# Gene editing policy and application to flowering modification in Eucalyptus

Steve Strauss / Oregon State University / USA

*Beijing Forestry University, China – September 2019*

# Agenda

- Definition of gene editing (GE) compared to GMO and conventional breeding
- The social environment that makes use of GE and GMO, and integration with breeding, difficult
  - A recent attempt by scientists to stimulate change
- A GE example – showing precision and stewardship possibilities
  - CRISPR for reproductive modification in Eucalyptus

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# What is genetic engineering (GMO) and gene editing (GE)?

- Direct modification of DNA
  - vs. indirect modification in breeding
- Asexually modified, usually in somatic cells
  - Then regenerated into whole organisms, usually starting in Petri dishes
- <u>Specificity</u> of modification, common use of modified native genes vs. new genes, differentiates GE from GMO

Overview of steps to create a GMO or GE plant

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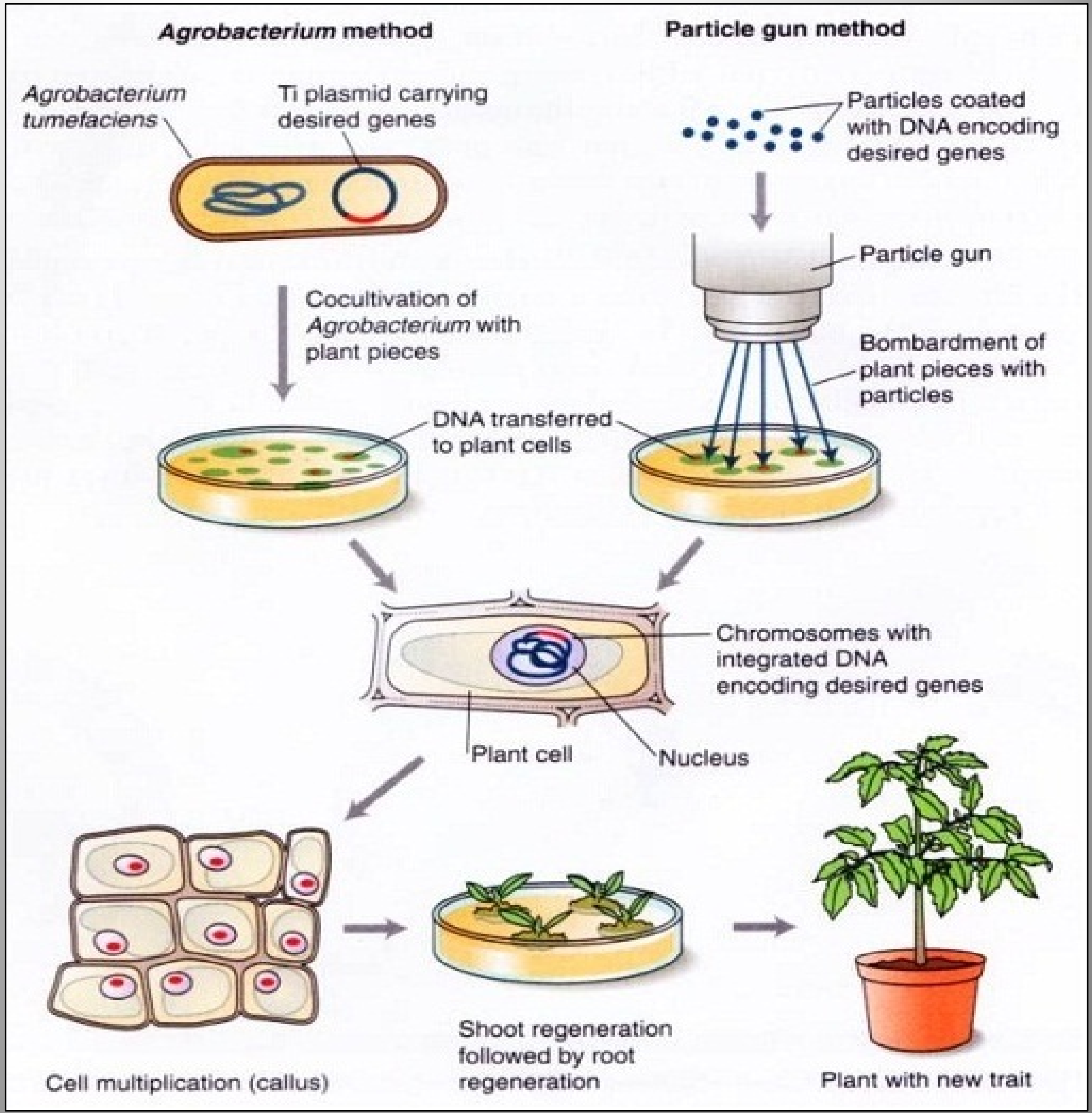
New genes usually removed or deactived after GE



# Many forms of GE, but CRISPR gene editing technology considered a major scientific breakthrough

## Science magazine names CRISPR 'Breakthrough of the Year'

By Robert Sanders | DECEMBER 18, 2015

In its year-end issue, the journal *Science* chose the CRISPR genome-editing technology invented at UC Berkeley 2015's Breakthrough of the Year.

A runner-up in 2012 and 2013, the technology now revolutionizing genetic research and gene therapy "broke away from the pack, revealing its true power in a series of spectacular achievements," wrote *Science* correspondent John Travis in the Dec. 18 issue. These included "the creation of a long-sought 'gene drive' that

CRISPR a very general technology – it works well wherever it's been tested

# Overview of CRISPR gene edit machinery

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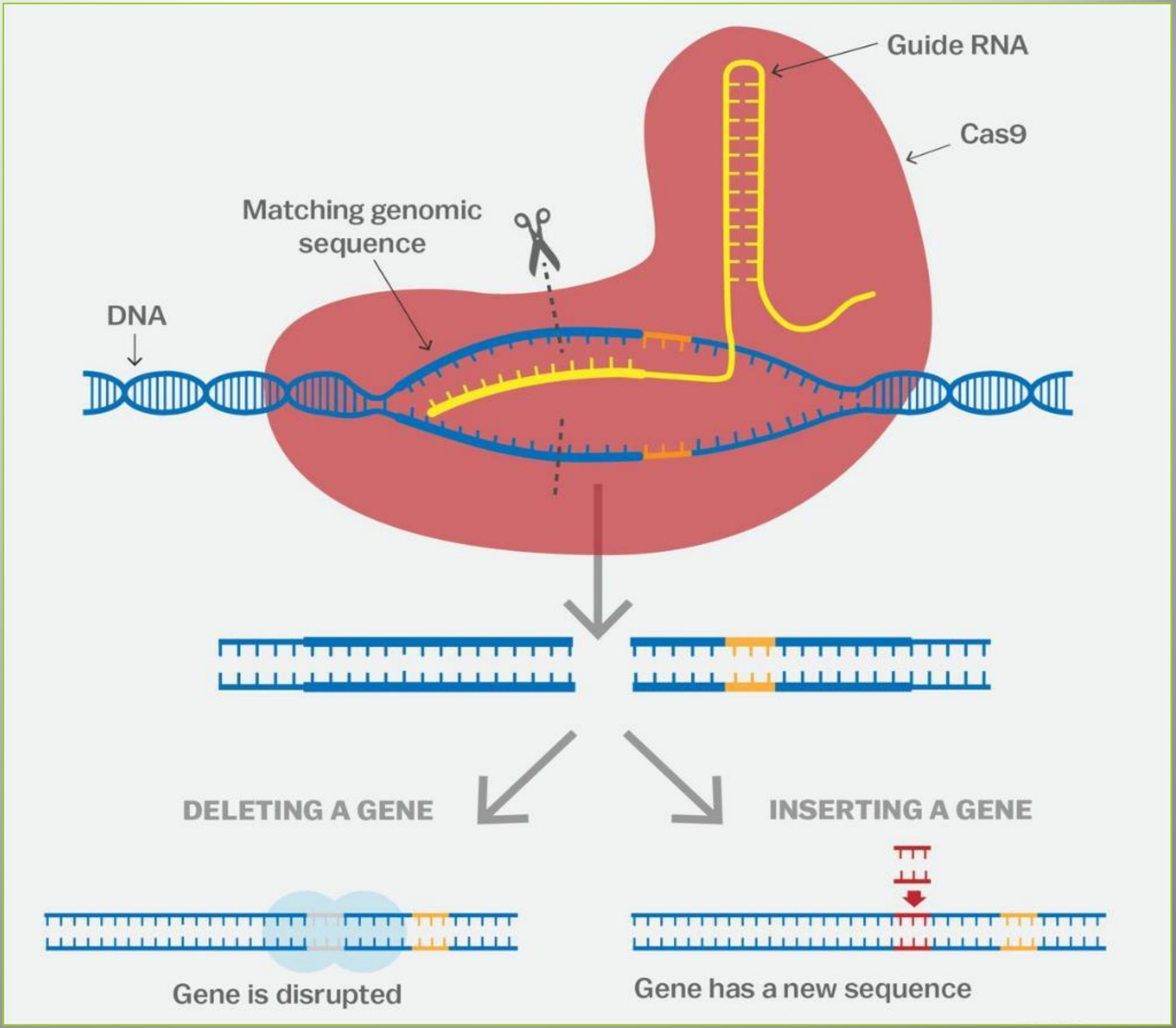
Two parts: Nuclease and guide RNAs to direct it in genome



# A big deal for plants and trees?

Ability to modify native genes efficiently makes growing science knowledge of gene trait-relationships actionable

**The formerly theoretical becomes practical**

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

**Editing plant genomes with CRISPR/Cas9**

Khaoula Belhaj[1], Angela Chaparro-Garcia[1], Sophien Kamoun, Nicola J Patron and Vladimir Nekrasov

CrossMark

“CRISPR/Cas9 is a game-changing technology that is poised to revolutionize basic research and plant breeding.”

