

Development of *Agrobacterium* strain resources for plant transformation



Society for In Vitro Biology

PlantGENE NextGen *Agrobacterium* workshop

Presented by Chris Willig

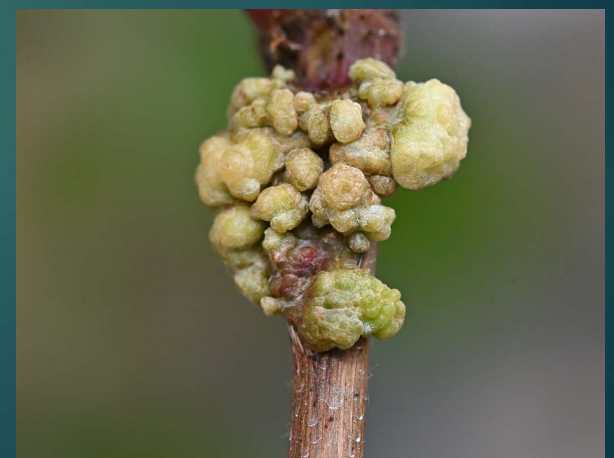
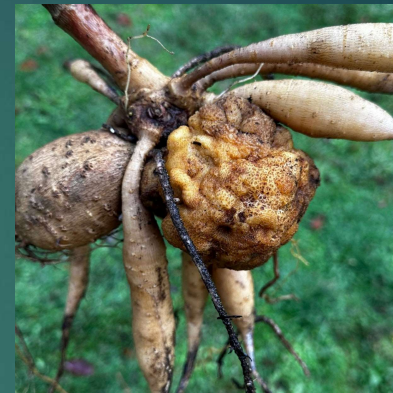
June 6th, 2026



Topics covered

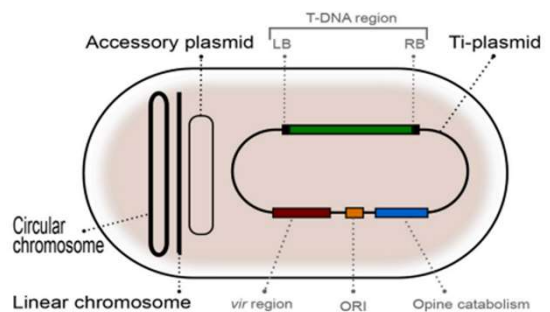
- ▶ Overview of conventional *Agrobacterium* strains used in biotech
- ▶ Natural diversity of agrobacteria
- ▶ Transformation-related problems associated with agrobacteria
- ▶ Pioneering strain modifications to address transformation-related problems
- ▶ Potential for utilizing novel strain backgrounds as biotech tools

Agrobacterium gall/root formation phenotypes

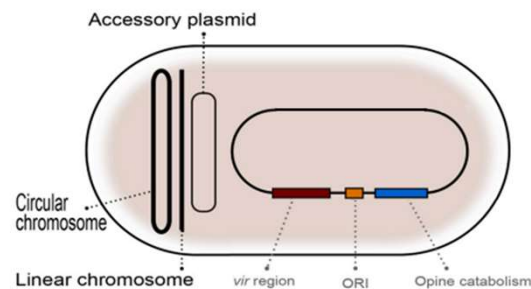


Agrobacterium genome structure and basic modification

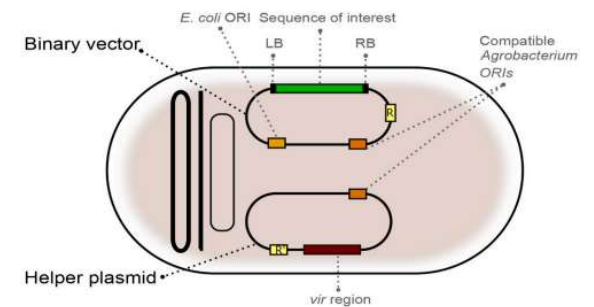
- Wild strain C58 has a circular chromosome, a linear chromid, a Ti virulence plasmid, and a single accessory plasmid. Wild strains vary widely.
- The virulence plasmid carries both oncogenes (T-DNA region) and DNA transfer machinery (*vir* region)
- Disarmament requires deleting the native T-DNA segment while retaining the *vir* region, leaving intact the strain's natural ability to transmit DNA to plant cells



Wild-type *Agrobacterium* strain



Disarmed strain



Transformation-ready binary strain

All *Agrobacterium* cell configuration schemes adapted from De Saeger *et al.* (2021)

