Just *rol* with it: A transgene excision system for rapid transformation and gene-editing in plants

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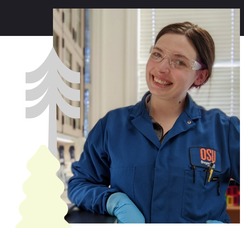
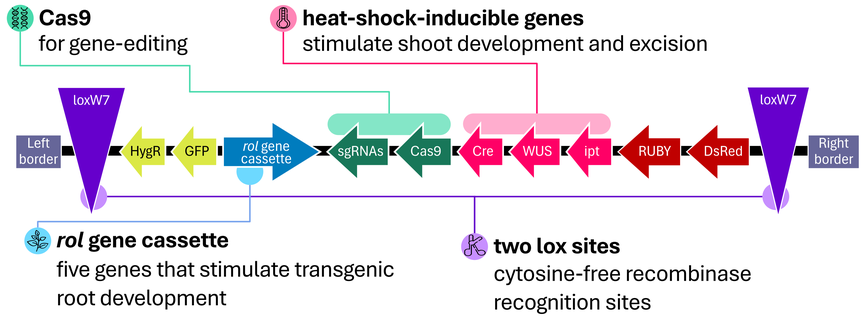


INTRODUCTION

Plant genetic transformation is a useful tool for improving resistance to disease and abiotic stressors like drought and heat, but remains difficult or impossible in the large majority of plant species. Insertion of *Rhizobium rhizogenes* genes reliably produces transgenic hairy roots in many plant species, but generating transgenic shoots from hairy root tissue is challenging. In addition, the presence of the *rol* gene cassette in the Ri plasmid after shoot production leads to phenotypic abnormalities. We therefore developed a heat-inducible system for removal of undesired genes, including the *rol* gene cassette, using the Cre/lox recombinase system. The system appears to be working well in transgenic poplars.

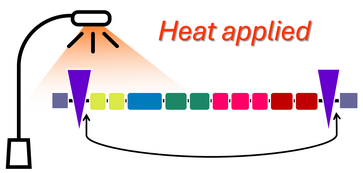
Hairy root from a single plated explant

VECTOR ASSEMBLY

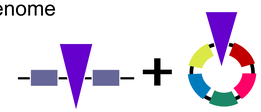


- To create **a heat-shock-inducible Cre/lox recombinase system**, we assembled a complex **transformation vector** between two **recombinase recognition sites** which undergo **excision** upon a heat shock treatment.
- Constructs were generated using the **Golden Gate method**, which allows rapid and seamless assembly of plant vectors, and further assembled using **GAANTRY**, a system for stably stacking genes in the *Agrobacterium* T-DNA.
- Gene editing via CRISPR/Cas9 aims to produce pollen and seed-free poplars with reduced stature

