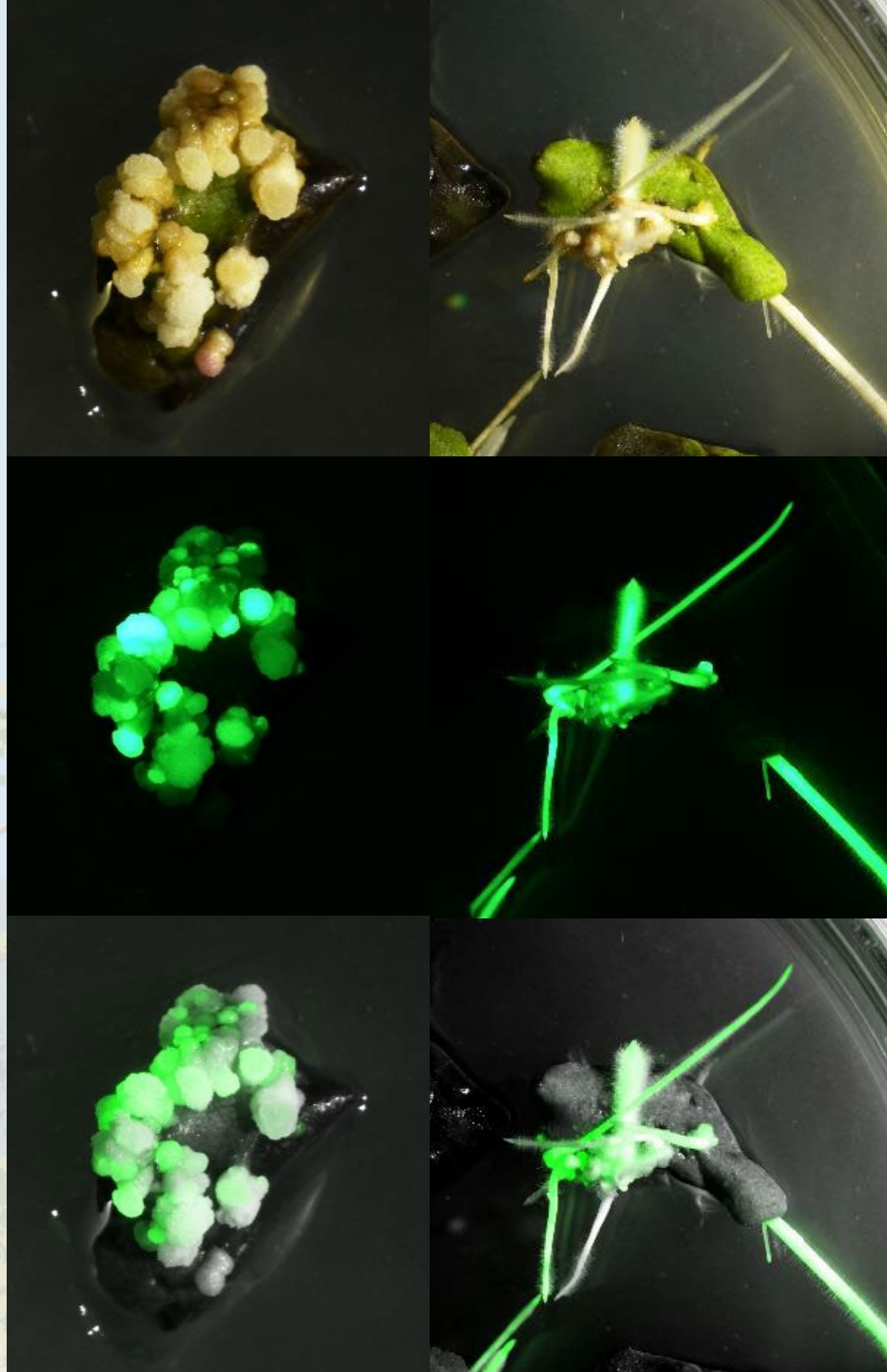
AGROSCAN

Screening & domesticating wild *Agrobacterium* strains to promote transgene delivery and regeneration in woody plants



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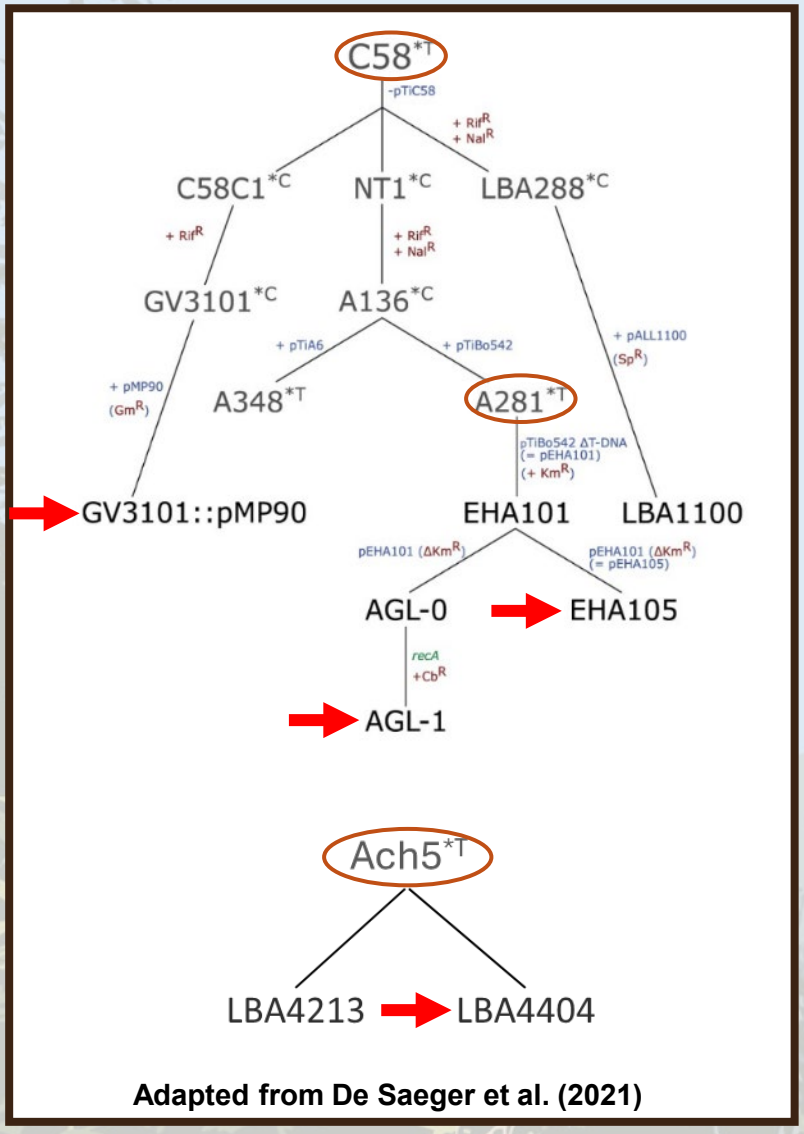
Woody plant research & development is enabled by genetic transformation