**Even more powerful for trees? The long generation time, and inability to inbreed, make specific genome modifications by conventional breeding ~ impossible**

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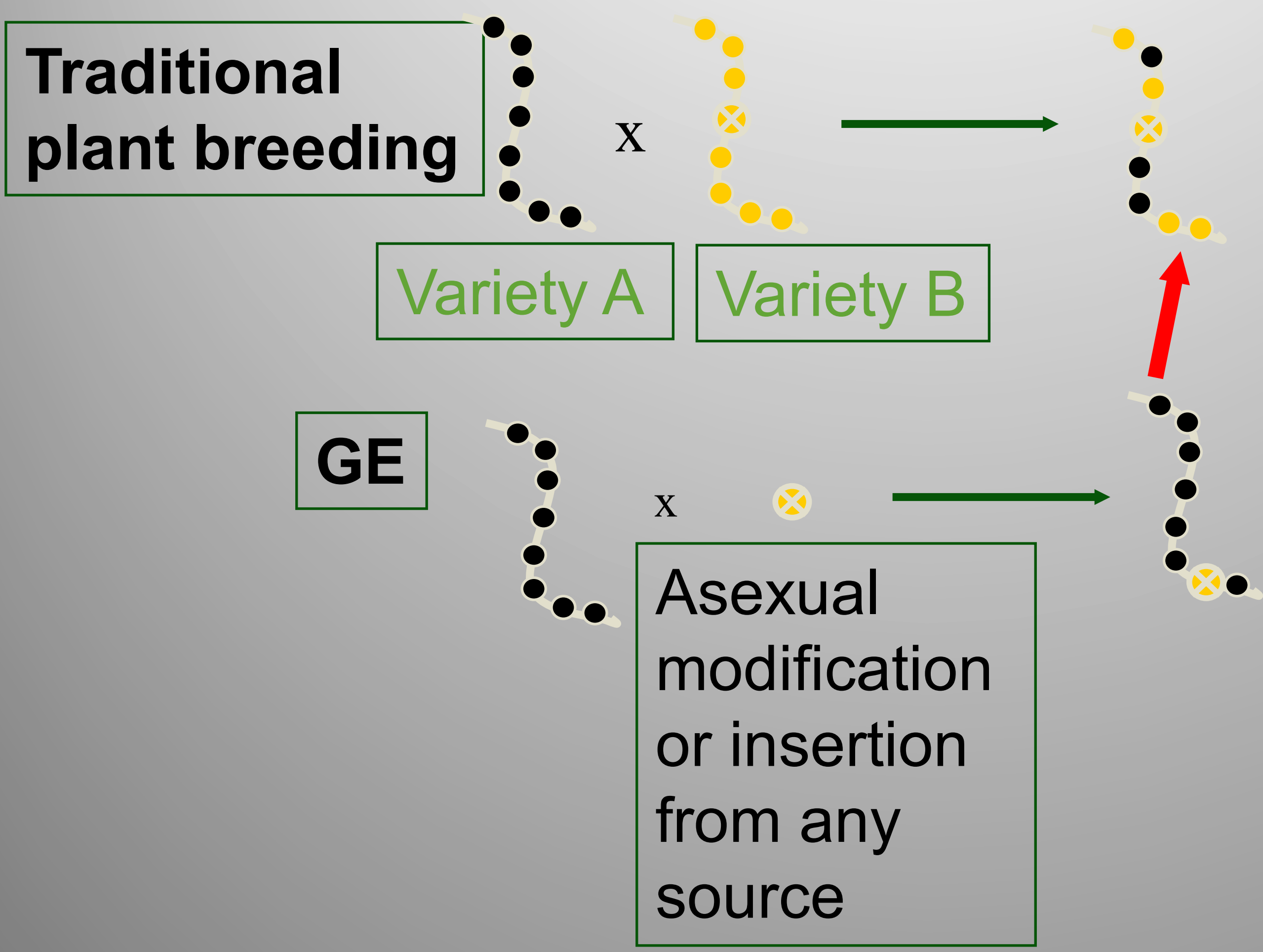
# But efficient integration with conventional breeding is critical




Integration with conventional breeding is very difficult

# Efficient integration nearly impossible with regulations that presume the method (each insertion) is a hazard until proven innocent

*Articles*

**Far-reaching Deleterious Impacts of Regulations on Research and Environmental Studies of Recombinant DNA-modified Perennial Biofuel Crops in the United States**

STEVEN H. STRAUSS, DREW L. KERSHEN, JOE H. BOUTON, THOMAS P. REDICK, HUIMIN TAN, AND ROGER A. SEDJO

*BioScience, October 2010*

- Rapid pace of breeding, many genotypes
- Increasingly complex traits like drought, salt, and pest resistances / wood structure & chemistry
- Complex and rapidly changing environments

# Also many complex ecological and social concerns for trees

- Wild/feral populations
- Record of invasiveness of many exotic trees/shrubs
- Long distance pollen and/or seed movement
- Limited domestication
- Keystone species / Larger role in providing ecosystem services
- Scientific uncertainty - Introgression experiments costly or impossible to do, models speculative
- Public view of forests as natural or wild: “contamination, impurity”

# “Green certification” of forests a reflection of these concerns, and creates severe barriers to field research, markets

**<u>A big deal</u>:**
**Many of the most highly managed forests and their products are certified**

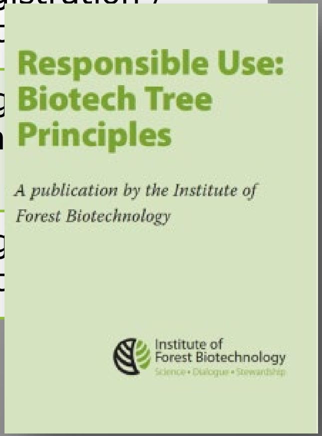
**500 million hectares, 13% global forest area**

FSC

Started by the Forest Stewardship Council, major principle: ***“genetically modified trees are prohibited”***

# All major forest certification systems now ban all GE trees – no research exemptions

| System | Region | GM Tree Approach / Reason |
|---|---|---|
| **PEFC :** Programme for Endorsement of Forest Certification | International | **Banned** / Precautionary approach based on lack of data |
| **FSC** : Forest Stewardship Council | International | **Banned** / Precautionary approach based on lack of data |
| **CerFlor :** Certificação Florestal | Brazil | **Banned** via PEFC registration / No additional rationale |
| **CertFor** : Certficación Forestal | Chile | **Banned** via PEFC registration / No additional rationale |
| **SFI** : Sustainable Forestry Initiative | North America | **Banned** via PEFC registration / Awaiting risk-benefit data |
| **ATFS** : American Tree Farm System | USA | **Banned** via PEFC registration / No additional rat |
| **CSA** : Canadian Standards Association | Canada | **Banned** via PEFC reg Allows public to determ |
| **CFCC** : China Forest Certification Council | China | **Banned** via PEFC reg No additional rat |




**Adam Costanza, Institute for Forest Biotechnology**

In 2001 and 2015, forest genetic and biotech scientists publicly criticized FSC for their complete ban – <u>no field research</u> on certified lands

…with little effect

## Plantation Certification & Genetic Engineering

## FSC's Ban on Research Is Counterproductive

Steven H. Strauss, Malcolm M. Campbell, Simon N. Pryor, Peter Coventry, and Jeff Burley

Genetic engineering, also called genetic modification (GM), is the isolation, recombinant mod-

Plantations can relieve pressure on nat-

Traces of the emerald ash borer on the trunk of a dead ash tree in Michigan, USA. This non-native invasive insect from Asia threatens to kill most North American ash trees.

BIOTECHNOLOGY

# Genetically engineered trees: Paralysis from good intentions

## Forest crises demand regulation and certification reform

By Steven H. Strauss[1*], Adam Costanza[2], Armand Séguin[3]

Intensive genetic modification is a long-standing practice in agriculture, and, for some species, in woody plant horticulture and forestry (1). Current regulatory systems for genetically engineered

recently initiated an update of the Coordinated Framework for the Regulation of Biotechnology (2), now is an opportune time to consider foundational changes.

Difficulties of conventional tree breeding make genetic engineering (GE) methods relatively more advantageous for forest trees than for annual crops (3). Obstacles

Although only a few forest tree species might be subject to GE in the foreseeable future, regulatory and market obstacles prevent most of these from even being subjects of translational laboratory research. There is also little commercial activity: Only two types of pest-resistant poplars are authorized for commercial use in small areas in China and two types of eucalypts, one approved in Brazil and another under lengthy review in the USA (5).