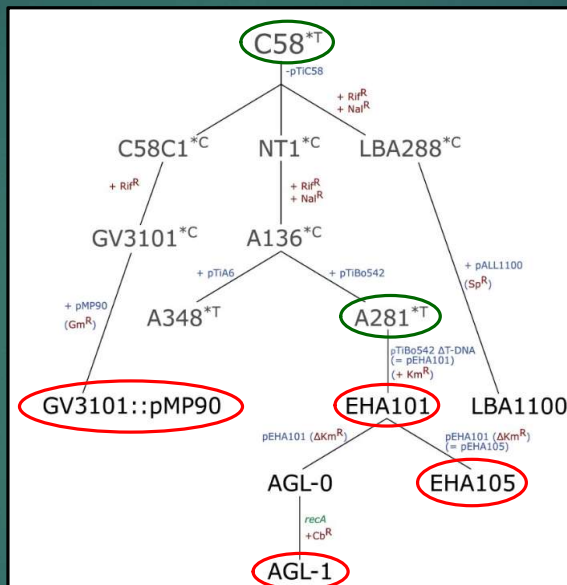
Landscape of *Agrobacterium* strains used in plant biotech

- ▶ The following strains primarily constitute the “conventional” agro toolset for plant biotech (used in 95%+ of stable plant transformation protocols):

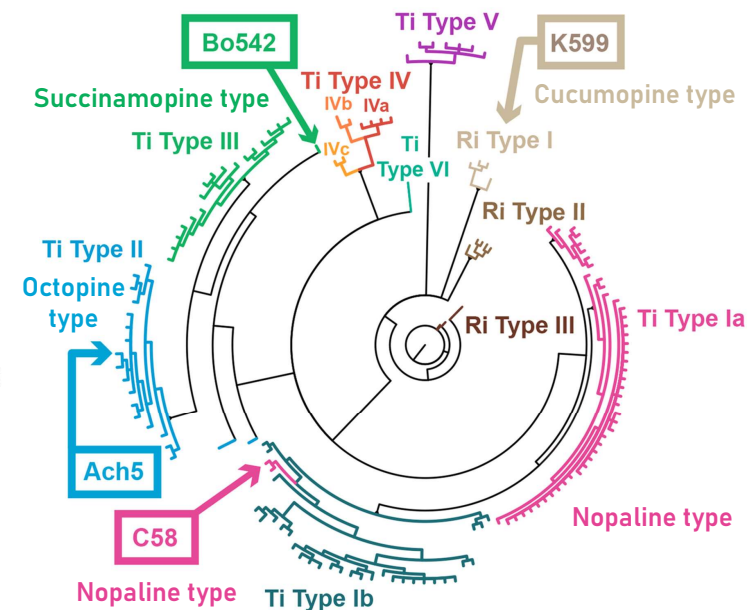
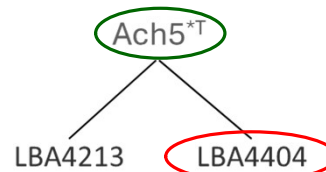
- ▶ AGL-1
- ▶ LBA4404
- ▶ EHA101/5
- ▶ GV3101(pMP90)

- Other minor ones used, including for hairy root induction:

- K599
- A4 or ATCC15834



Adapted from De Saeger et al. (2021)



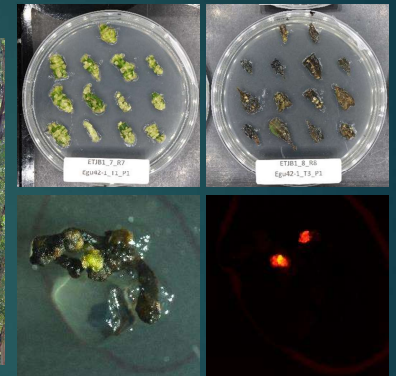
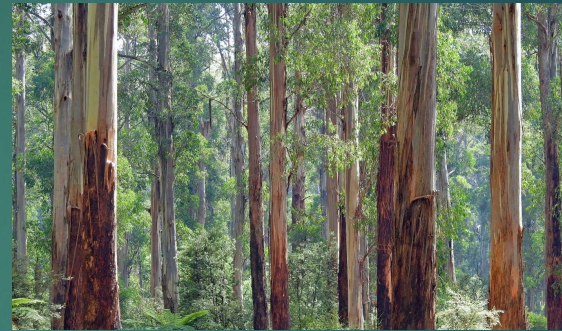
From Goralogia et al. (2025)

Reconstituted phylogenetic tree showing relationships between virulence plasmids of sequenced strains reported in Weisberg et al. (2020)

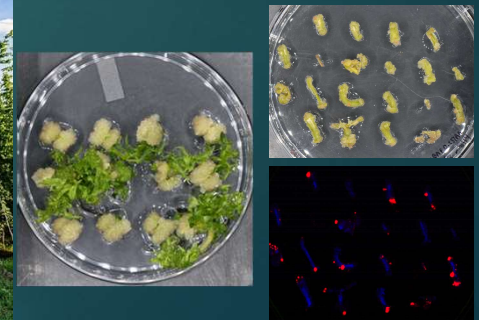
Why engineer *Agrobacterium*?