- Lengthy timeline for tree breeding operations
- Genetic modification allows for precise development
  - Introduction of new traits not found in breeding population
  - Nuclease technologies for precise gene editing
- **Low transformation efficiency and regeneration common in many woody species**

Transgenic purple poplar

Limitations in transformation tools may be contributing to transformation bottlenecks



Adapted from De Saeger et al. (2021)

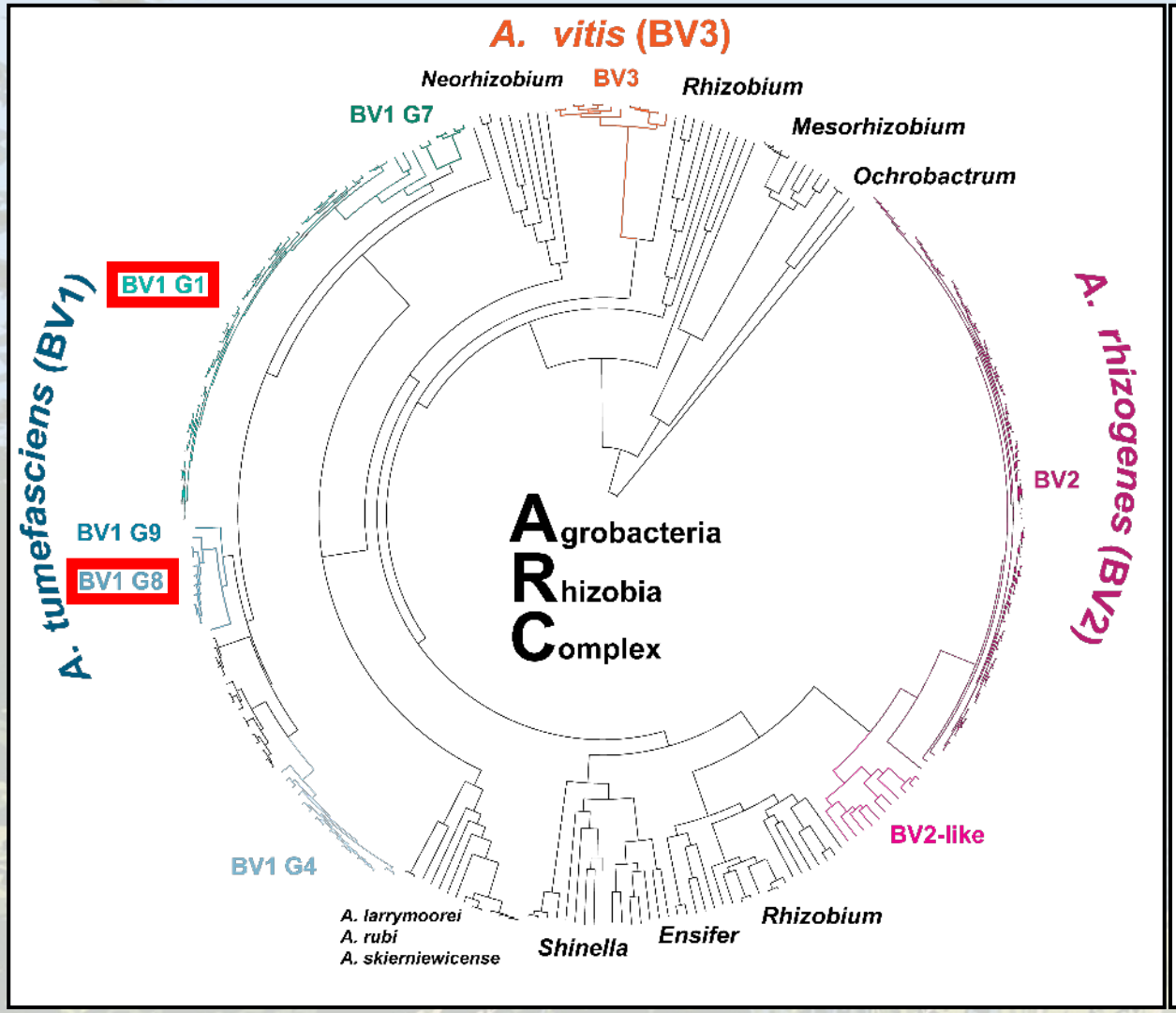
- Low diversity in the most common lab strains
- Extensive diversity of plasmid types and chromosomal backgrounds in wild strain collections around the world

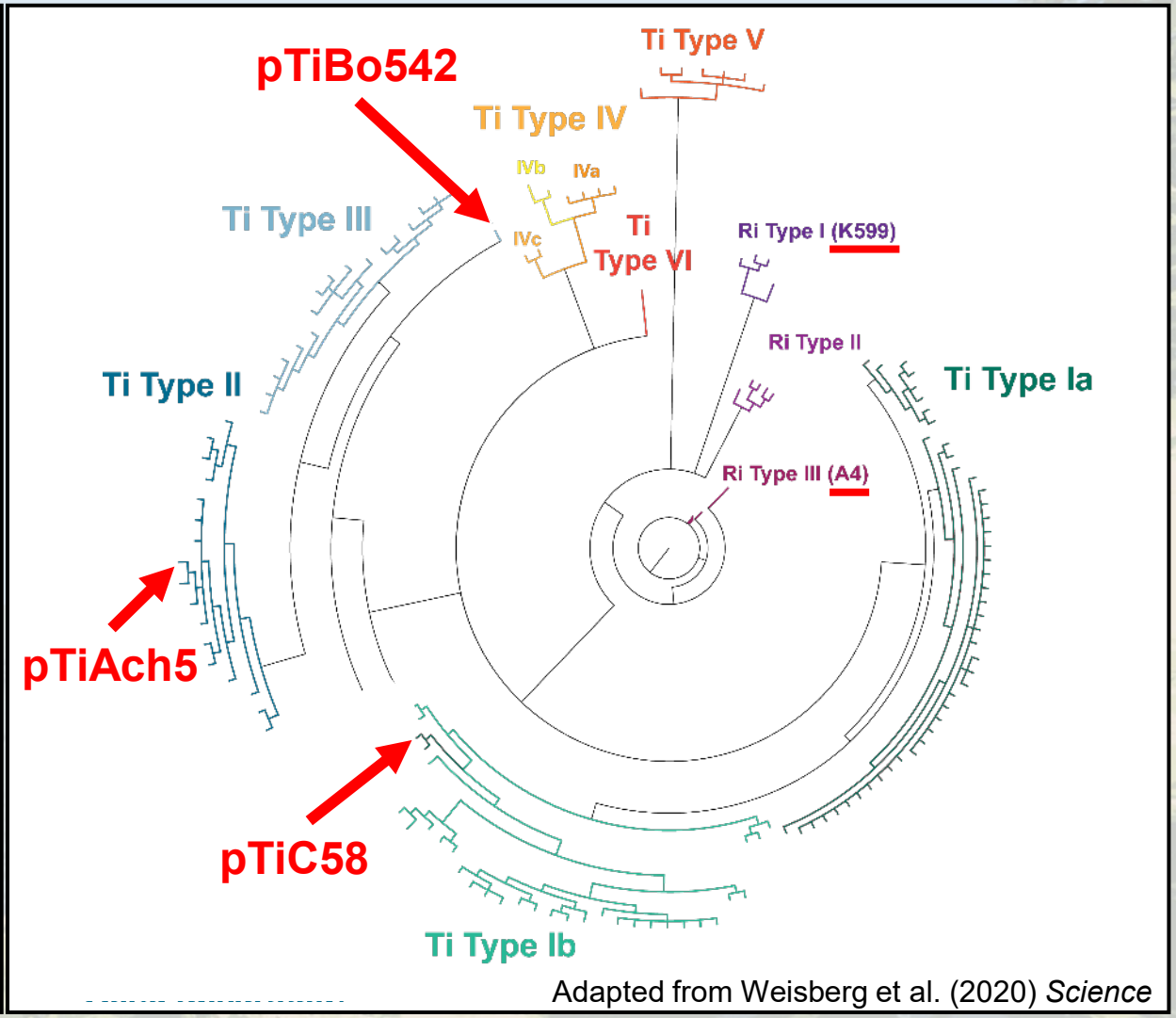
- **Can we identify wild strains with improved transformation and regeneration responses?**