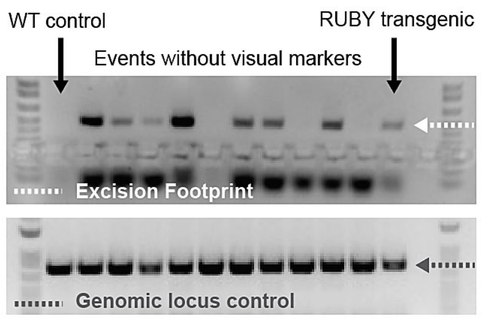
TRANSGENE EXCISION



1. Transgene is excised as a non-chromosomal DNA fragment and a small "footprint" is left in the genome

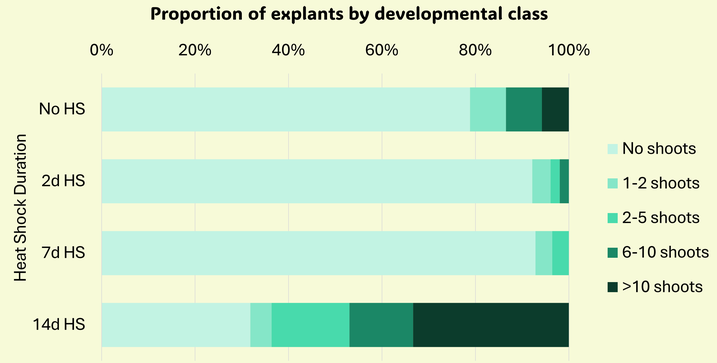


2. Shoot development is induced and Cre recombinase initiates recombination between lox sites

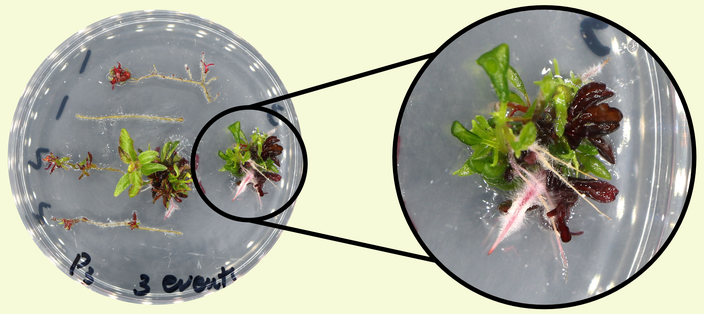


Presence of the excision "footprint" verified by PCR and gel electrophoresis

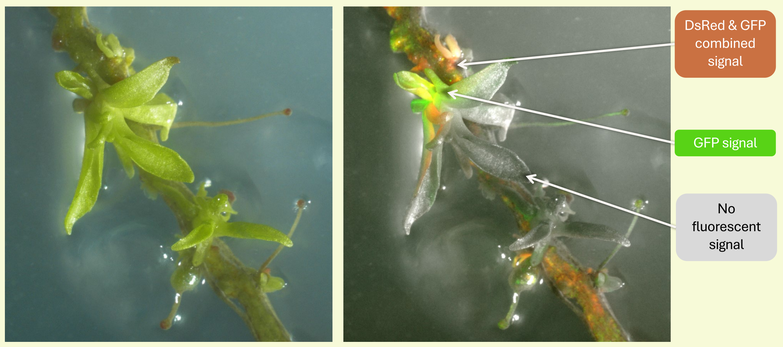
Longer heat-shock (HS) duration improved shoot regeneration rates per explant (segments of hairy roots)



Three transformation markers (one magenta pigment as shown below, and two fluorescent markers) allowed for easy identification of transgenic, excised tissue

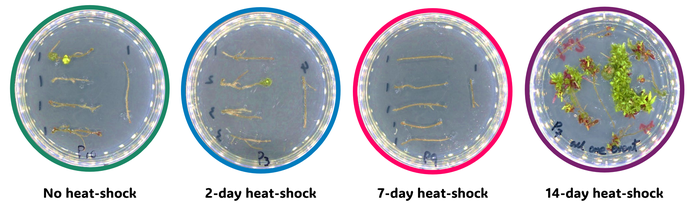


- Hairy roots expressing the RUBY phenotype (magenta) are transgenic
- Explants were exposed to three different heat shock treatments to induce excision via Cre recombinase
- Absence of fluorescent transformation markers GFP and DsRed (below) indicate that excision was successful

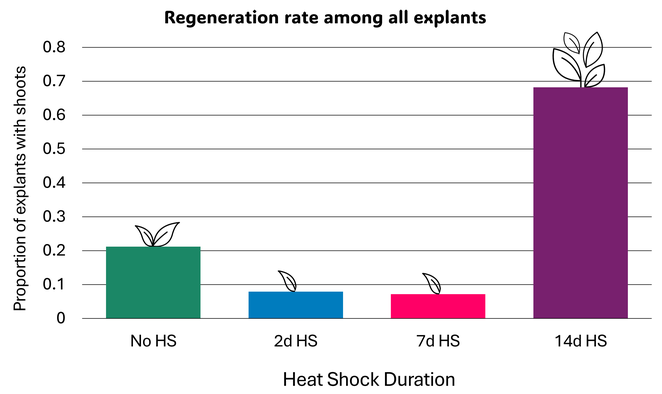


An explant in full-color (left) and overlaid fluorescent signals (right)

The 14-day heat-shock (HS) resulted in highest rate of shoot regeneration



One representative plate from each heat shock treatment from below



RESULTS SUMMARY

- 48% of events had at least one propagatable, visually excised shoot, most had many more

Of the shoots we screened at the DNA level:

- 50% were excised
- 36% were still transgenic or chimeric
- 14% were WT escapes

CURRENT RESEARCH

We are testing the efficacy of the novel construct in multiple poplar genotypes and investigating the relative importance of its genetic components in isolation (WUS, ipt, Cre).

ACKNOWLEDGEMENTS

We thank the Genetic Research, Engineering and Advanced Transformation of Trees (GREAT Trees) Consortium, and the USDA-NIFA Biotechnology Risk Assessment Program. I also thank my in-lab mentor Greg S Goralogia for his major contributions to this project, and extensive knowledge and patience.

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