~15bp or greater

b. Use restriction enzyme digest to identify smaller insertions or deletions

Selected *A. thaliana* 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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