Strain collection at OSU contains extensive chromosomal and plasmid type diversity


Alexandra Weisberg


Jeff Chang





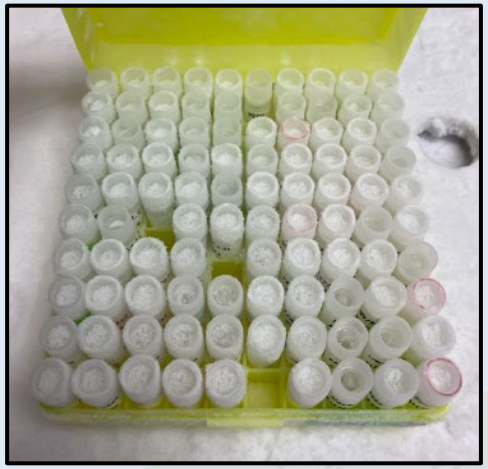
Adapted from Weisberg et al. (2020) *Science*

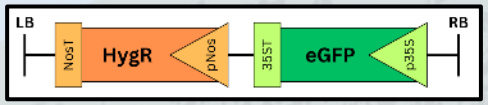
A high throughput phenomics system was used to screen transformation and regeneration responses

Selected ~100 diverse *Agrobacterium* strains

Selection criteria to maximize plasmid & chromosomal diversity

Inserted binary vector



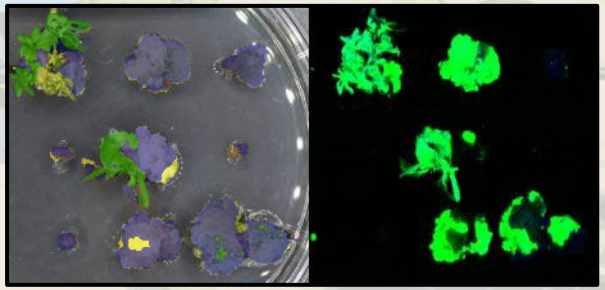


Kanamycin selection for binary vector in *Agrobacterium*

Transformed plants

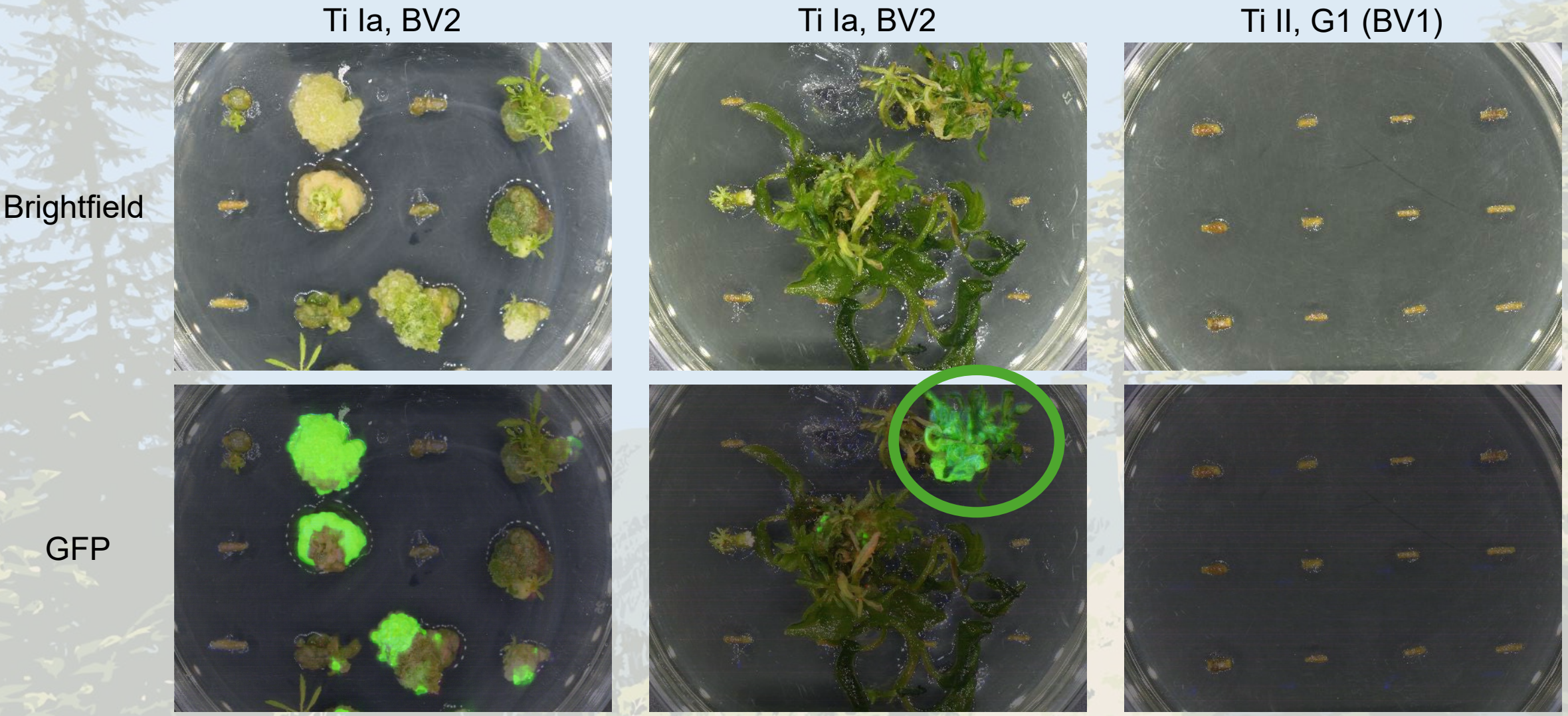
- Hybrid poplar (easy)
- Black cottonwood (moderate)
- Eucalyptus (hard)

