Excisable gene editing systems

Generation of dwarf and sterile poplars using a developmental and chemical-controlled CRISPR/recombinase excision system

Greg S. Goralogia and Steven H. Strauss

Department of Forest Ecosystems and Society

Oregon State University

Thanks to postdoc Greg Goralogia who is the force behind this work

Please see our poster

Excisable gene editing systems: Generation of dwarf and sterile poplars using a developmental and chemical-controlled CRISPR/recombinase excision system

Greg S. Goralogia, Anna Brousseau, Isabella Andreatta, Daniel Casey-Hain, Cathleen Ma and Steven H. Strauss
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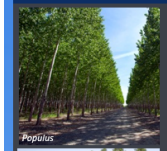
Oregon State University

PB21 PLANT BIOLOGY

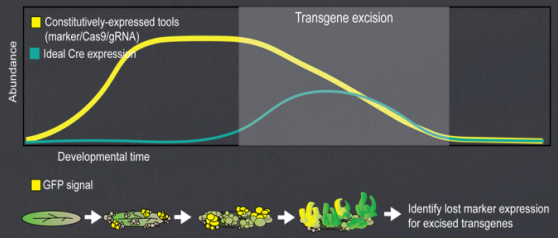
Editing machinery is difficult to remove from trees and clonally propagated plants



- Most CRISPR/Cas9 editing systems in plants remove transgenes through sexual segregation
- This method does not work for clonally propagated or highly heterozygous plants, or in induced or naturally sterile plants, including many forest trees
- Transformation aids like "DEV" genes that help recalcitrant species also need to be removed to recover usable events
- Excision of editing transgenes after insertion through Cre/lox is an attractive method around these roadblocks
- May be categorically accepted under new USDA SECURE regulatory system

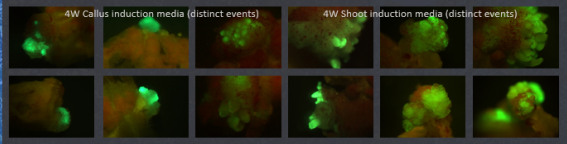
Initial goal: Developmental excision during regeneration using meristem-specific promoters

- Common Cre / FLP recombinase induction systems use stress stimuli like heat shock, which can harm plant health and impede regeneration
- Developmental triggers would be an alternative method to express Cre, when developmental shifts occur as plants are regenerated into shoots
- Ideally, we could then quickly recover edited events with transgenes removed

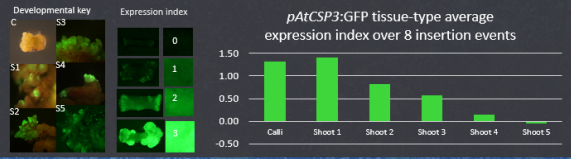


Meristem-dominant promoters tested through promoter:GFP fusions – all strongly callus active

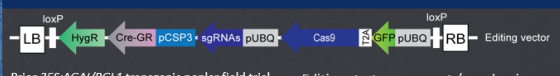
AtCSP3 promoter, 1.3kb fragment, drives strong GFP expression in meristems and callus



Scoring of tissue specificity & expression level showed high callus expression in all events

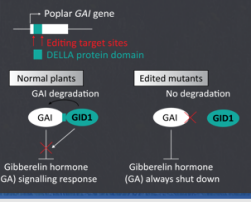


Final vectors included GR/dex induction, tested as in gene edited, dwarf / sterile tree prototype



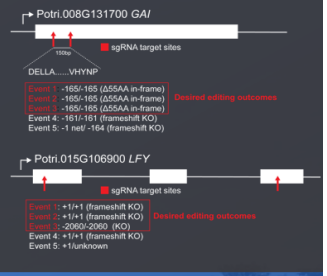
Prior *35S:ΔGAI/RGL1* transgenic poplar field trial showing range of dwarf phenotypes possible

Editing strategy concept / mechanism

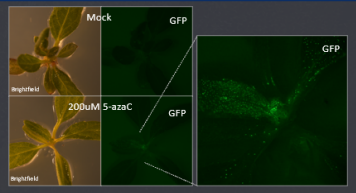


Dwarf / sterile gene-edited poplars were produced, but with low rate of transgene excision, very strong dwarf phenotypes