- The Strauss lab works on some plant species which are broadly difficult to transform
- In some genotypes, regeneration alone can be achieved at high frequency, but T-DNA delivery with selection is inefficient
- Thus we're exploring methods to potentially boost T-DNA transfer on the *Agrobacterium* side

EUCALYPTUS



HOPS



A great review paper exploring wide possibilities in *Agrobacterium* engineering

Research review paper

Agrobacterium strains and strain improvement: Present and outlook

Jonas De Saeger^{a b c}, Jihae Park^{a d}, Hoo Sun Chung^{b c}, Jean-Pierre Hernalsteens^e, Mieke Van Lijsebettens^{b c}, Dirk Inzé^{b c}, Marc Van Montagu^{b c}, Stephen Depuydt^{a b c}  



Biotechnology Advances

Volume 53, December 2021, 107677

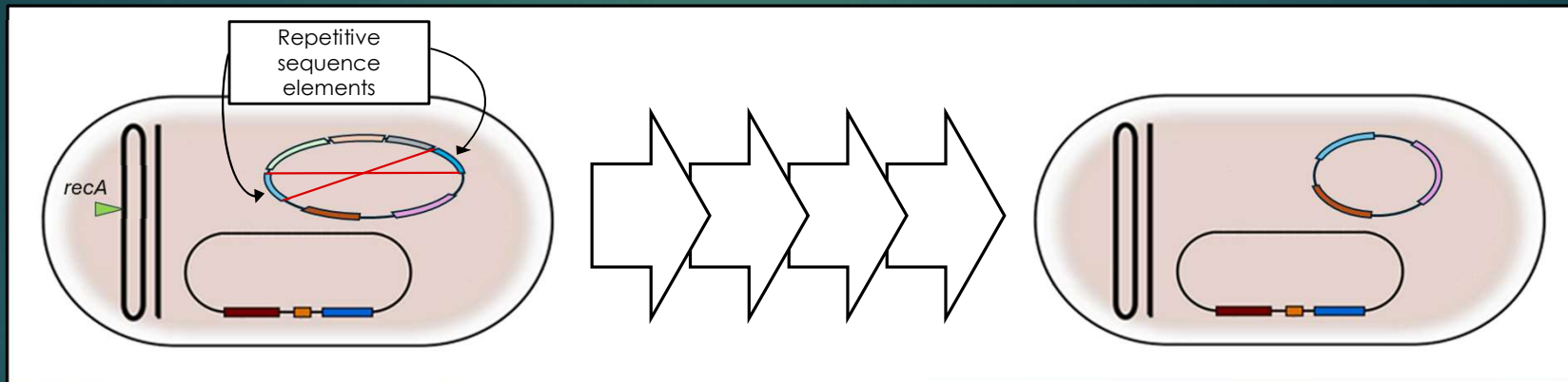


Select target traits for strain enhancement

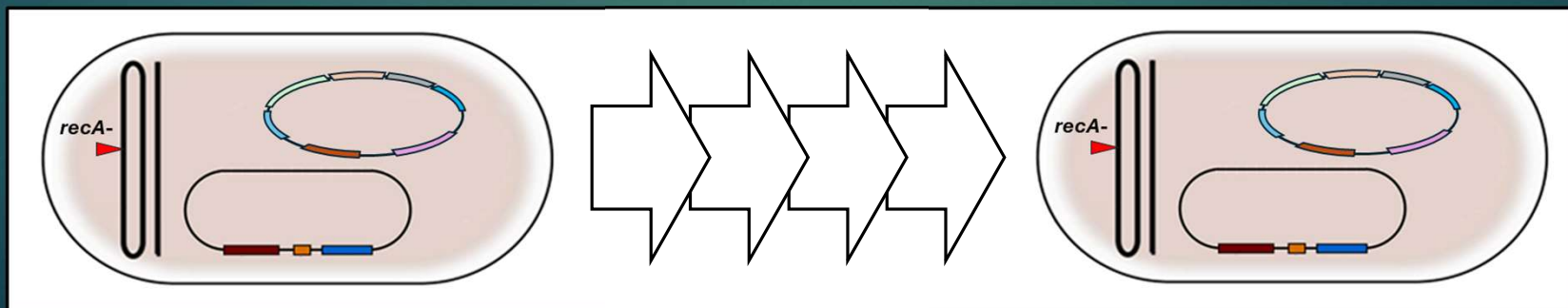
1. Convert plant pathogenic strain into useful biotech vector (disarmament)
2. Reducing construct instability
3. Containment of bacterium / preventing overgrowth
4. Enhanced function of DNA transfer machinery
5. Improved transgene insertion copy number/quality
 - For generating optimal transgenic lines
6. Conferring low/no transgene insertion
 - For generating transgene-free gene edited lines

2. Reducing construct instability

- ▶ *recA* is a key gene enabling DNA repair by homologous recombination
 - ▶ Can catalyze undesirable rearrangements in large and/or repetitive constructs



- *recA* deficient strains are desirable for increased stability of binary plasmids