Imaged phenotypes

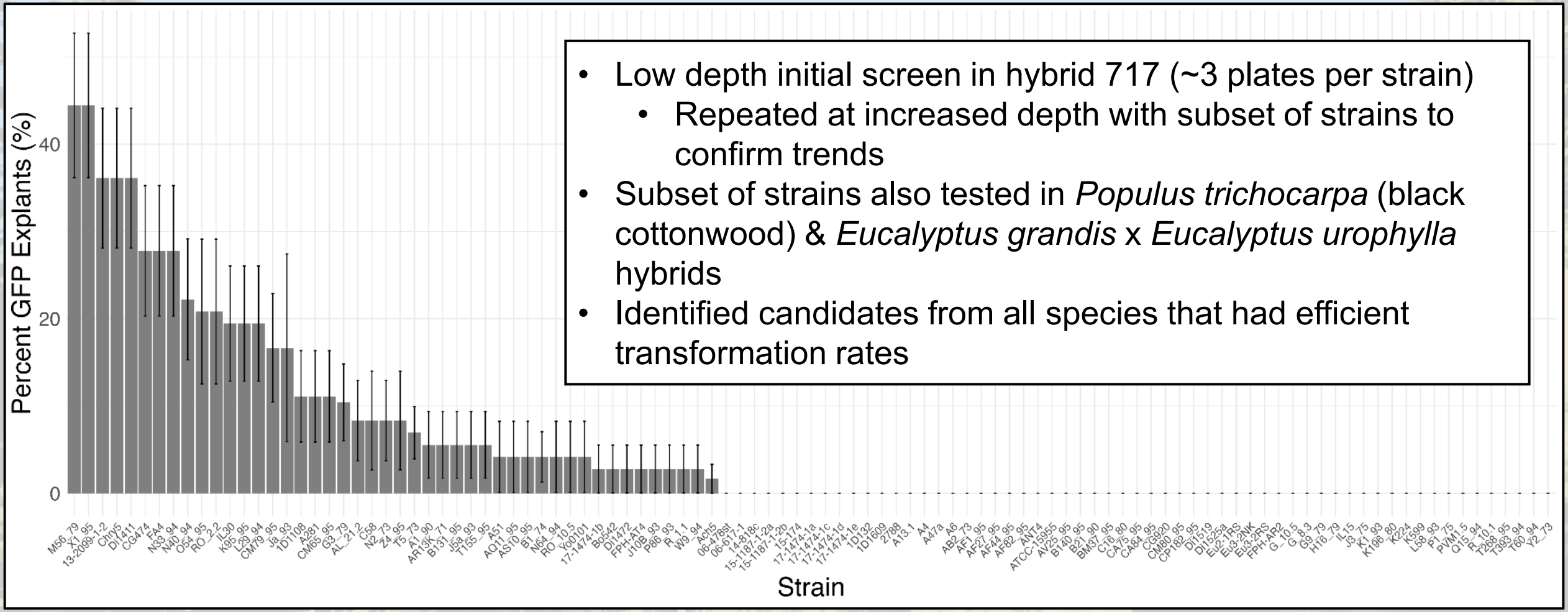


- Transformation efficiency
- Regeneration

Wild Agrobacterium strains showed high variation in transformation & regeneration responses in hybrid 717 (P. tremula x P. alba)



Less than half of 100 wild strains tested in hybrid 717 gave any transgenic signal