**METHOD-FOCUSED AND MISGUIDED.** Many high-level science reports state that the GE method is no more risky than conventional breeding, but regulations around the world essentially presume that GE is hazardous and requires strict containment

# A new strategy in 2019: A petition to certifiers to allow field research

## Petition in Support of Forest Biotechnology Research

Petition | Committee of Scientists | Examples of Biotech Trees | Background Literature | FAQ | Pubs-Press

Drone image of an rDNA-modified poplar plantation in the USA

***The goal of this petition is to urge forest certification systems to better align their certification criteria with scientific findings in biotechnology.***

http://biotechtrees.forestry.oregonstate.edu

# Impemented by the Alliance for Science at Cornell University, USA

# Endorsed by the largest scientific society of plant biologists in the world

American Society of Plant Biologists

**ASPB has studied and endorsed the petition.**

members to support a petition to change certification rules for forests to enable field research on biotech (gene edited, genetically engineered) trees. Amazingly, all of the private certification systems have a complete ban in place that extends to research, at a time when forest health is in growing crisis due to expanding pests and climate change. Biotech is not a panacea, but its also too powerful to ignore—and can sometimes provide powerful solutions where other approaches fail.The petition follows the release of a major report on The Potential for Biotechnology to Address Forest Health from the USA National Academy of Sciences that has identified biotechnologies as a key tool for helping to manage forest health and associated pest epidemics.

ASPB has studied and endorsed the petition.

# Alerts to tens of thousands of scientists sent by American Association for the Advancement of Science - AAAS (worlds largest general scientific society)

AAAS | Policy Alert

**Petition Launched to Change Certification of Biotechnology Forest Research**

A committee of forest biotechnologists from around the world, which includes several AAAS honorary fellows, have launched a petition to change certification rules for forests to enable field research on gene-edited and genetically engineered trees. Currently, private certification systems include a ban on research using biotechnology tools in forest research. The petition comes on the heels of a recent report from the National Academies that discusses the importance of biotechnology research to help improve forest health. For additional background, visit the petition website. (**BACK TO THE TOP**)

# 1,161 signatures, majority PhDs

## Support modern forest biotechnology research

May 30 2018 · Cornell Alliance for Science · Closed on Jun 11 2019


https://www.gopetition.com/petitions/petition-in-support-of-modern-forest-biotechnology.html

# Letter published in Science about it (September 2019)

## *Certification for gene-edited forests*

Forest certification bodies were established to provide consumers with confidence that they are purchasing
sourced wood products. Over
hectares of forests, or about
l forest area, are certified
rgest certification systems
ver, certification bodies have
excluded all genetically
or gene-edited (GE) trees from
, including from field research
lands that is essential for
ng local benefits and impacts
ing forest biotechnology
om around the world, with
of more than 1000 globally
atories to a recent detailed
call for all forest certification
romptly examine and modify
s.
ce mounting stresses posed
pests and climate change (*6*).



Engineering, and Medicine recently completed an in-depth study on forest health and biotechnology, concluding that the potential benefits are numerous and rapidly increasing (*12*). Our forests are in dire need of assistance, and GE trees hold tremendous potential as a safe and powerful tool for promoting forest resilience and sustainability.

**Steven H. Strauss[1]*, Wout Boerjan[2], Vincent Chiang[3], Adam Costanza[4], Heather Coleman[5], John M. Davis[6], Meng-Zhu Lu[7], Shawn D. Mansfield[8], Scott Merkle[9], Alexander Myburg[10], Ove Nilsson[11], Gilles Pilate[12], William Powell[13], Armand Seguin[14], Sofia Valenzuela[15]**

[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331, USA. [2]Department of Plant Biotechnology and Bioinformatics, Ghent University and Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium. [3]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA. [4]Chapel Hill, NC 27517, USA. [5]Department of Biology, Syracuse University, Syracuse, NY 13244, USA. [6]School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, USA. [7]State Key Laboratory of Subtropical Silviculture, School of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou 311300, China. [8]Forest Sciences Centre, University

standard-pefc-st-2002-2013.

Gene-edited and genetically engineered trees, such as these poplars, should be allowed in certified forests.

# News article also published in Science

AAAS Become a Member

Science Contents News Careers Journals

Productivity of eucalyptus plantations could be increased with trees genetically modified for faster growth.
CASADAPHOTO/SHUTTERSTOCK.COM

## Scientists say sustainable forestry organizations should lift ban on biotech trees

By Erik Stokstad | Aug. 23, 2019 , 5:45 PM

# Key petition arguments

- Forest health crises growing, need biotech tools to help
- Extensive research and field trials show promise and safety for many kinds of traits
- Gene editing of natural genes more precise than conventional breeding
- Local, site specific research as part of breeding programs are needed to understand value, economics
- The ban contradicts scientific opinion that the trait, not the method, is of significance
- Details here: http://biotechtrees.forestry.oregonstate.edu/

# GE trees: Reliable in the field

**Lessons from Two Decades of Field Trials with Genetically Modified Trees in the USA: Biology and Regulatory Compliance**

**Steven H. Strauss, Cathleen Ma, Kori Ault and Amy L. Klock**

**Abstract** We summarize the many field trials that we have conduc
beginning in 1995 and continuing to this day. Under USDA APHI
latory notifications and permits, we have planted nearly 20,000 tre
approximately 100 different constructs in more than two dozen fie
The large majority of the trials were in *Populus* and included hybri

# Many studies done -- show value and promise for diverse traits (2018 review)

In Vitro Cellular & Developmental Biology - Plant (2018) 54:341–376
https://doi.org/10.1007/s11627-018-9914-1

**INVITED REVIEW**

CrossMark

## Genetic engineering of trees: progress and new horizons

**Shujun Chang[1] · Elizabeth L. Mahon[2] · Heather A. MacKay[2] · William H. Rottmann[3] · Steven H. Strauss[4] · Paula M. Pijut[5] · William A. Powell[6] · Vernon Coffey[6] · Haiwei Lu[4] · Shawn D. Mansfield[2] · Todd J. Jones[1]**

Received: 5 February 2018 / Accepted: 20 June 2018 / Editor: Marie-Anne Lelu-Walter / Published online: 5 July 2018


**Abstract**
Genetic engineering of trees to improve productivity, wood quality, and resistance to biotic and abiotic stresses has been the primary goal of the forest biotechnology community for decades. We review the extensive progress in these areas and their current status with respect to commercial applications. Examples include novel methods for lignin modification, solutions for long-standing problems related to pathogen resistance, modifications to flowering onset and fertility, and drought and freeze tolerance. There have been numerous successful greenhouse and field demonstrations of genetically engineered trees, but commercial application has been severely limited by social and technical considerations. Key social factors are costly and uncertain regulatory hurdles and sweeping market barriers in the form of forest certification systems that disallow genetically modified trees. These factors limit and, in many cases, preclude field research and commercial adoption. Another challenge is the high cost and uncertainty in transformation efficiency that is needed to apply genetic engineering and gene editing methods to most species and genotypes of commercial importance. Recent advances in developmental gene-based transformation systems and gene editing, if combined with regulatory and certification system reform, could provide the foundation for genetic engineering to become a significant tool for coping with the increasing environmental and biological stresses on planted and wild forests.

# Forest health a major and growing concern – we can benefit from biotech tools

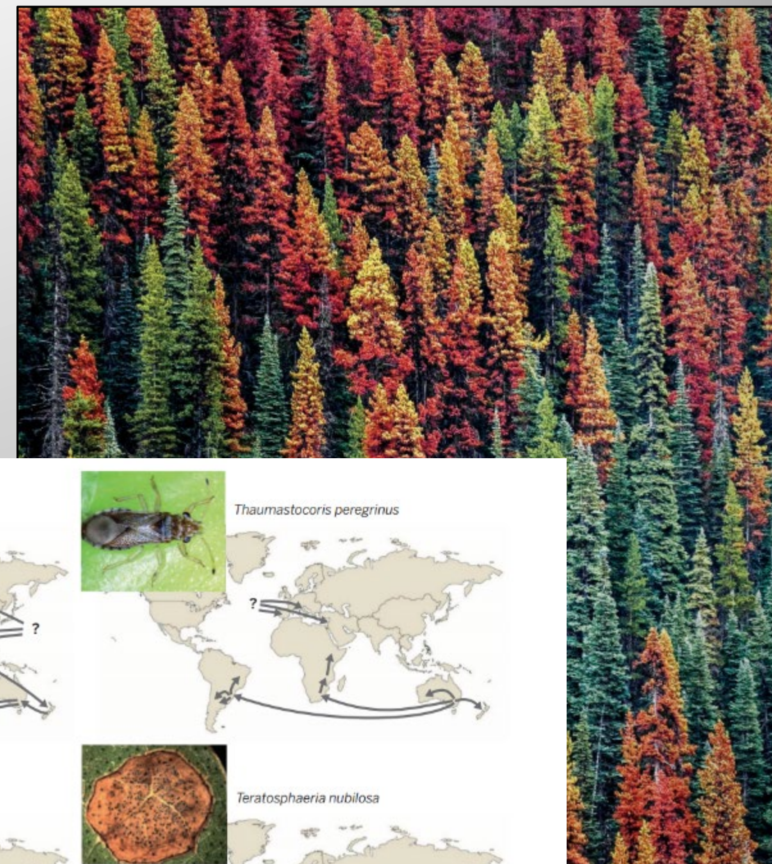
REVIEW

**Planted forest health: The need for a global strategy**

M. J. Wingfield,[1]* E. G. Brockerhoff,[2] B. D. Wingfield,[1] B. Slippers[1]

ed forests worldwide, and these represent valuable
reasingly threatened by insects and microbial
ntally and/or have adapted to new host trees.
ergence, despite a growing awareness of the
costs, and an increased focus on the importance of
ntial of planted forests, innovative solutions and a
needed. Mitigation strategies that are effective only in
s elsewhere in the world, ultimately leading to global
ns in the future should mainly focus on integrating
than single-country strategies. A global strategy to
nd urgently needed.