- Explants treated with DEX during shoot regeneration (3WCIM/3WSIM + 1mo SIM + DEX (20uM)) and removed from selection during dex treatment
- High escape rate: 13/87 (15%) of shoots transgenic
- 5 events had a *gai* deletion detected by PCR
- 3 in-frame deletions had a severe dwarf phenotype



Reawakening of GFP expression with 5-azacytodine suggests that DNA methylation may explain low excision rate



- A recent study showed strong DNA methylation in recombinase target region inhibits excision, but can be relieved by demethylase treatments (Liu et al., New Phytologist 2021)
- In a test event, 8/8 propagated shoots showed a recovery of GFP expression with 5-azacytidine
- Currently studying effects of demethylation chemicals and demethylase induction on excision rates, resolution of transgenes from complex insertions, and plant health and genetic integrity

Conclusions

- A meristem-enriched promoter + GR/Dex system was functional in triggering excision of CRISPR-Cas9 editing transgenes in poplar, but at a low frequency
- CRISPR editing using multiplexed gRNAs was successful at inducing dwarfism and obtaining loss-of-function edits in the key flowering gene *LEAFY*
- Impairment of methylation appears to improve GFP expression and likely excision; this and other improvements to excision efficiency are under study
- Similar approaches could be used for other plant species for control of plant form and elimination of pollen, seeds, or fruits

Acknowledgements

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We also thank Corteva Agriscience for technical expertise related to excision vectors and Agrobacterium strains that were used in this study.

USDA NSF J. Frank Schmidt Family Charitable Foundation

We need a somatic excision system for trees and clonal crops

Sexual segregation and transformation very difficult

Editing machinery is difficult to remove from trees and clonally propagated plants

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- Transformation aids like "DEV" genes that help recalcitrant species also need to be removed to recover usable events
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- May be categorically accepted under new USDA SECURE regulatory system

Initial goal: Developmental excision during regeneration using meristem-specific promoters

Basic concepts “out there” but not tools for routine use

Plant Cell Rep (2009) 28:1509–1520
DOI 10.1007/s00299-009-0750-y

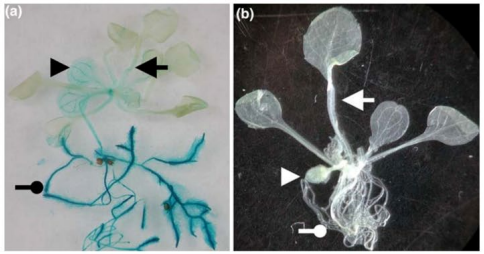
ORIGINAL PAPER

Evaluation of seven promoters to achieve germline directed Cre-*lox* recombination in *Arabidopsis thaliana*

Frédéric Van Ex · Dimitri Verweire · Martine Claeys · Ann Depicker · Geert Angenon

LoxP-35S-GUS-LoxP T_0

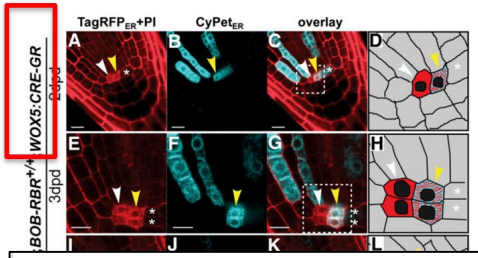
LoxP-35S-GUS-LoxP T_1 X *pCLV3*:Cre



The Plant Cell, Vol. 23: 2581–2591, July 2011, www.plantcell.org © 2011 American Society of Plant Biologists. All rights reserved.

Distinct Cell-Autonomous Functions of *RETINOBLASTOMA-RELATED* in *Arabidopsis* Stem Cells Revealed by the Brother of Brainbow Clonal Analysis System

Guy Wachsman, Renze Heidstra, and Ben Scheres[1]
Department of Biology, Utrecht University, 3584 CH Utrecht, The Netherlands



The Plant Journal (2000) **24**(2), 265–273

TECHNICAL ADVANCE

An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants

Jianru Zuo, Qi-Wen Niu and Nam-Hai Chua
Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, N

Received 2 May 2000; revised 1 August 2000; accepted 1 August 2000.
*For correspondence (fax +1 212 327 8327; e-mail chua@rockvax.rockefeller.edu).