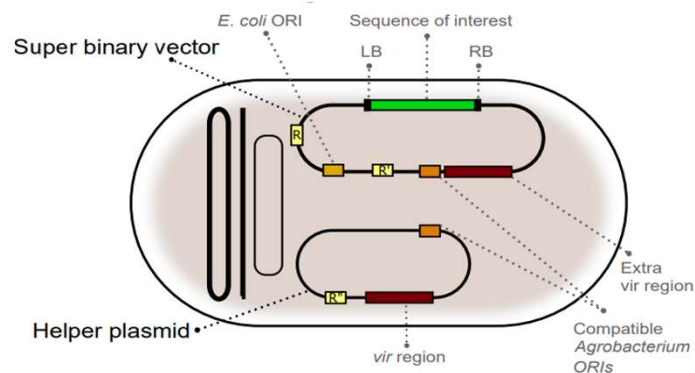
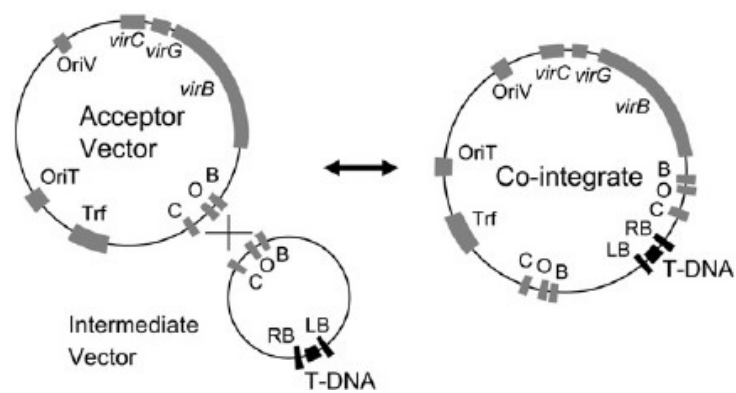
3. Controlling overgrowth in tissue culture

- Auxotrophic mutants are desirable for containment of strain growth
 - *thyA*⁻ is most frequently-used knockout in agro (Ranch et al., 2012)
- Methionine biosynthesis (*metA*⁻) has also been targeted (Prías-Blanco et al., 2022)
- Threonine, cysteine, and tryptophan auxotrophs also produced (Tamzil et al., 2021)

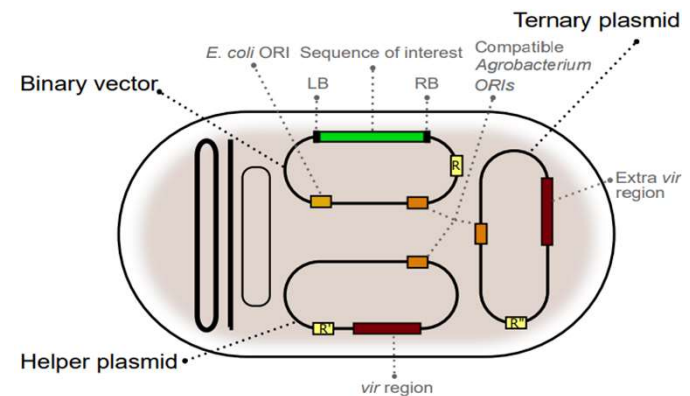
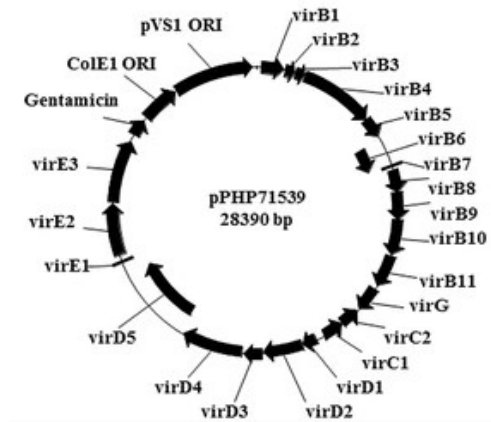


Prías-Blanco et al. (2022) Transgenic Res.

4. Enhancing T-DNA transfer with supplemental *vir* genes



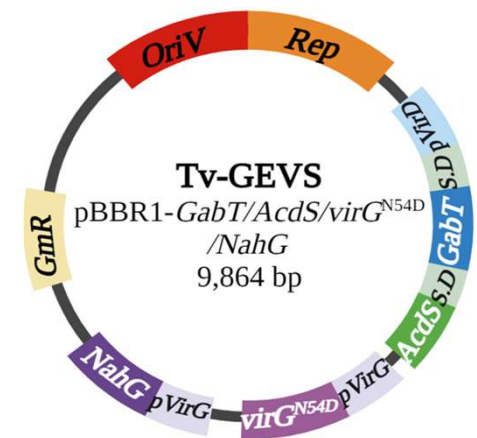
Strain with "superbinary" vector configuration