r 8, 2015

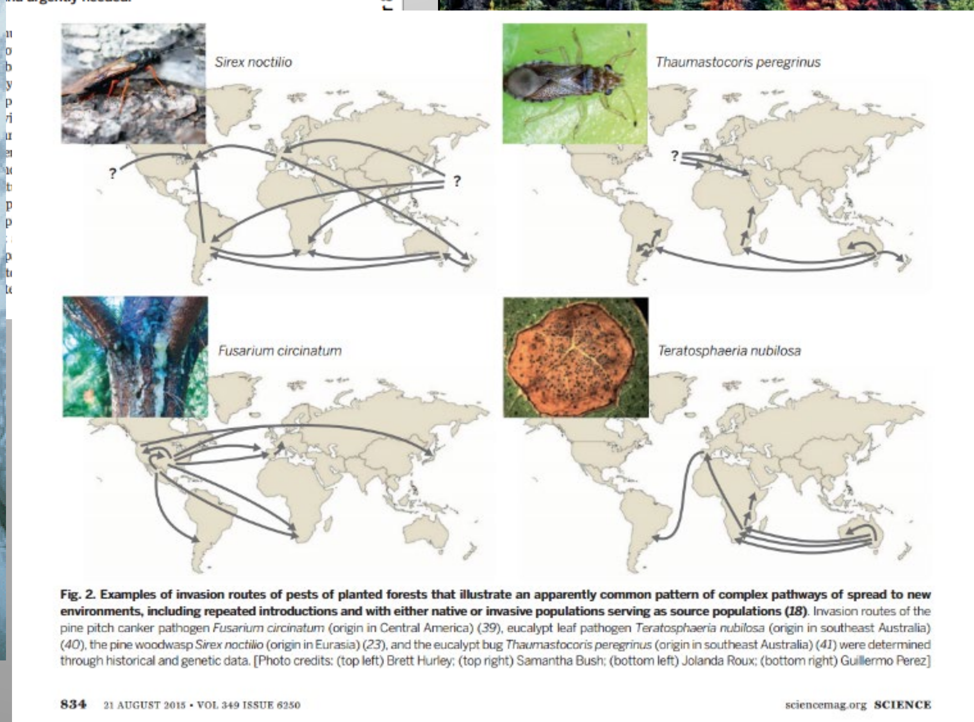



**Fig. 2. Examples of invasion routes of pests of planted forests that illustrate an apparently common pattern of complex pathways of spread to new environments, including repeated introductions and with either native or invasive populations serving as source populations (18).** Invasion routes of the pine pitch canker pathogen *Fusarium circinatum* (origin in Central America) (39), eucalypt leaf pathogen *Teratosphaeria nubilosa* (origin in southeast Australia) (40), the pine woodwasp *Sirex noctilio* (origin in Eurasia) (23), and the eucalypt bug *Thaumastocoris peregrinus* (origin in southeast Australia) (41) were determined through historical and genetic data. [Photo credits: (top left) Brett Hurley; (top right) Samantha Bush; (bottom left) Jolanda Roux; (bottom right) Guillermo Perez]

834 21 AUGUST 2015 • VOL 349 ISSUE 6250 sciencemag.org SCIENCE

# Wild trees also can benefit from GE/GMO: American Chestnut restoration – genetic engineering a key solution?

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SCIENTIFIC AMERICAN — New Century of the Brain — See Inside

**The American Chestnut's Genetic Rebirth**

A foreign fungus nearly wiped out North America's once vast chestnut forests. Genetic engineering can revive them

By William Powell

In 1876 Samuel B. Parsons received a shipment of chestnut seeds from Japan and decided to grow and sell the trees to orchards. Unbeknownst to him, his shipment likely harbored a stowaway that caused one of the greatest ecological disasters ever to befall eastern North America. The trees probably concealed spores of a pathogenic fungus, *Cryphonectria parasitica*, to which Asian chestnut trees—but not their American cousins—had evolved resistance. *C. parasitica* effectively strangles

More In This Article

**A New Generation of American Chestnut Trees May Redefine America's Forests**

March 2014 issue - Scientific American

Most effective gene is oxalate oxidase from wheat – which breaks down the fungal toxin oxalic acid

# What next?

- The petition one part of larger efforts by companies to gain access to biotech while under certification
- The stigma, poor reputation of GMO crops and foods to many in public a key barrier to a policy change
- Is this a good model for scientific advocacy for primacy of science in business and policy decisions ?
- What else or what next?
- What can China and Chinese scientists do?

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# Gene flow: A major reason for strict regulation and market barriers to GMOs

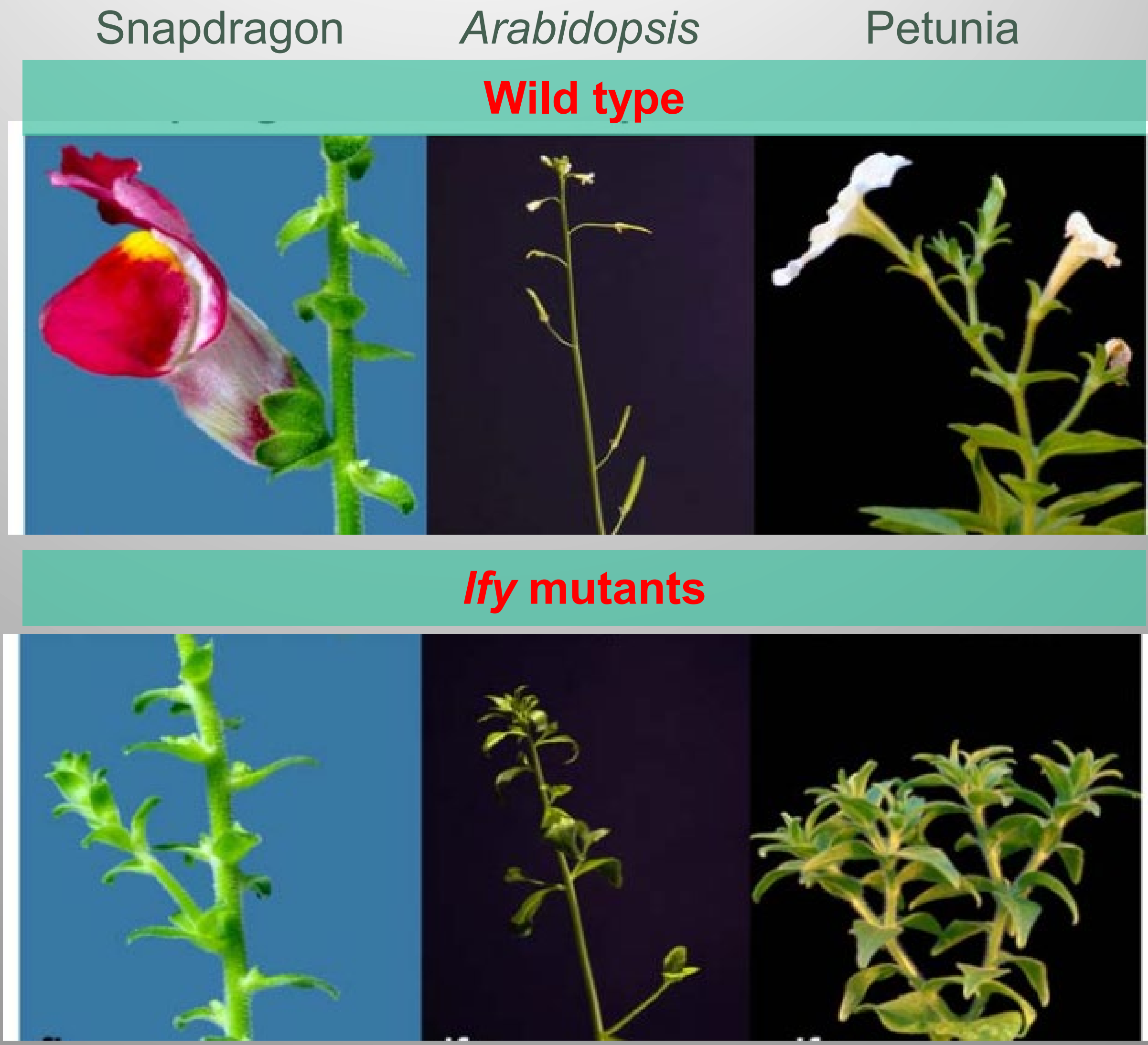
- Bigger for forest trees than most ag crops – for many reasons
  - Wild/feral populations
  - Record of invasiveness of many exotic trees/shrubs
  - Keystone roles in ecosystems
  - Long distance pollen and/or seed movement
  - Limited domestication
  - Larger role in providing ecosystem services
  - Public view of forests as natural or wild
  - Scientific uncertainty - Introgression experiments costly or impossible to do, models speculative
- Gene flow prevention an essential tool, especially for more novel and high impact GMOs? For highly sensitive countries?
- Gene flow a major concern with GMO eucalypts in the USA

# Need to curtail male and female reproduction in poplar? Potential for wide dispersal of pollen and seed

# Many containment options

- Non-GE:   Ploidy changes / irradiation / hybrids
- Cytotoxins / barnase driven by floral promoters
- Disruption of essential genes for flowering
  - Dominant interfering proteins
  - Suppressing expression
  - **Physical mutation**
- Various options for control: Male vs. female, induction & restorer
- Our focus has been on <u>bisexual</u> and <u>permanent</u> sterility for vegetatively propagated species
  - CRISPR mutation likely to be most reliable, predictable, and efficient