Vitis **49** (4), 201–208 (2010)

Comparing 17-β-estradiol supply strategies for applying the XVE-*Cre/loxP* system in grape gene transfer (*Vitis vinifera* L.)

L. Dalla Costa, M. Mandolini, V. Poletti and L. Martinelli

Research and Innovation Centre, Fondazione Edmund Mach-IASMA, San Michele all'Adige, Italy

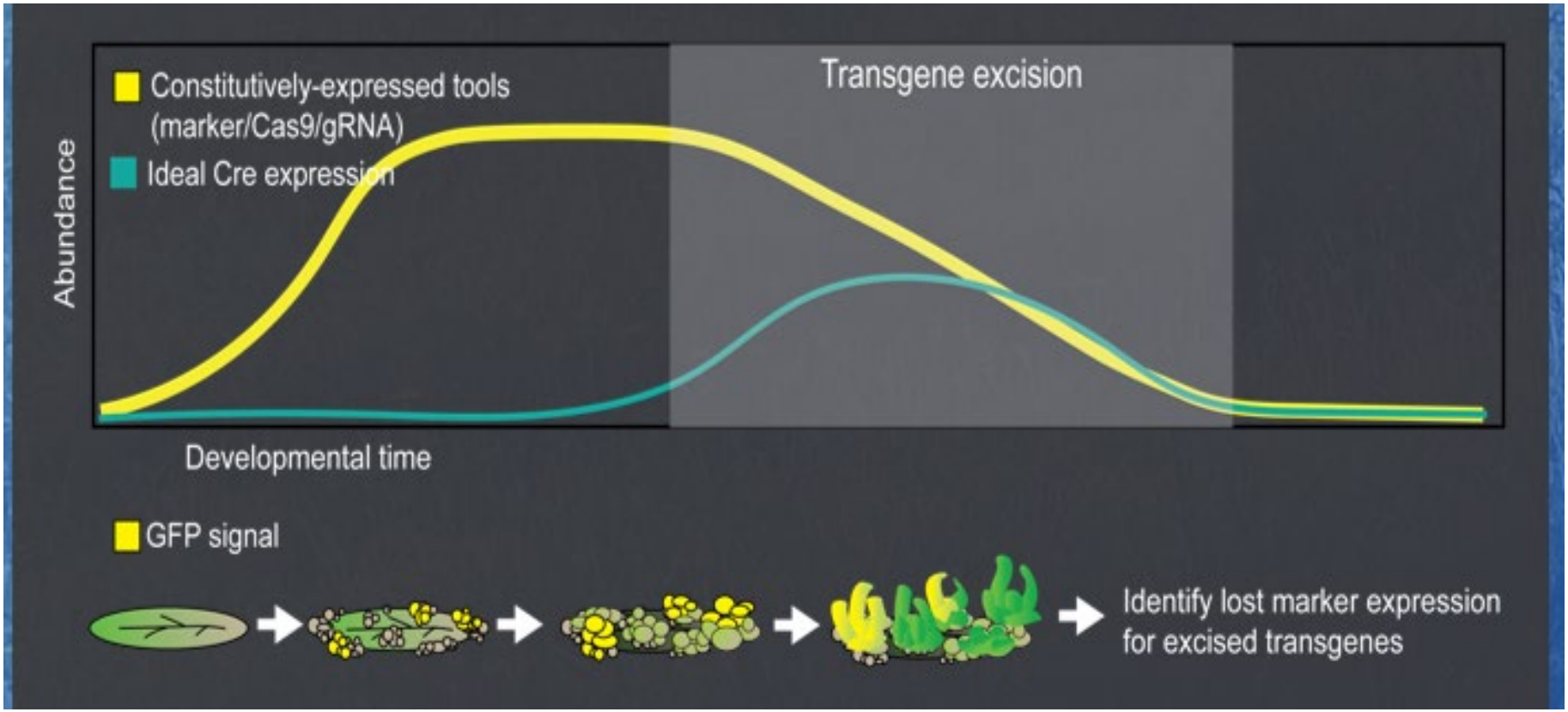
Plant Cell Tiss Organ Cult (2016) 124:471–481
DOI 10.1007/s11240-015-0907-z