Strain with ternary vector configuration

4. Enhancing T-DNA transfer with plant hormone-modulating genes

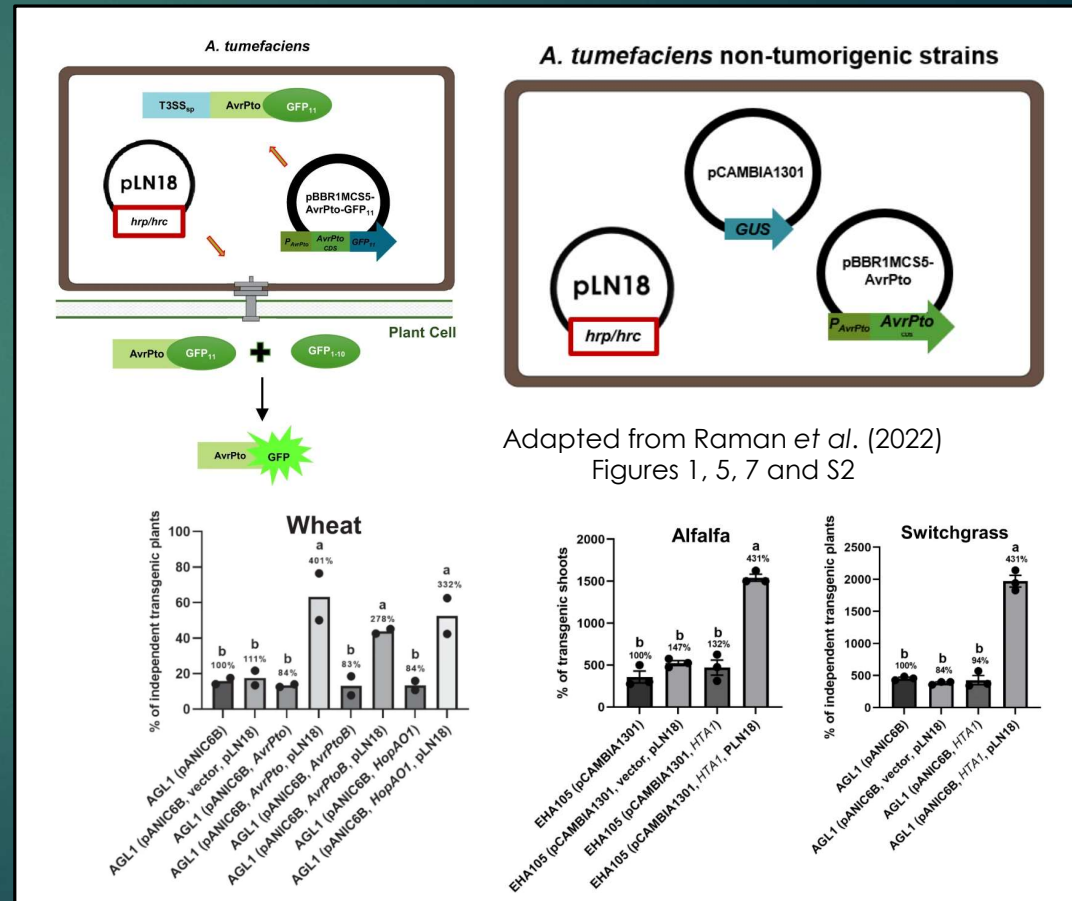
- *trans*-zeatin synthase (*tzs*) gene from pTiC58
 - enhances T-DNA delivery in some plant species when ectopically expressed in strains that lack it (Sardesai *et al.*, 2013; Ye *et al.*, 2016)
- Ethylene degrading enzyme (*acdS*)
 - improves melon, Arabidopsis stable transformation rates (Nonaka *et al.*, 2008)
- GABA degradation (*gabT*)
 - Improves stable transformation in tomato and sweetcane. Additive effect when expressed on ternary vector with *acdS* (Nonaka *et al.*, 2014; 2019)
- ▶ SA-reducing enzyme (*nahG*)
 - ▶ *nahG* transgenic Arabidopsis/tomato lines are much more transformation susceptible (Rosas-Díaz *et al.*, 2017; Anand *et al.*, 2008)