# *LEAFY* gene target for bisexual sterility: Strong mutants appear to have no flowers




Parcy et al. 2002; Moyroud et al. 2010

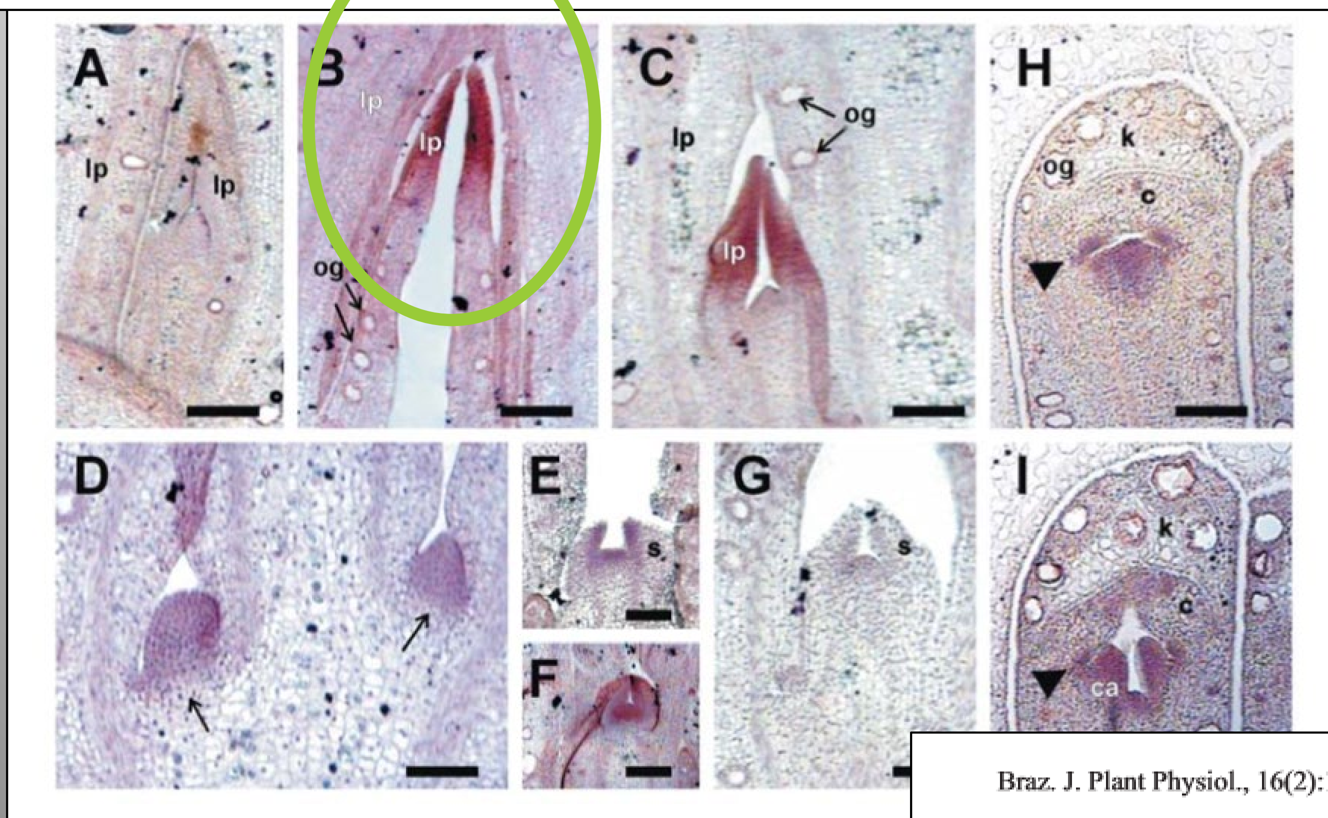
# The full roles of *LFY* unclear

- Critical that *LFY* mutation does not depress tree productivity, though might increase it
  - Studies in model plants did not conduct significant analyses of vegetative/productivity effects
  - An absence of gene knock-out studies in the field
- No studies in the very divergent floral types of important forest tree taxa
  - *LFY* might have evolved different functions in species like poplar and eucalypts
- Found to have vegetative as well as floral expression in poplars and eucalypts, thus worrisome
  - Meristematic vegetative cell expression

# Eucalyptus *LFY* vegetative expression

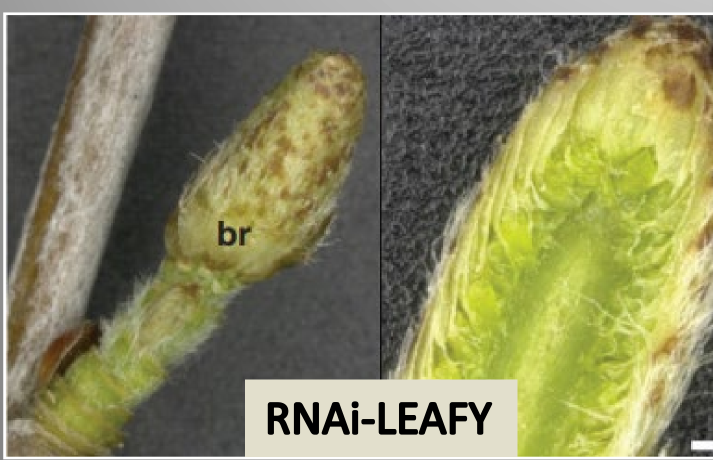
**_EgLFY_, the _Eucalyptus grandis_ homolog of the _Arabidopsis_ gene _LEAFY_ is expressed in reproductive and vegetative tissues**

**Marcelo Carnier Dornelas[1*], Weber A. Neves do Amaral[2] and Adriana Pinheiro Martinelli Rodriguez[1]**




Braz. J. Plant Physiol., 16(2):105-114, 2004

# Why not RNAi? Poplar sterility using RNAi against *LEAFY* works in the field, is stable, but only two of 15 events sterile in *P. alba* 6K10


## 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

…ity has been …ucalyptus …s by …gene in …n reduce …nd vegetative …xpression … sterility …ispersal, … exotic trees, …tion[9]. We report the use of RNA interference (RNAi) to suppress expression of the single-copy *LEAFY* (*LFY*) gene to produce sterility in poplar.

*Amy L Klocko[1], Amy M Brunner[1,3], Jian Huang[2], Richard Meilan[1,3], Haiwei Lu[1], Cathleen Ma[1], Alice Morel[1], Dazhong Zhao[2], Kori Ault[1], Michael Dow[1], Glenn Howe[1], Olga Shevchenko[1,3] & Steven H Strauss[1]*

*[1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon, USA. [2]Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA. [3]Present addresses: Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, Virginia, USA (A.M.B.), Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana, USA (R.M.), and Delaware Biotechnology Institute, Newark, Delaware, USA (O.S.).*
*e-mail: steve.strauss@oregonstate.edu*

…4 NUMBER 9 SEPTEMBER 2016 NATURE BIOTECHNOLOGY

# Two other LFY-RNAi poplar clones tested

No sterile trees obtained among ~30 insertion events in a 4 ha field trial in Oregon, USA

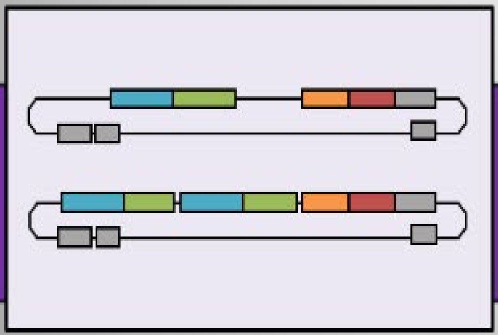
**Clone 717 female**

**Clone 353 male**

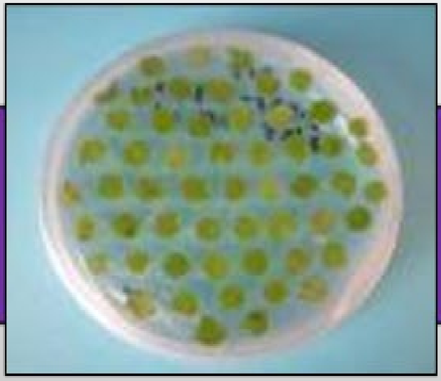
# Eucalypt RNAi-LFY also tested in a field trial in Israel: No floral modified trees seen in ~30 events


Thus need a much more efficient, and complete, gene knock-out method – **CRISPR to the rescue**?