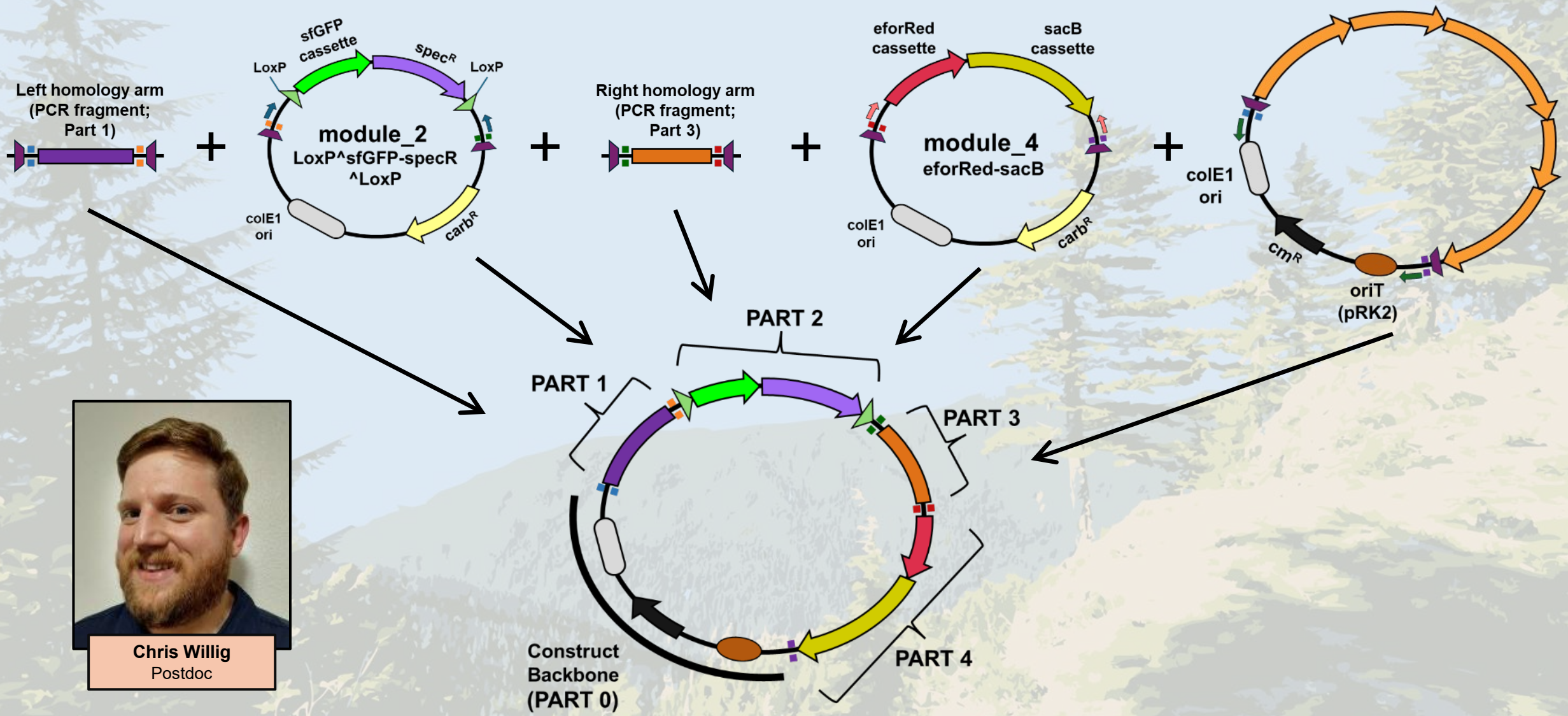


- Low depth initial screen in hybrid 717 (~3 plates per strain)
  - Repeated at increased depth with subset of strains to confirm trends
- Subset of strains also tested in *Populus trichocarpa* (black cottonwood) & *Eucalyptus grandis* x *Eucalyptus urophylla* hybrids
- Identified candidates from all species that had efficient transformation rates

Screening *Agrobacterium* strains identified candidates with efficient transformation in different woody species

How do we turn these wild strains into plant biotechnology tools?

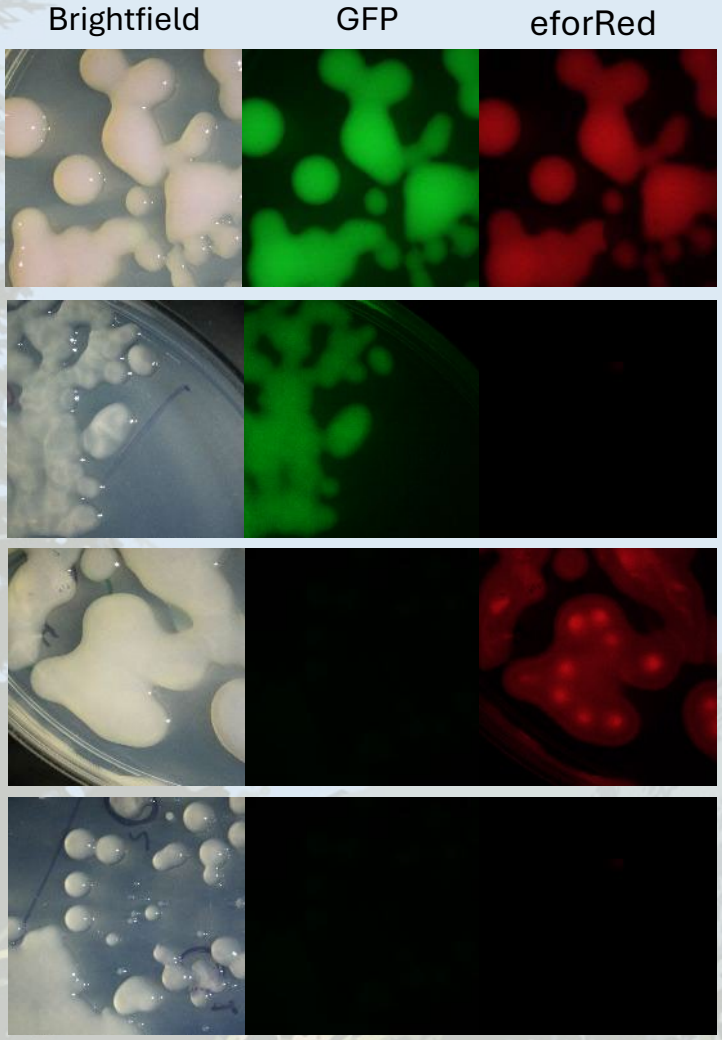
A cloning toolkit for *Agrobacterium* engineering enables robust, precise genomic modifications



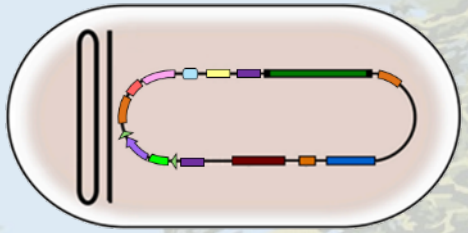
Chris Willig
Postdoc

Visual reporters assist with tracking molecular configuration of colonies

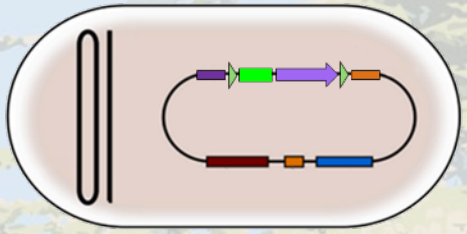
Chris Willig
Postdoc



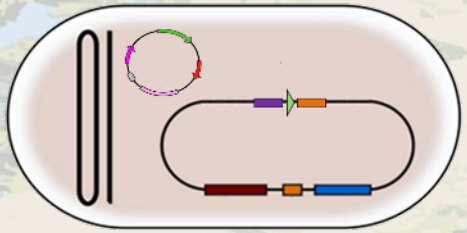
GFP and eforRed indicates a single-crossover event



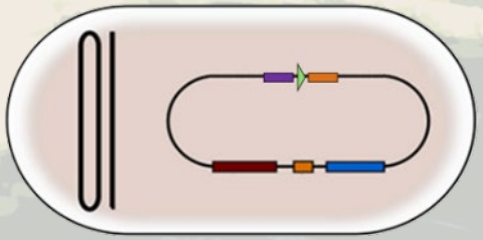
GFP only indicates a double-crossover event.



eforRed only indicates the presence of the Cre-recombinase containing construct.



Expression of neither indicates removal of the Cre-recombinase containing construct