ORIGINAL ARTICLE

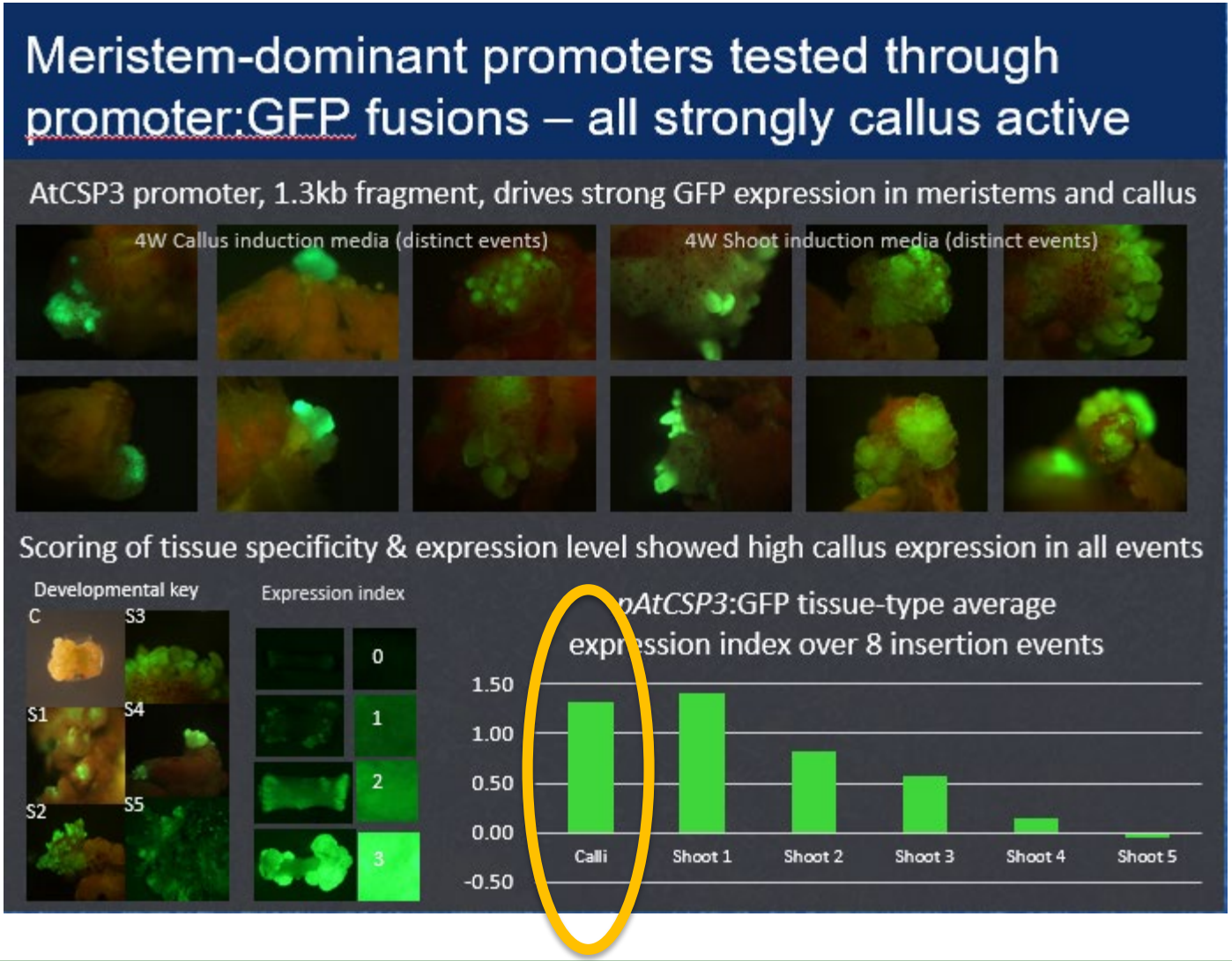
Efficient heat-shock removal of the selectable marker gene in genetically modified grapevine