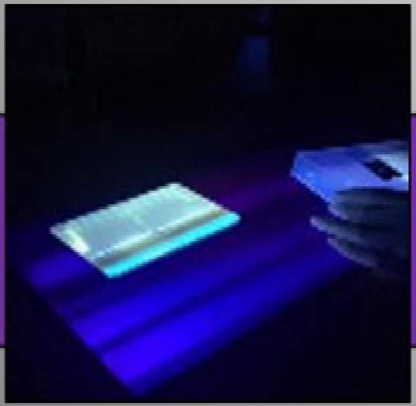
# CRISPR pipeline

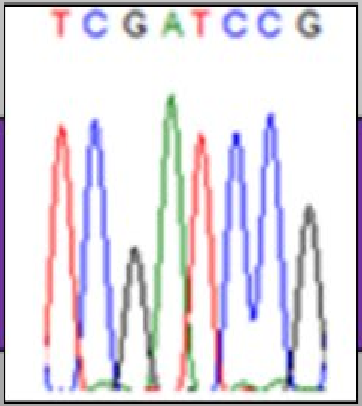


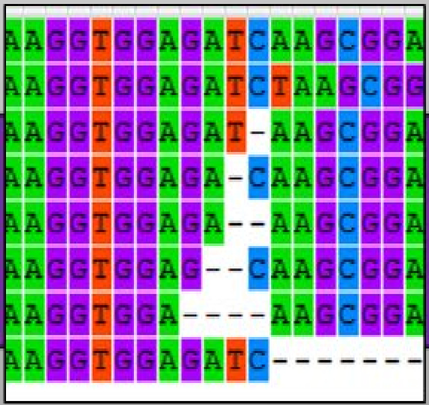
Construct



Transformation and regeneration



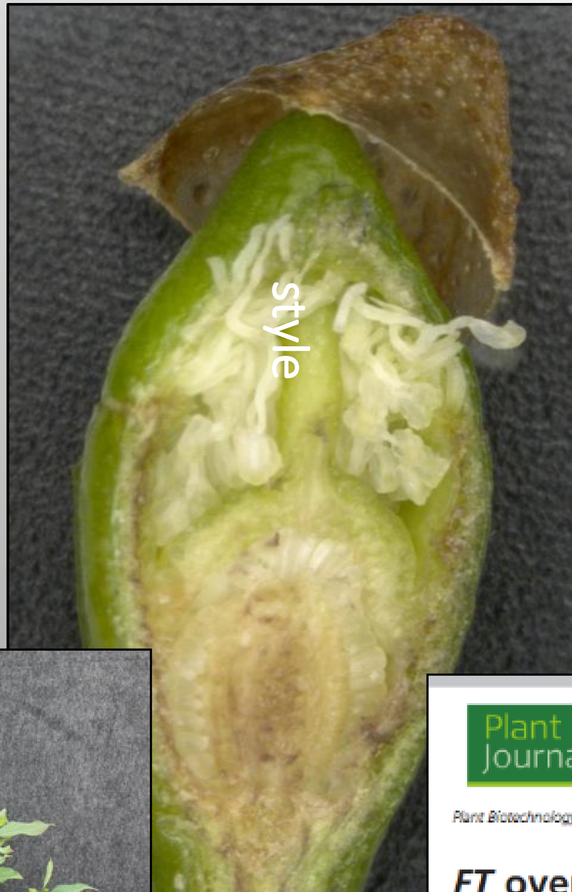


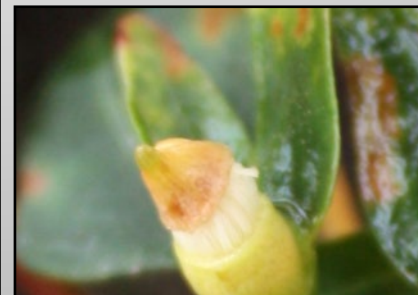



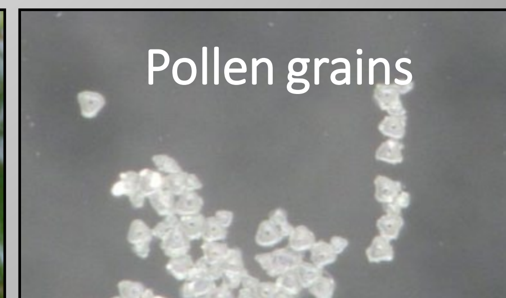

PCR and gel analysis (allele specific)

Sequencing of targets, alignment, and phenotyping

# Early flowering *FT*-eucalypts to speed floral phenotyping






Plant Biotechnology Journal

aab SEB Society for Experimental Biology

Plant Biotechnology Journal (2016) 14, pp. 808–819 doi: 10.1111/pbi.12431

## *FT* overexpression induces precocious flowering and normal reproductive development in *Eucalyptus*

Amy L. Klocko[1], Cathleen Ma[1], Sarah Robertson[1], Elahe Esfandiari[1], Ove Nilsson[2] and Steven H. Strauss[1,*]

[1]Department Forest Ecosystems & Society, Oregon State University, Corvallis, OR, USA
[2]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden

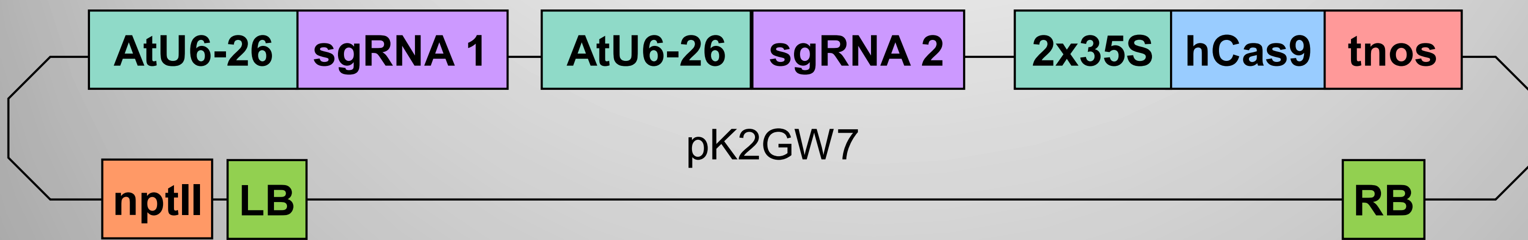
Received 8 April 2015;
revised 29 May 2015;
accepted 10 June 2015.

**Summary**

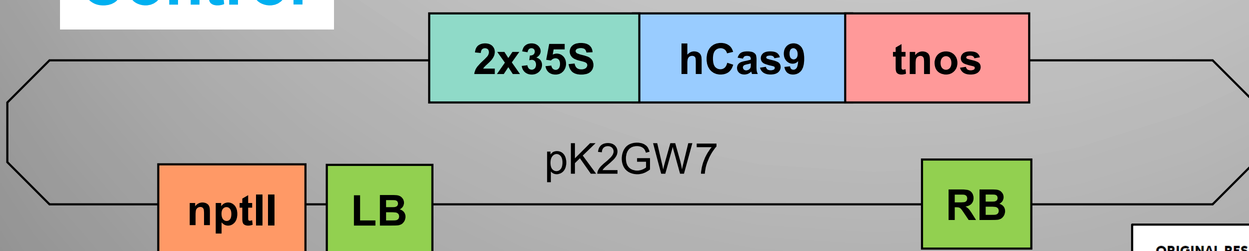
*Eucalyptus* trees are among the most important species for industrial forestry worldwide. However, as with most forest trees, flowering does not begin for one to several years after

# Constructs employed: Two sgRNA targets in 5’ protein coding region of the *LEAFY* gene gave a high rate of biallelic mutation and large deletions

## CRISPR




## Control




ORIGINAL RESEARCH ARTICLE
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**Variation in Mutation Spectra Among CRISPR/Cas9 Mutagenized Poplars**