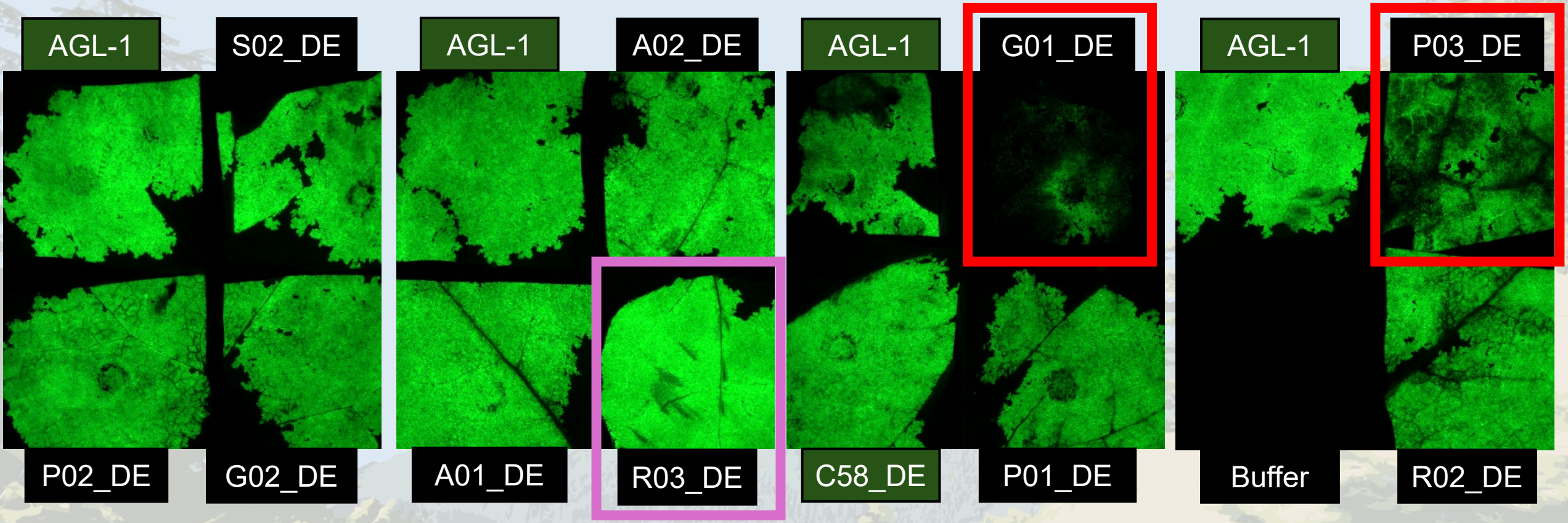


This cloning toolkit has enabled the disarmament of 16 new Agrobacterium strains to date

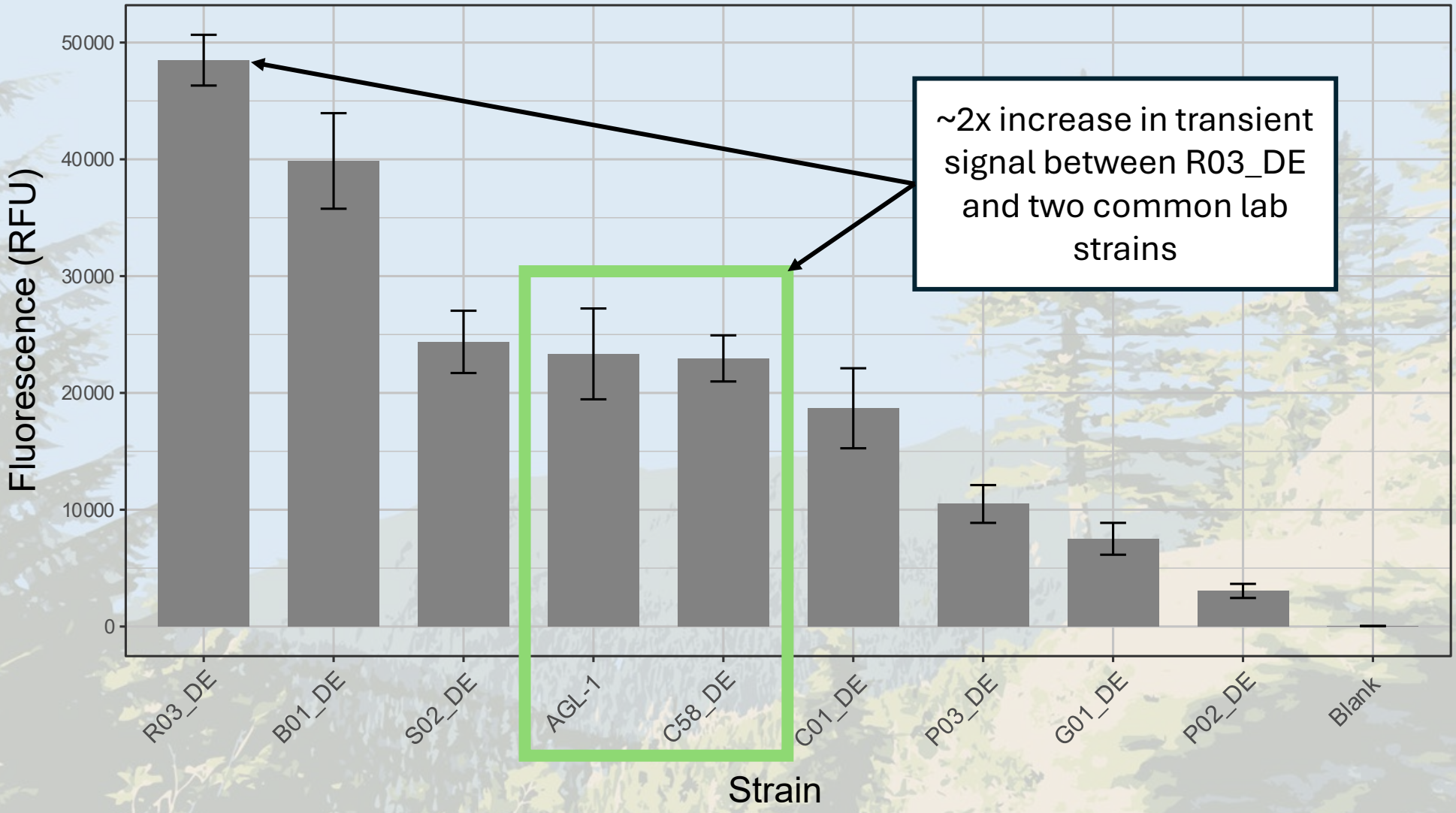
Strain designation	Host plant source	Virulence plasmid type	Chromosomal background / taxonomy
STR_H01_DE	Hop (*Humulus lupulus*)	Ti Ia	*Agrobacterium fabrum* (biovar 1)
STR_L01_DE	Pear (*Pyrus betulifolia*)	Ti Ia	*Rhizobium rhizogenes* (biovar 2)
STR_R01_DE	Rose (*Rosa* sp.)	Ti Ia	*Rhizobium rhizogenes* (biovar 2)
STR_P01_DE	Peach (*Prunus persica*)	Ti Ia	*Rhizobium rhizogenes* (biovar 2)
STR_P02_DE	Apricot (*Prunus siberica*)	Ti Ia	*Rhizobium rhizogenes* (biovar 2)
STR_P03_DE	Cherry (*Prunus avium*)	Ti Ia	*Agrobacterium radiobacter* (biovar 1)
STR_A01_DE	Quaking aspen (*Populus tremuloides*)	Ti Ib	*Agrobacterium tumefaciens* (biovar 1)
STR_A02_DE	Cottonwood (*Populus* sp.)	Ti Ib	*Agrobacterium tumefaciens* (biovar 1)
STR_G02_DE	Grape (*Vitis vinifera*)	Ti Ib	*Rhizobium rhizogenes* (biovar 2)
STR_R02_DE	Rose (*Rosa* sp.)	Ti Ib	*Agrobacterium tumefaciens* (biovar 1)
STR_S02_DE	Red raspberry (*Rubus idaeus* x *strigosus*)	Ti Ib	*Agrobacterium fabrum* (biovar 1)
STR_Y01_DE	Yarrow (*Achillea* sp.)	Ti II	*Agrobacterium tumefaciens* (biovar 1)
STR_B01_DE	Lilac (*Syringa vulgaris*)	Ti II	*Agrobacterium tumefaciens* (biovar 1)
STR_C01_DE	Chrysanthemum (*Chrysanthemum* sp.)	Ti III	*Agrobacterium tumefaciens* (biovar 1)
STR_G01_DE	Grape (*Vitis vinifera*)	Ti IVa	*Allorhizobium vitis* (biovar 3)
STR_R03_DE	Rose (*Rosa* sp.)	Ti Ia	*Rhizobium rhizogenes* (biovar 2)