Ternary vector map from Jeong *et al.* (2024),
Figure S1 panel G

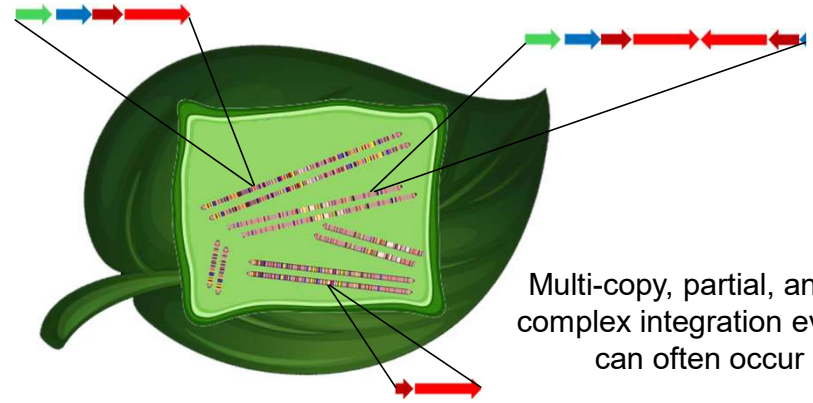
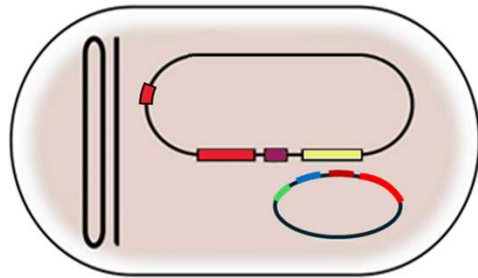
4. Enhancing T-DNA transfer via host immune evasion

- A type III secretion system (T3SS) from *Pseudomonas* spp. can be expressed in agro
- This enables transmission of T3 effector proteins into host cells, thereby suppressing immune responses
- Strains combining T3SS with a binary vector can confer substantial increases in transformation rates over binary-only strains
- Expressing plant-derived protein HTA1 in agro fused to a T3 secretion peptide also enabled large gains in transformation frequency



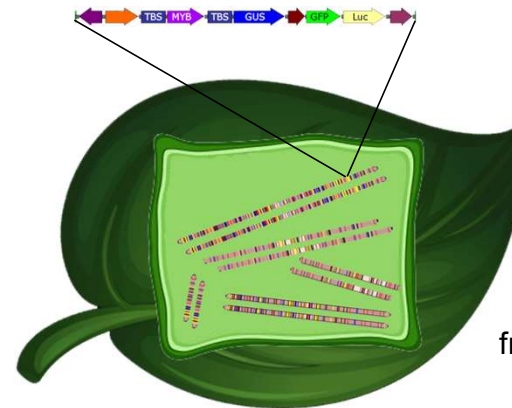
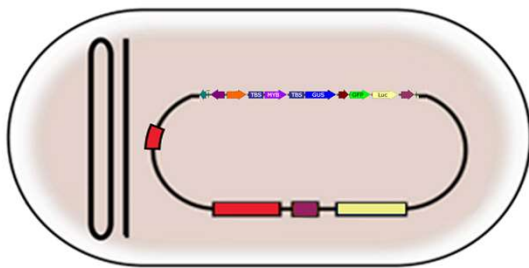
5. Improved transgene insertion copy number/quality

Binary strain



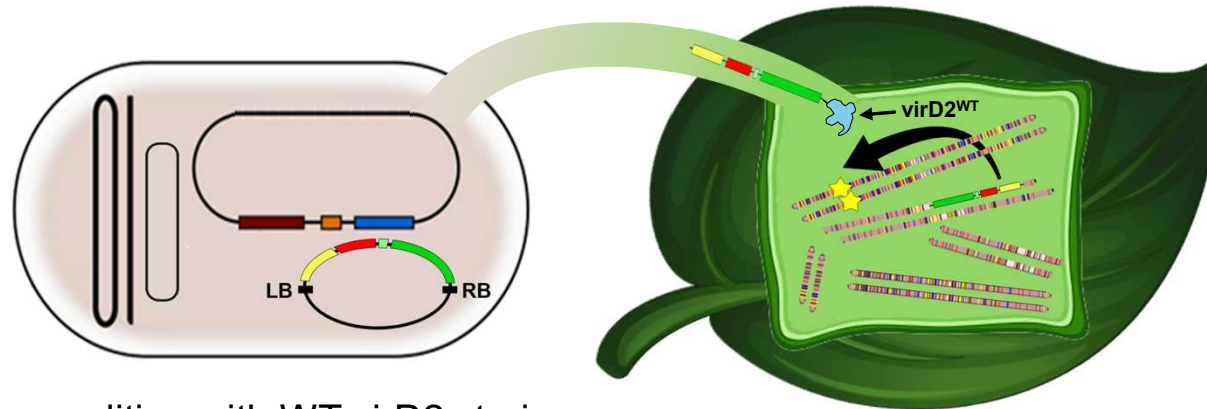
Multi-copy, partial, and/or complex integration events can often occur

