Determining the Function of T-DNA *rol* Genes in *Agrobacterium rhizogenes* Pathogenicity

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A. rhizogenes integrates a specific region of DNA into its plant host

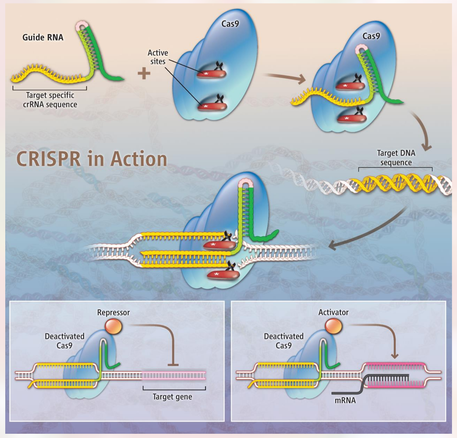
- Hairy root disease is characterized by the development of a thick, multi-branching root system
- A region of *A. rhizogenes* DNA (T-DNA) carries genes which cause hairy root disease
- The resulting phenotype provides useful metabolites for *A. rhizogenes*
- This process occurs naturally in many different plants

Biological Questions:

- Which genes are located within the T-DNA?
- In which cells are those genes expressed?
- Which genes are important for the resulting phenotype?
  - Which genes are sufficient for inducing hairy root growth?
  - Which genes are necessary for inducing hairy root disease?
- **What is the function of the *rol* genes in the development and maintenance of hairy roots in tomatoes?**

4. Are individual *rol* genes necessary for hairy root disease?

Using CRISPR/Cas9 gene editing, each of the four *rol* genes can be mutated while leaving the others intact to test if the individual genes are necessary for inducing hairy root disease.



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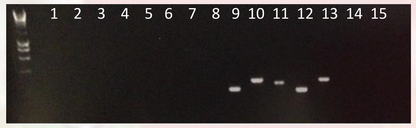
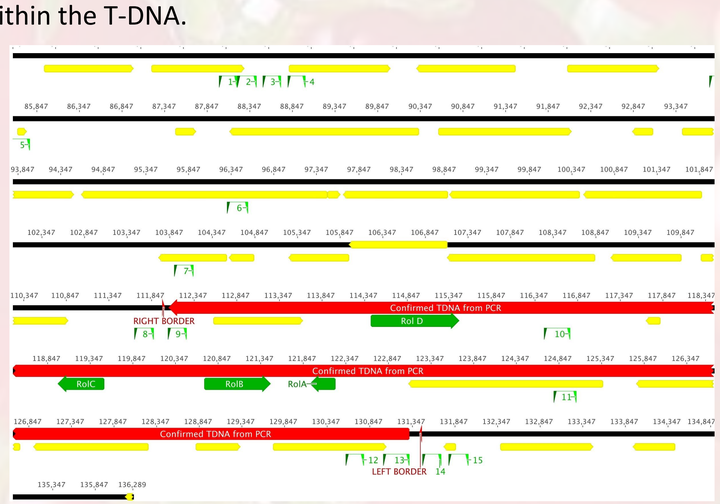
1. T-DNA and *rol* genes were identified using bioinformatics and the T-DNA was confirmed using PCR

Various primer pairs were designed 100-300bp apart at locations upstream and downstream of the theoretical left and right borders of the T-DNA. These primers were tested using *A. rhizogenes* infected M82 tomato roots.



The location of the *rol* genes was deduced through the use of sequence homology to known gene sequences. These known sequences were aligned to the sequenced *A. rhizogenes* genome, revealing the location of each *rol* gene within the T-DNA.



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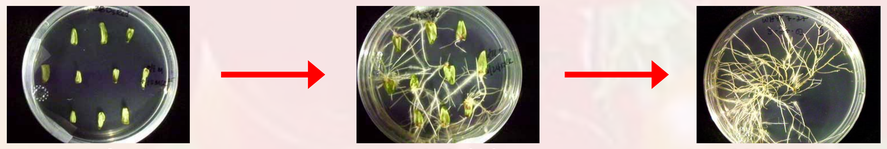
5. Plant Infection and Propagation of Tissue Cultures

The constructs are transformed into *Agrobacterium* which is then introduced into tomatoes through wounded cotyledons. This process allows us to:

- Analyze the resulting phenotypes of each construct
- Visualize cell-type specific gene expression using confocal microscopy
- Propagate the tissue line for further research



Day 1: Infection → **Day 14: Subclone** → **Day 28: Propagation**

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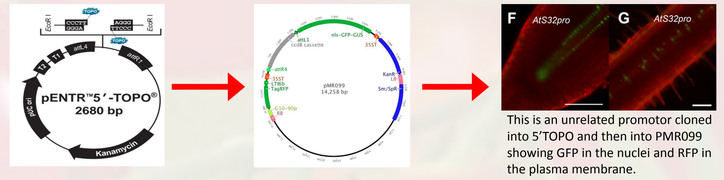
2. Cell-type Specific Gene Expression

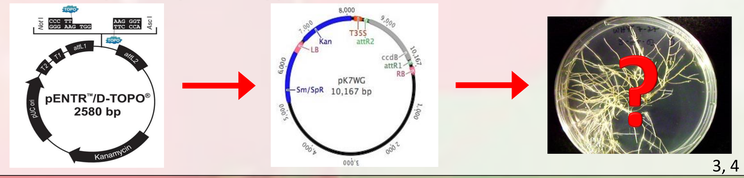
The *rol* gene promotors can be cloned into a series of vectors, which ultimately allows each promotor to drive expression of Green Fluorescent Protein (GFP).



This is an unrelated promotor cloned into 5'TOPO and then into PMR099 showing GFP in the nuclei and RFP in the plasma membrane.

3. Which *rol* genes are sufficient for inducing hairy root disease?

By cloning single *rol* genes into the proper vector and then transforming it into a strain of *Agrobacterium* which lacks T-DNA, we can test for the ability of that single gene to induce the hairy root phenotype



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Conclusion

- The region of *A. rhizogenes* DNA which is transferred into the plant genome during infection has been identified.
- The identity and location of genes within the T-DNA have been identified based on homology to known genes.
- Further work should be done to identify the role of these genes in hairy root development

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References

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