Lorenza Dalla Costa[1] · Stefano Piazza[1] · Manuela Campa[1,2] · Henryk Flachowsky[3] · Magda-Viola Hanke[3] · Mickael Malnoy[1]

Desired a meristem-dominant promoter for indirect regeneration system

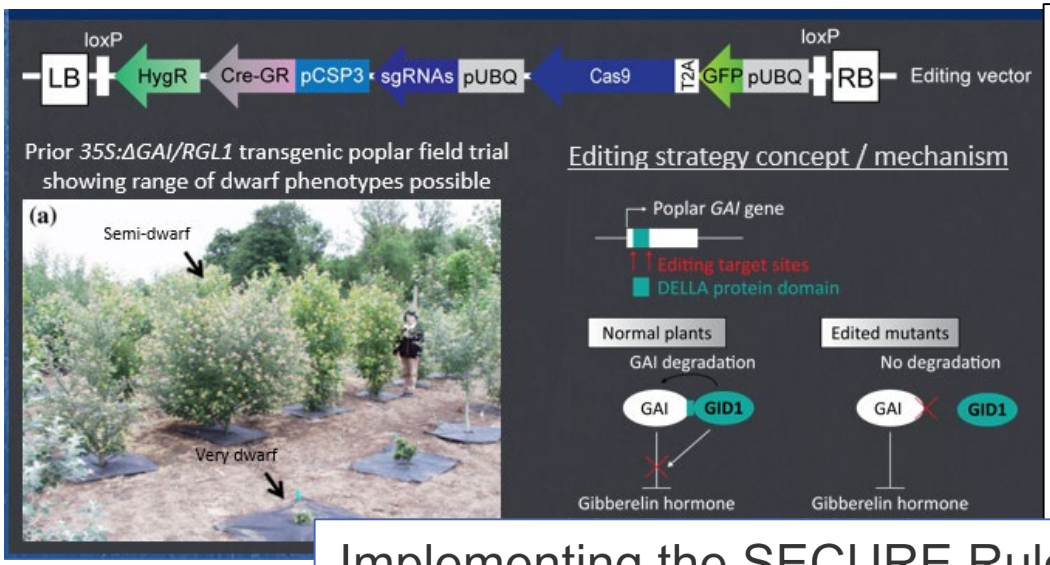


Screened promoters *WUS*, *STM*, *CSP*, others as triggers – **callus expression !**



Created a Cre-glucocorticoid-receptor fusion to overlay chemical induction on promoter system

Test case: Gene edited semi-dwarf + sterile trees



Containment of transgenic trees by suppression of *LEAFY*

To the Editor:

Field studies and commercial use of genetically engineered (GE) trees have been limited, in large part owing to concerns over transgene flow into wild or feral tree populations[1–4]. Unlike other crops, trees are long-lived, weakly domesticated and their propagules can spread over several kilometers[5]. Although male sterility has been engineered in pine, poplar, and eucalyptus trees grown under field conditions by expression of the barnase RNase gene in anther tapetal cells[6,7], barnase can reduce rates of genetic transformation and vegetative growth[6]. Furthermore, barnase expression

report the use of RNA interference (RNAi) to suppress expression of the single-copy *LEAFY* (*LFY*) gene to produce sterility in poplar.

RNAi has been used to reduce gene expression in many plant species[10,11], and the reduction in gene expression that RNAi confers is highly stable in trees under field conditions[12]. *LFY* is required for the early stages of male and female floral organ formation in plants, and encodes a transcription factor that promotes floral meristem identity[13,14]. In *Arabidopsis thaliana*, loss of *LFY* function results in the formation of vegetative structures instead of ...eristems, whereas reduction of *LFY* ...ion decreases floral abundance and ...n partial conversion of floral organs ...ike structures[13,14]. We selected *LFY*