*Classified according to biovar/genomospecies designations determined in Weisberg et al. (2020; 2022)

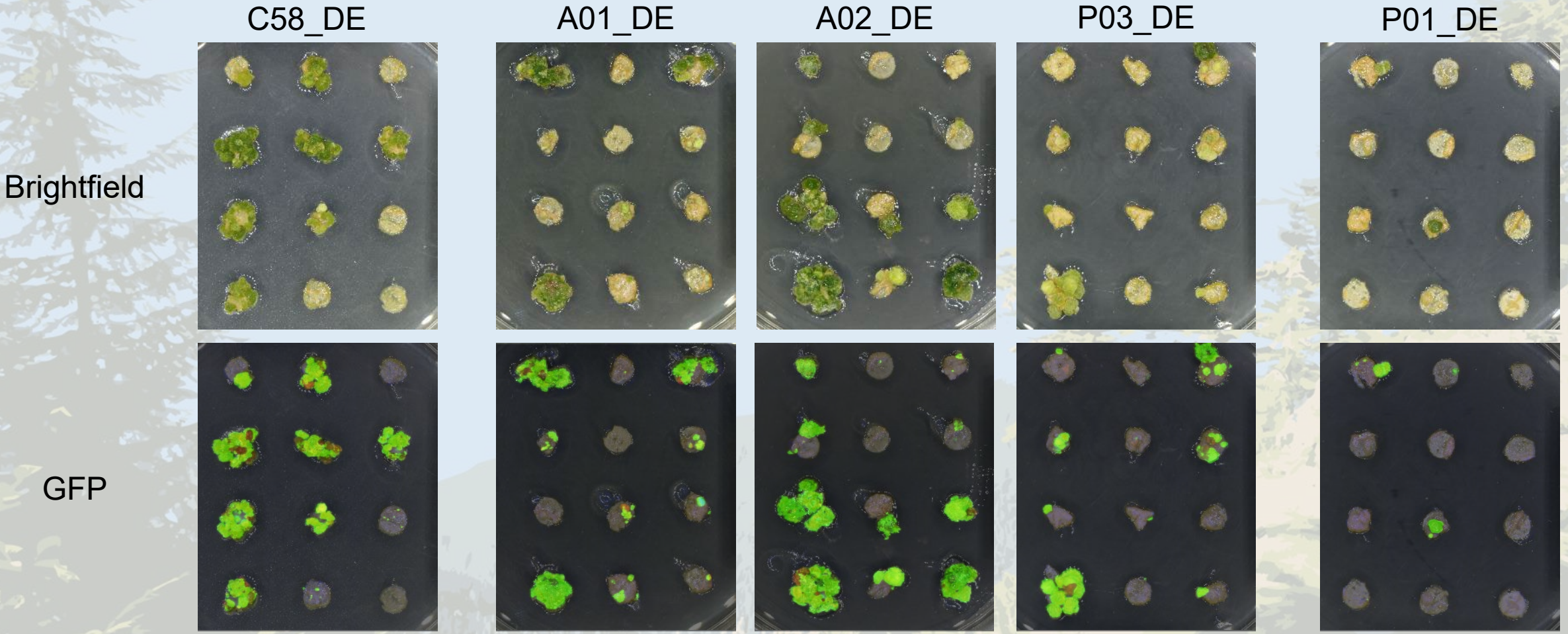
Testing transient T-DNA delivery in *Nicotiana benthamiana* showed qualitative variation in newly disarmed strains



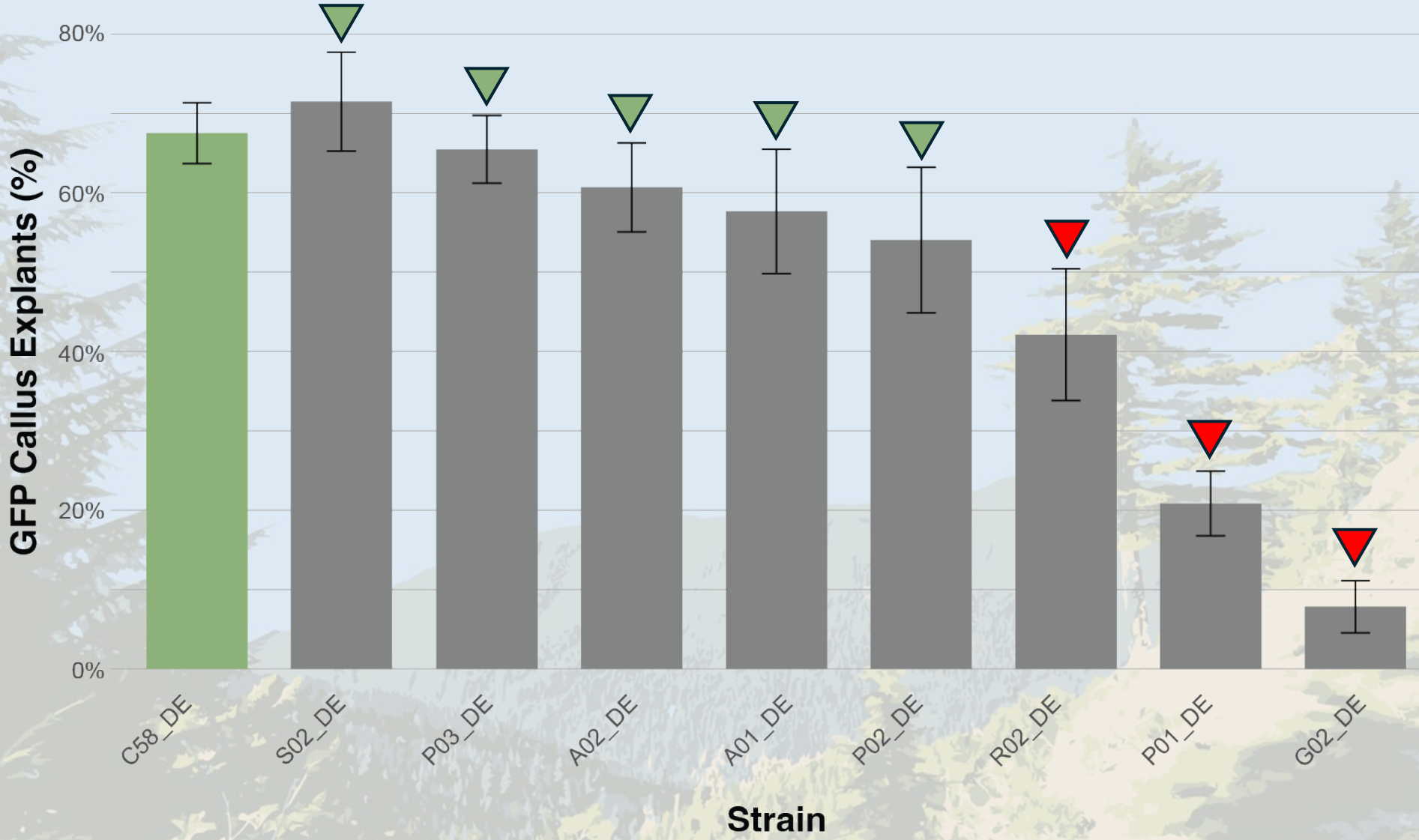
Quantifying transient T-DNA delivery using fluorescent plate reader shows differences in disarmed strains compared to common laboratory strains