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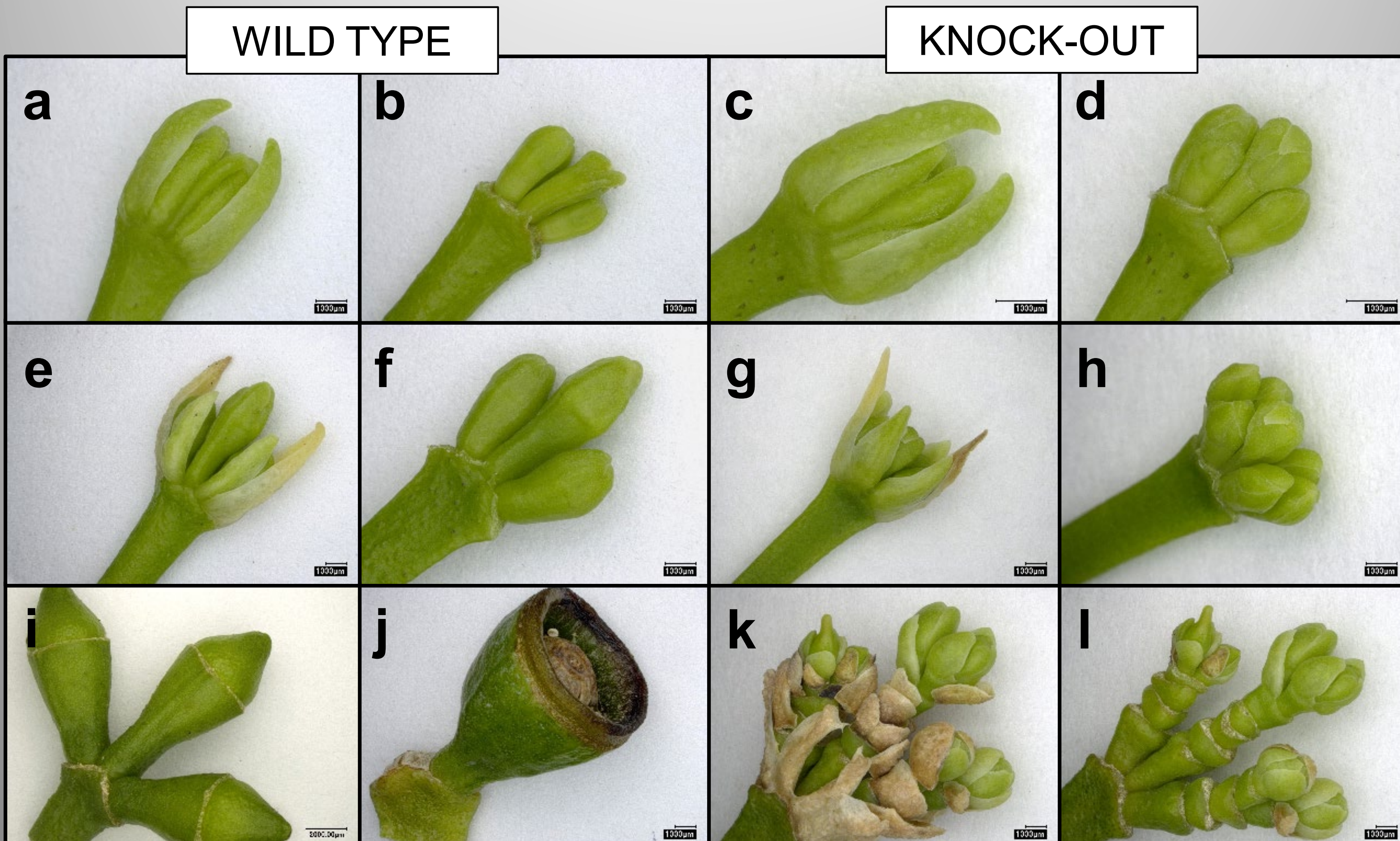
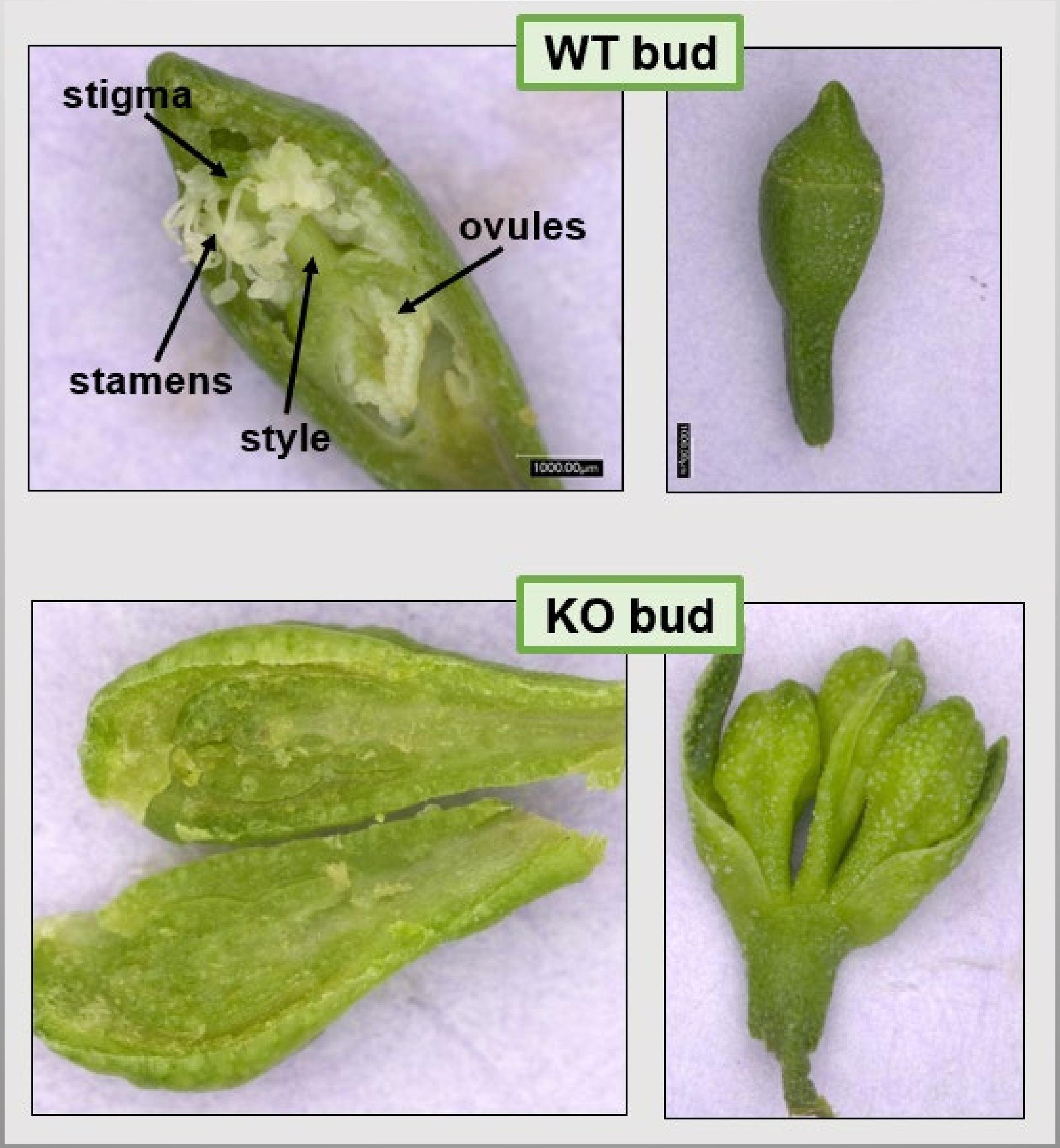
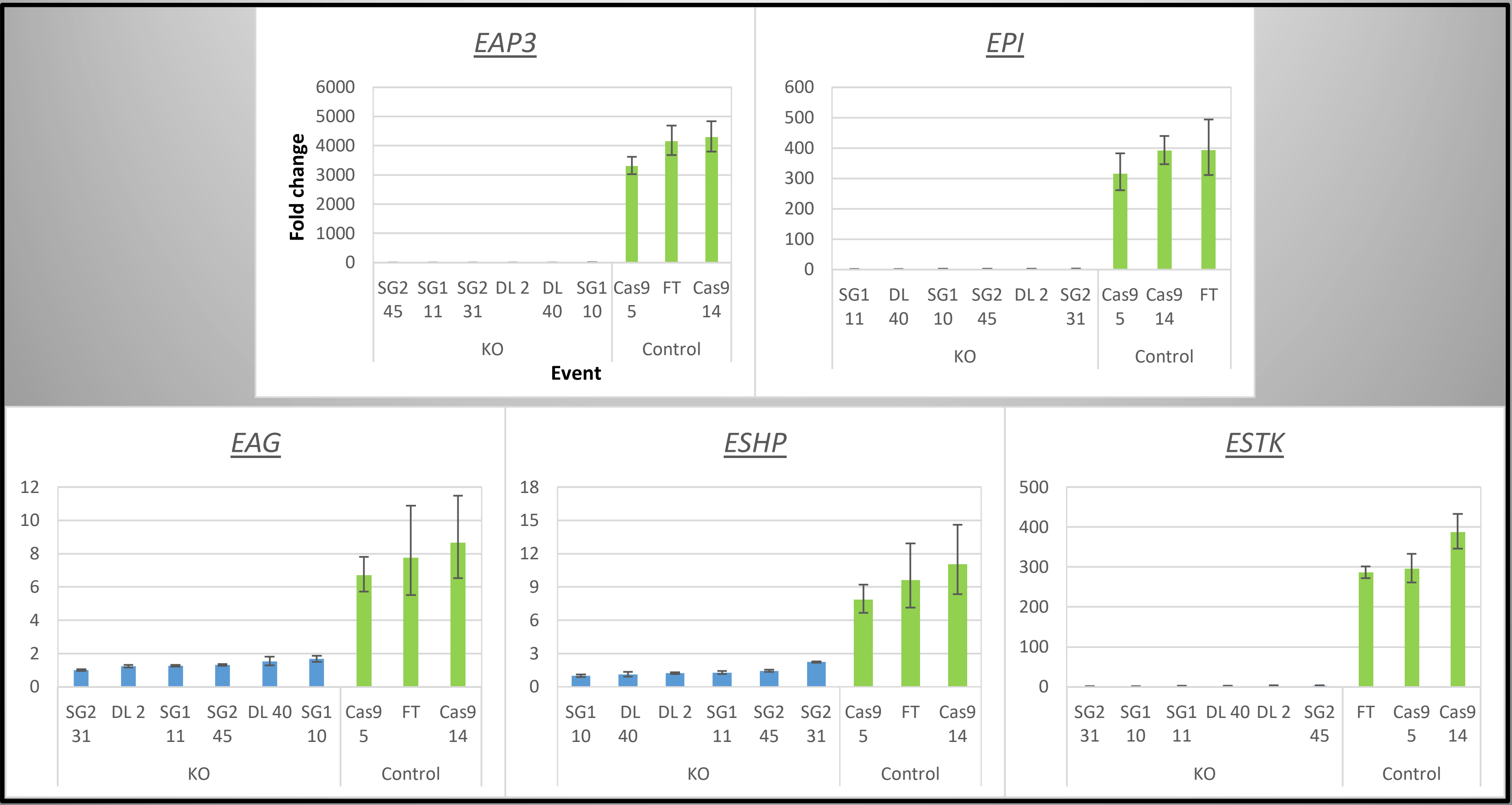
# Distinct trajectories for wild type vs. CRISPR knock-outs in developing floral shoots