...BER 2016 NATURE BIOTECHNOLOGY

Implementing the SECURE Rule

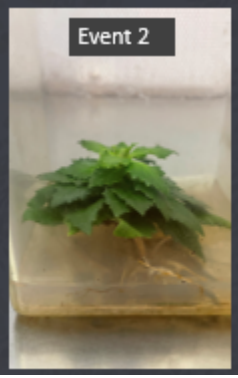
Last Modified: Jun 2, 2020

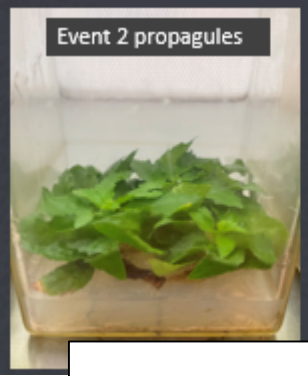
Print

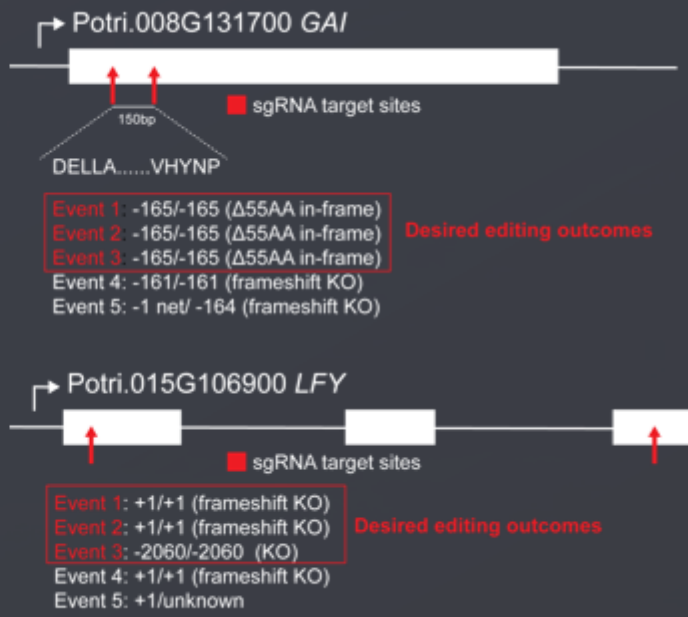
The SECURE rule is final on the day it is published in the Federal Register. The new rule's provisions become effective on key dates over the next 18 months. The biotechnology community will have to learn some new processes and meet new requirements in accordance with the implementation schedule. We are available to support you through this process. It is our goal to minimize regulatory burden and help you comply with our regulations.

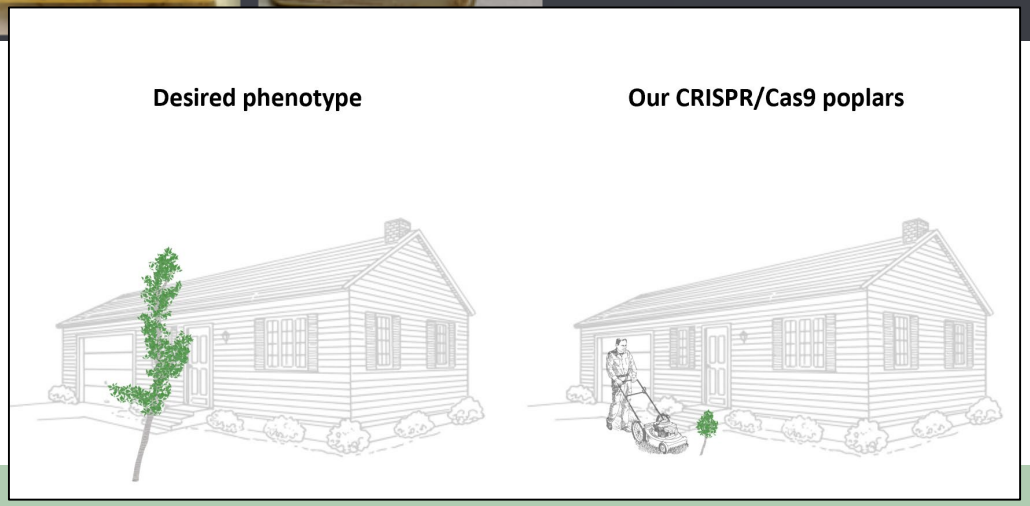
Editing worked, but dwarfism extreme

- Explants treated with DEX during shoot regeneration (3WCIM/3WSIM + 1mo SIM + DEX (20uM)) and removed from selection during dex treatment
- High escape rate: 13/87 (15%) of shoots transgenic
- 5 events had a *gai* deletion detected by PCR
- 3 in-frame deletions had a severe dwarf phenotype