Several disarmed strains showed similar rates of transformation to a common lab strain



Testing disarmed strains in hybrid 717 gave similar transformation efficiencies to a common lab strain

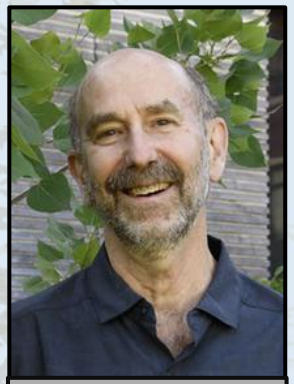


Summary & Future Research

- Screening wild strains in various woody species identified candidates with efficient transformation rates
- An *Agrobacterium* cloning toolkit enabled the development of 16 new disarmed strains
- Early testing of disarmed strain showed increased transient delivery in *N. benthamiana* and similar transformation rates in 717 to a common lab strain

- Finishing testing of disarmed strains in hybrid 717
- Beginning testing disarmed strains in more difficult woody species
- Looking to test strains in more species & genotypes in collaboration with other groups

Acknowledgements



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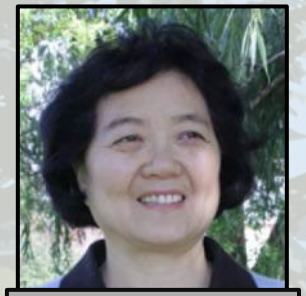

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Experiments

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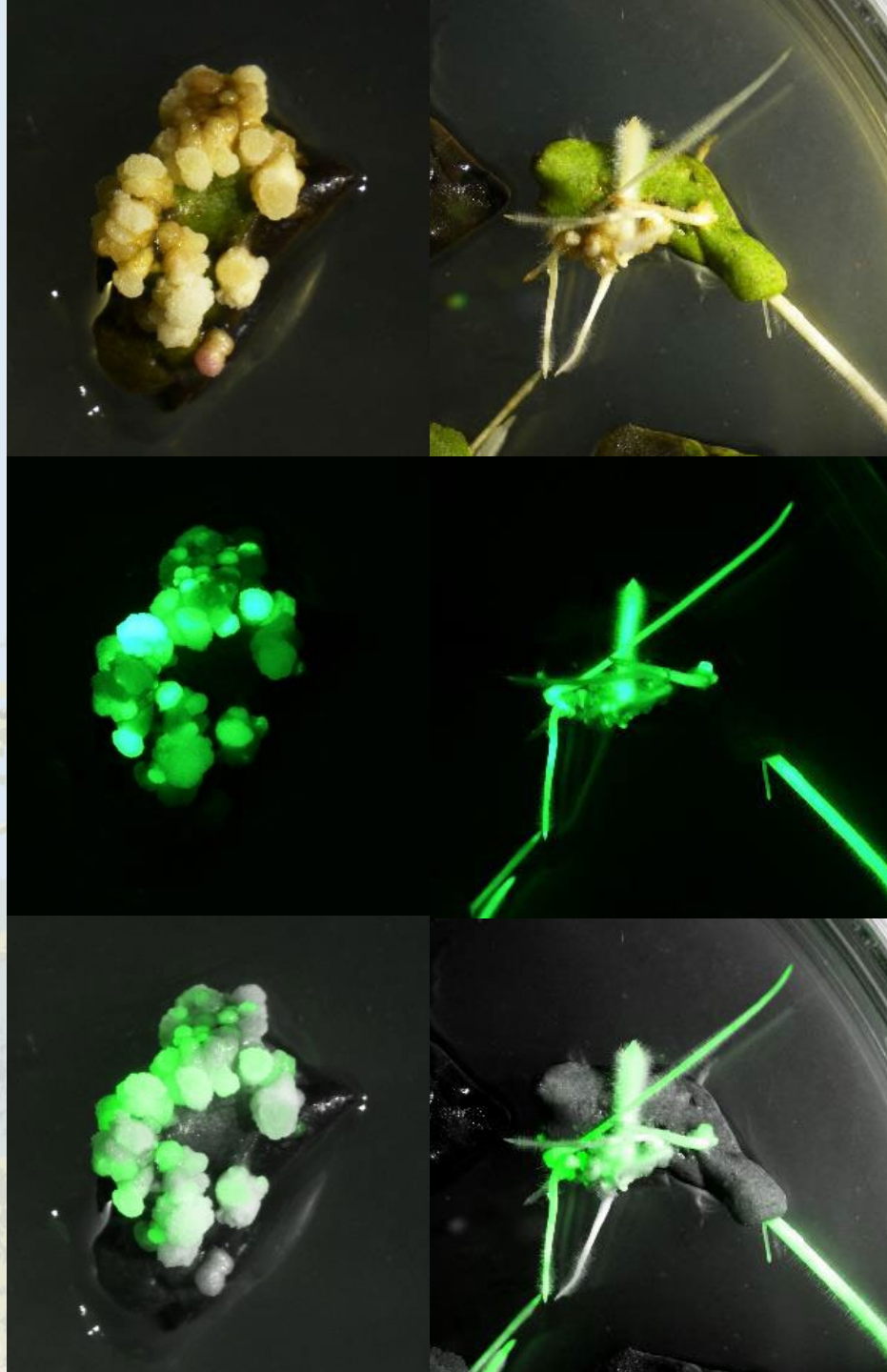
Oregon State University

Thank you!



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