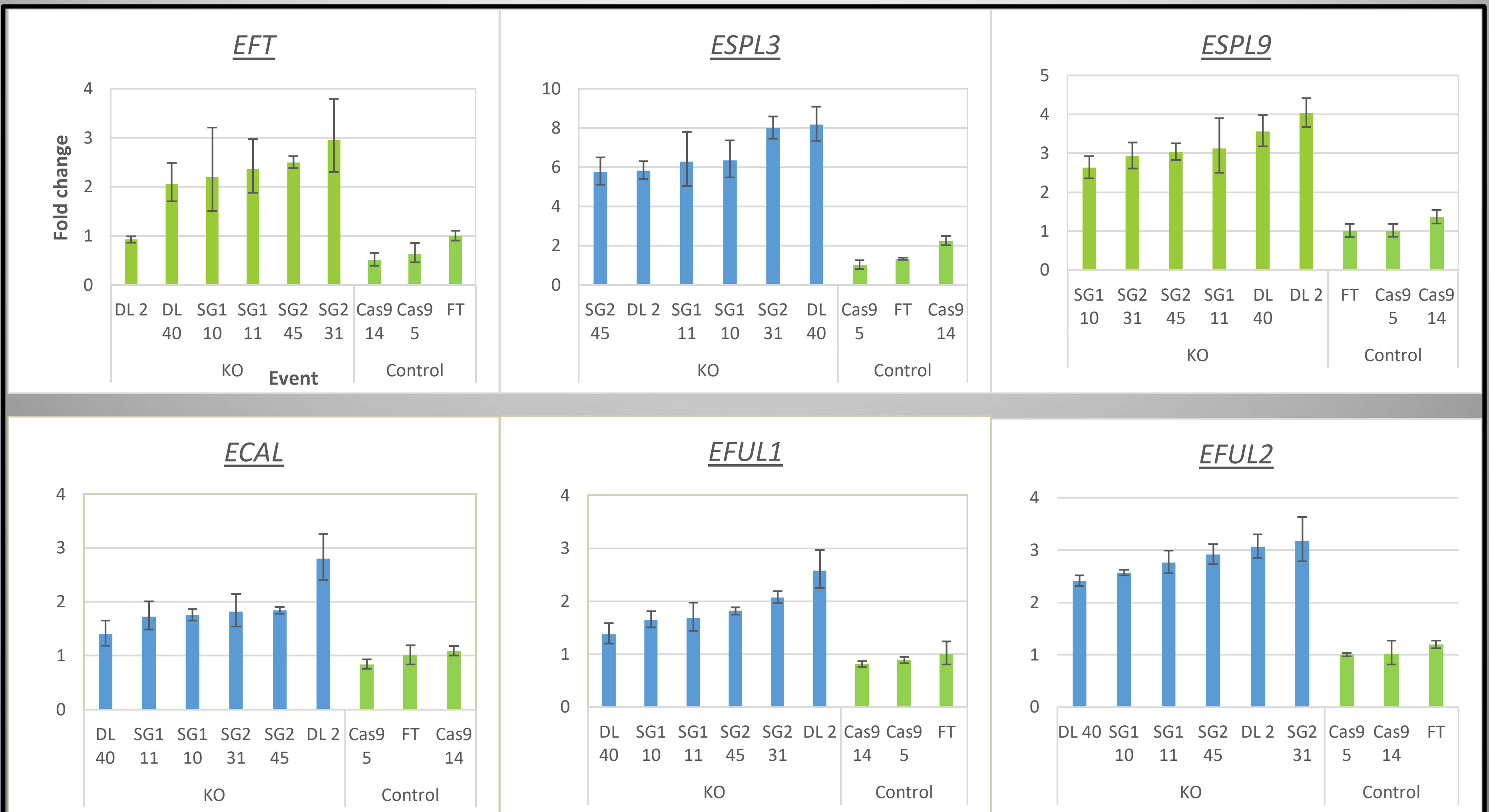
# Knockout buds appear devoid of floral organs



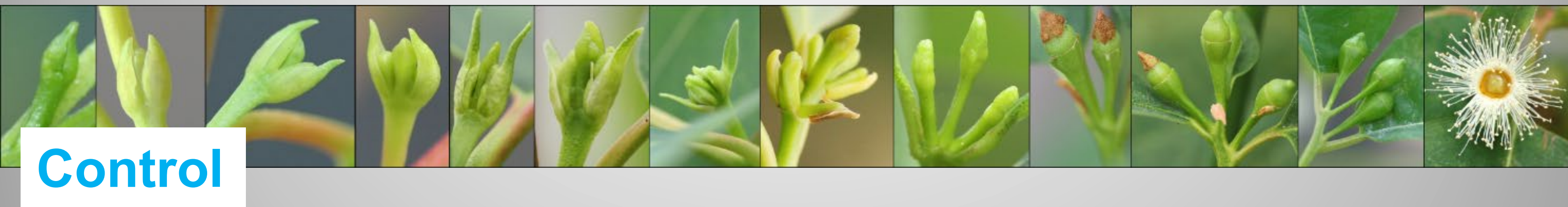
# Knock-out buds nearly devoid of floral meristem gene expression based on qPCR

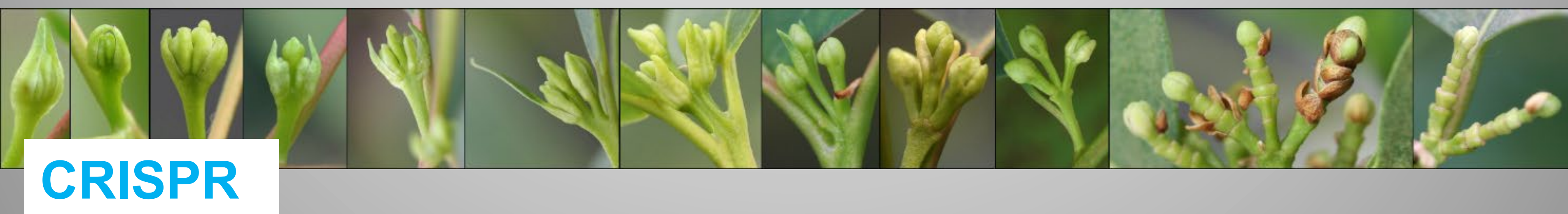


# In contrast, knock-outs bud with enhanced pre-floral, inductive gene expression



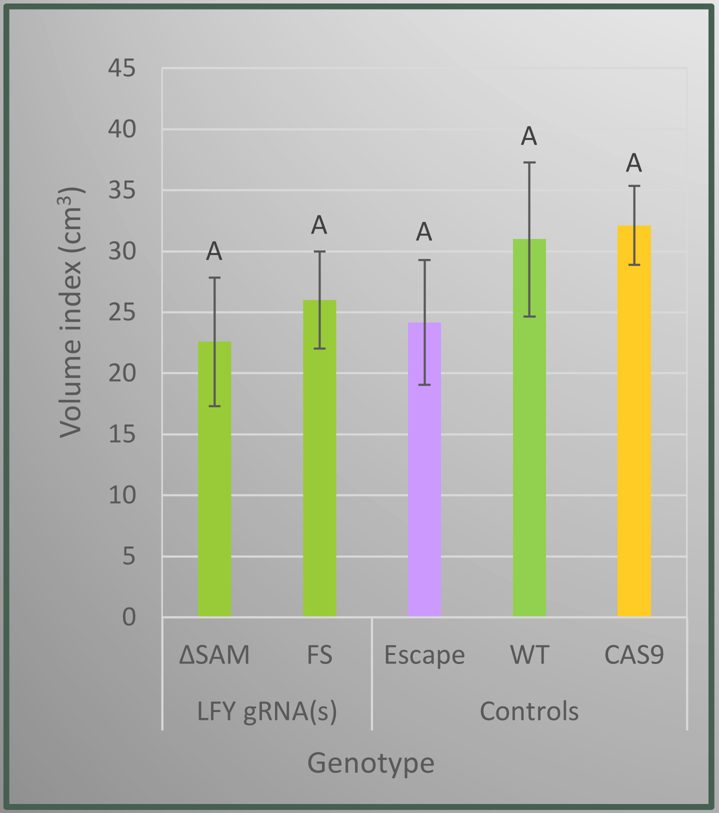
# Summary view of floral shoot development in knockouts vs. wild-type



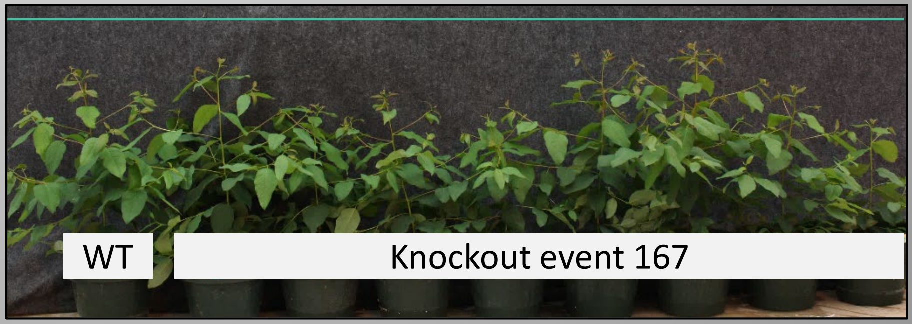





# Vegetative growth and morphology in greenhouse unaffected by knock-out







# Summary – *LFY* CRISPR in Eucalyptus

- Nearly 100% biallelic knockout rate
- Flower buds devoid of reproductive structures and lack nearly all floral organ transcription factor expression
  - Floral inductive genes hyperexpressed
- Partially indeterminate inflorescences
- No detectable vegetative effects on leaf morphology or biomass productivity in the greenhouse
- All CRISPR trees were transgenic: Work underway to develop CRISPR excision and other methods for “clean” knock-outs

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Cathleen Ma,
Transformation technician

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