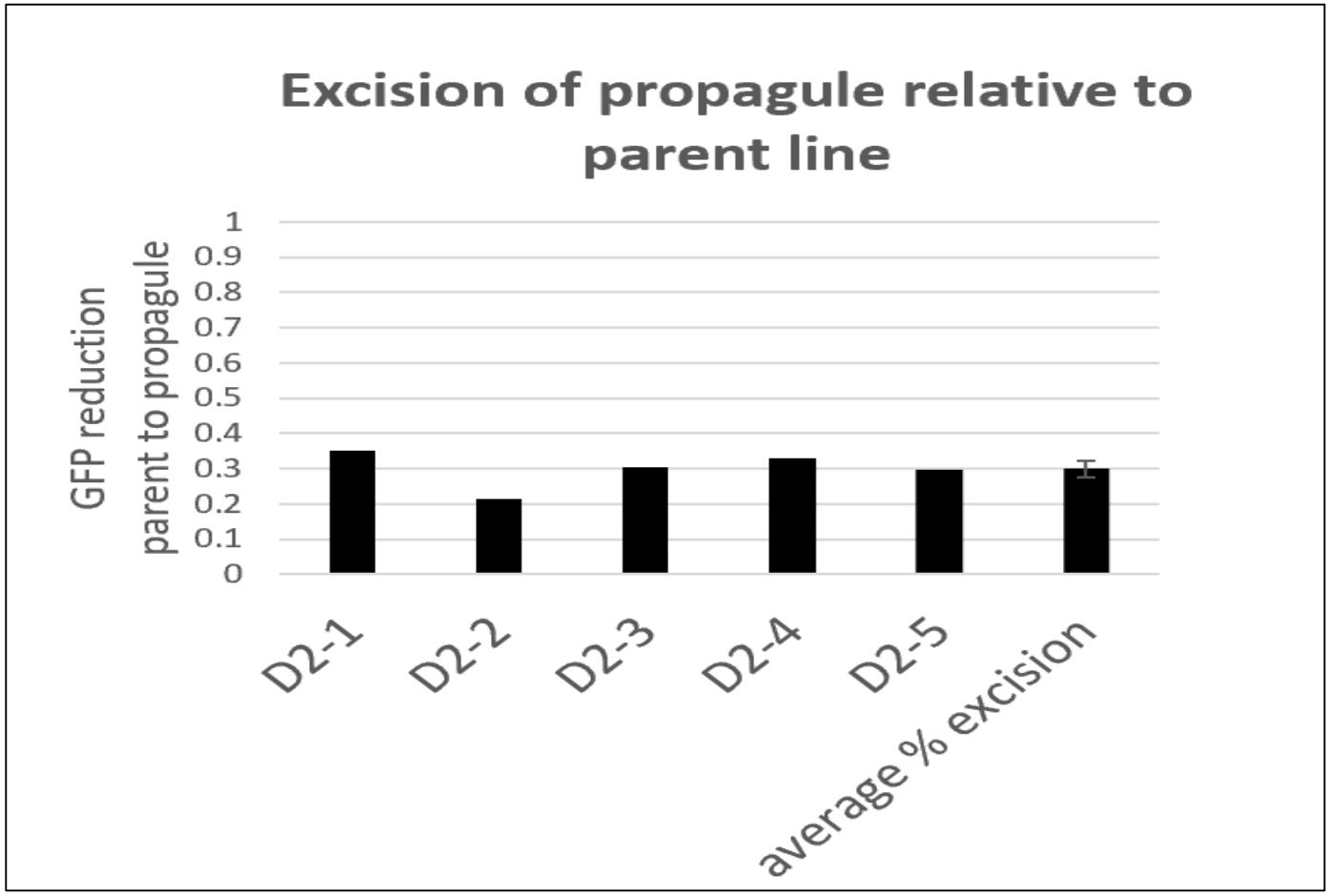








qPCR showed excision rate low even with repeated dex induction – trying other tweaks



Thank you

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Todd Jones

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NSF Plant Genome Research
No: 1546900

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GREAT TREES industrial cooperative at OSU