GAENTRY (unitary) strain

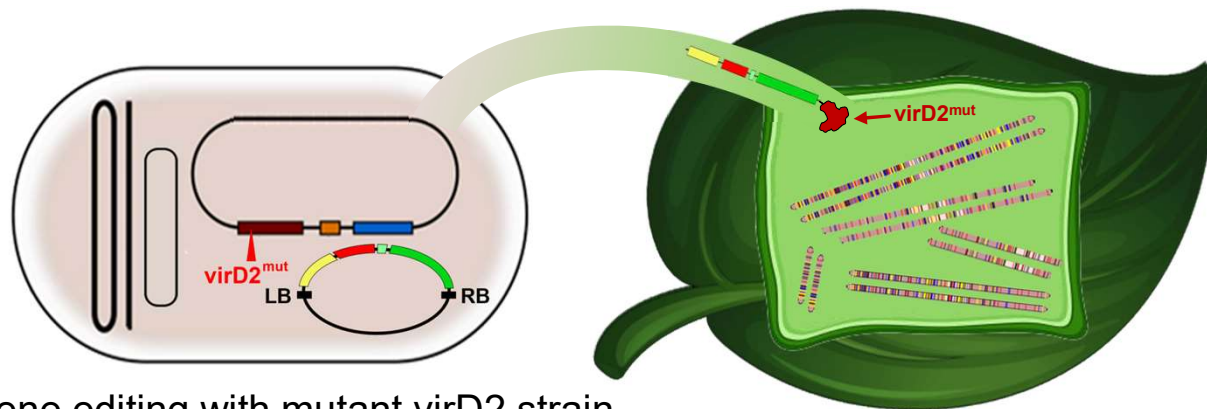


GAENTRY gives higher frequency of simple, single-copy integration events

6. Conferring low/no transgene insertion



Gene editing with WT *virD2* strain



Gene editing with mutant *virD2* strain



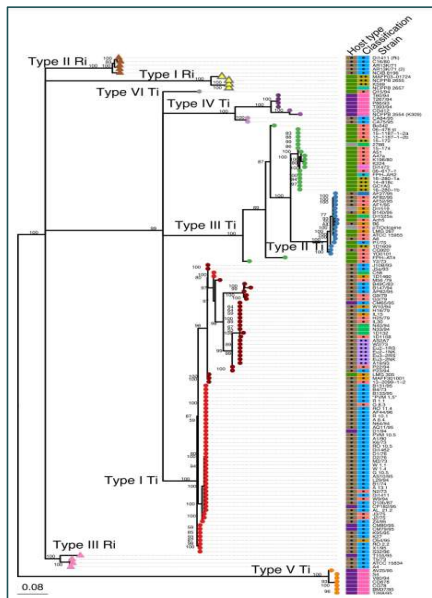
Greg Goralogia
Postdoctoral Researcher

Agrobacterium transformation research at Oregon State University in the Strauss lab



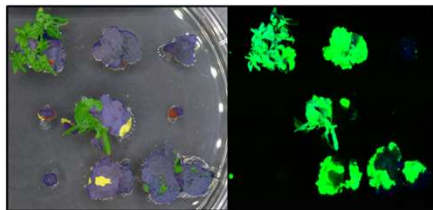
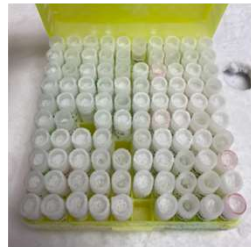
Zachary Heinhold
PhD student

Well-characterized strain collection at OSU

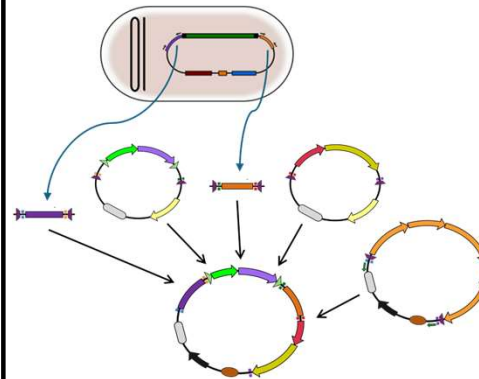


Adapted from Weisberg et al. (2020)

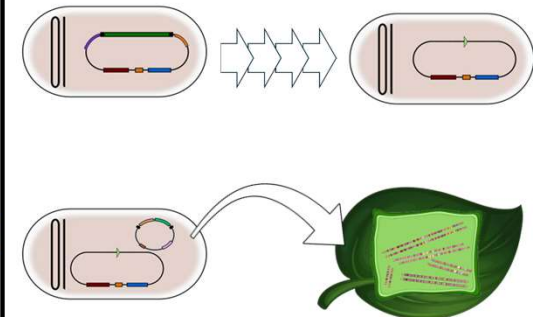
Screening wild strains for transformation phenotypes



Strain engineering toolkit construction



Disarmament and testing of novel strain backgrounds

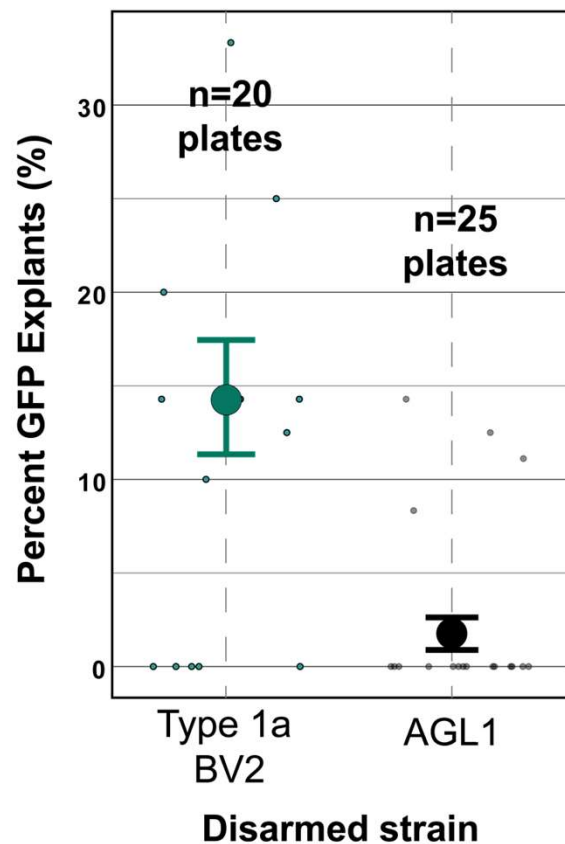


16 novel strain backgrounds disarmed (so far...)

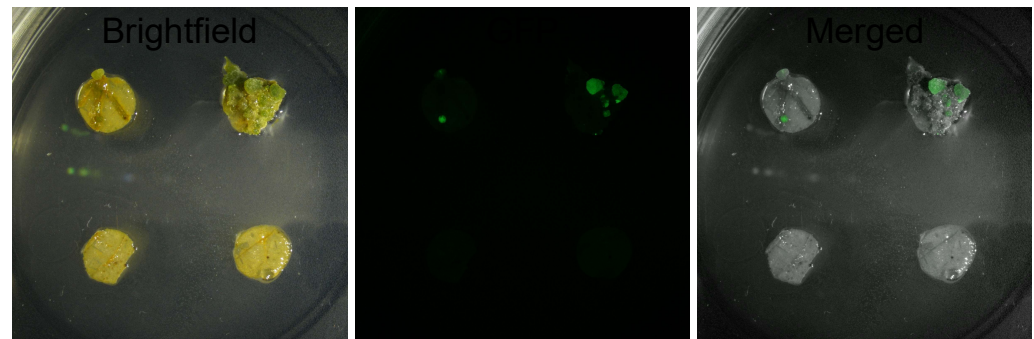
Strain designation	Host plant source	Virulence plasmid type	Chromosomal background / taxonomy*
STR_H01_DE	Hop (<i>Humulus lupulus</i>)	Ti Ia	<i>Agrobacterium fabrum</i> (biovar 1)
STR_L01_DE	Pear (<i>Pyrus betulifolia</i>)	Ti Ia	<i>Rhizobium rhizogenes</i> (biovar 2)
STR_R01_DE	Rose (<i>Rosa</i> sp.)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

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

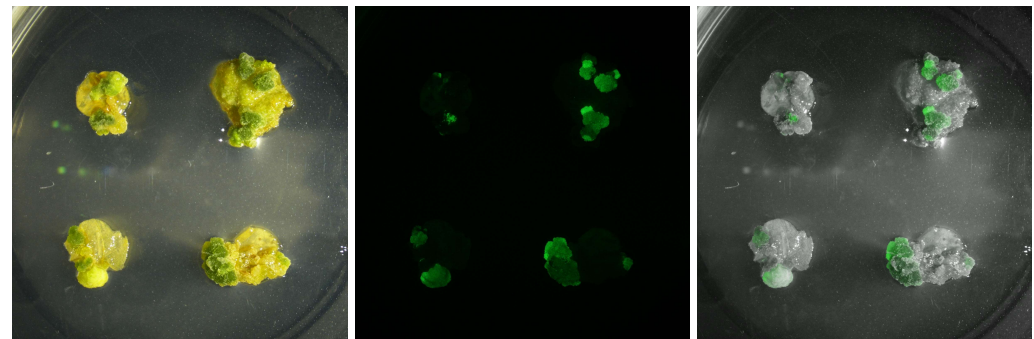
Benchmarking a novel disarmed Ti type Ia strain against AGL1 showed improved transformation in poplar



AGL1



T1aBV2



**Our AGL1 strain may not be everyone's AGL1 strain!*

Acknowledgements



Contributors:

- ▶ Zachary Heinhold
- ▶ Colette Richter
- ▶ Greg Goralogia
- ▶ Cathleen Ma
- ▶ Kate Peremyslova
- ▶ Parker Wheeler
- ▶ Bethani Sutliff
- ▶ Jay Adams
- ▶ Tanner Whiting
- ▶ James Warner
- ▶ Victoria Conrad
- ▶ Steve Strauss

Other Strauss lab members:

- Veerendra Sharma
- Anthony Marroquin
- Carter Mucken
- Sydney Gould
- Dana Howe

Support:

USDA-NIFA-AFRI
Postdoctoral Fellowship
program

