GAANTRY, a Versatile and Robust *Agrobacterium*-based Gene Stacking System.

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Thursday, May 9, 2019

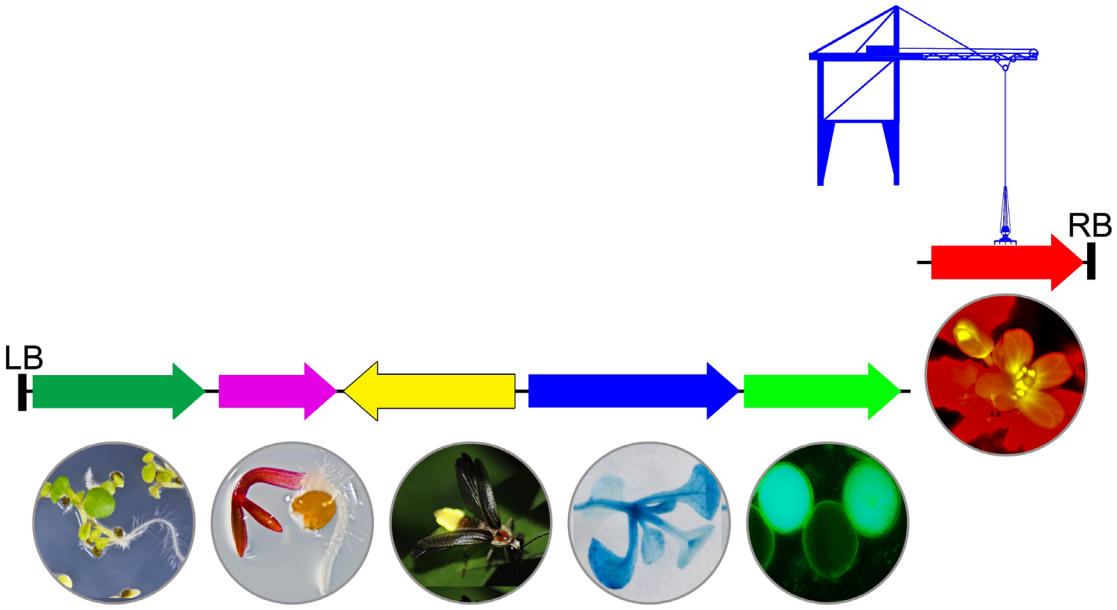
Seed Central FORUM

UC Davis Conference Center

GA*A*NTRY

Gene **A**ssembly in *A*grobacterium by **N**ucleic acid **T**ransfer using **R**ecombinase technolog**Y**

Shown is a gantry crane loading a large amount of cargo onto a container ship



Gene stacking

Our motivation for developing GA*A*NTRY:

- **The stacking of multiple traits into a biotech crop requires either**
  - multiple rounds of transformation potentially combined with target genome integration
  - or extensive plant breeding to bring individual transgenic loci together into a single genotype
  - or the assembly of a transformation construct containing multiple transgene cassettes

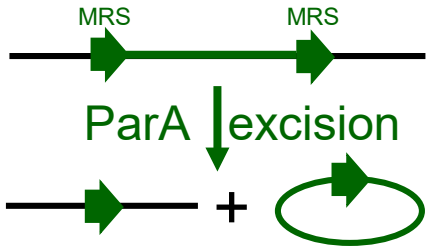
- **Stacking 5 or more transgene cassettes together within a single transformation construct T-DNA can be technically challenging**

The next generation of biotech crops requires an efficient and effective transgene stacking technology

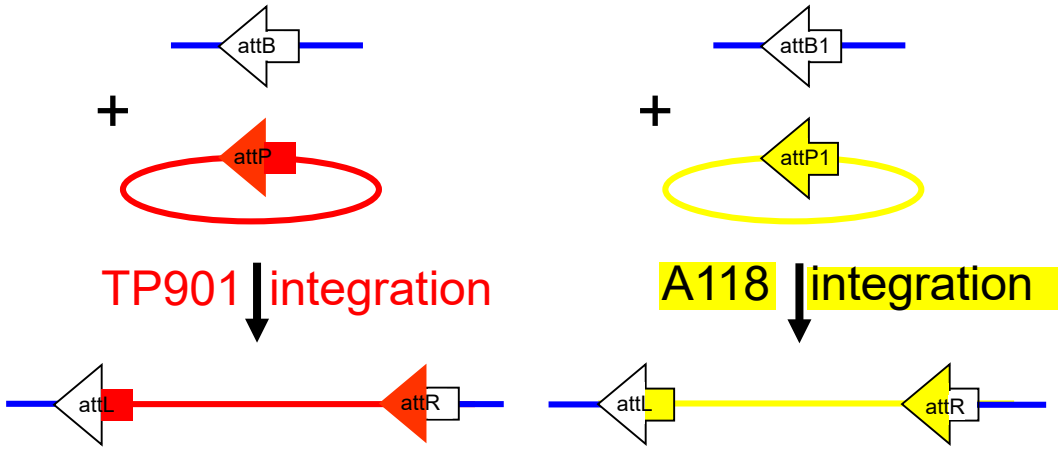
GAANTRY

Gene **A**ssembly in ***A****grobacterium* by **N**ucleic acid **T**ransfer using **R**ecombinase technolog**Y**

- Goal is to develop a method to efficiently stack transgenes into a plant transformation construct.
- The transgene stacking is performed *in vivo* (within *Agrobacterium*).
- This method enables the sequential stacking of transgene cassettes within a T-DNA on the *Agrobacterium* virulence plasmid.
- This technology utilizes the **ParA**, **TP901** and **A118** unidirectional site-specific recombinases.

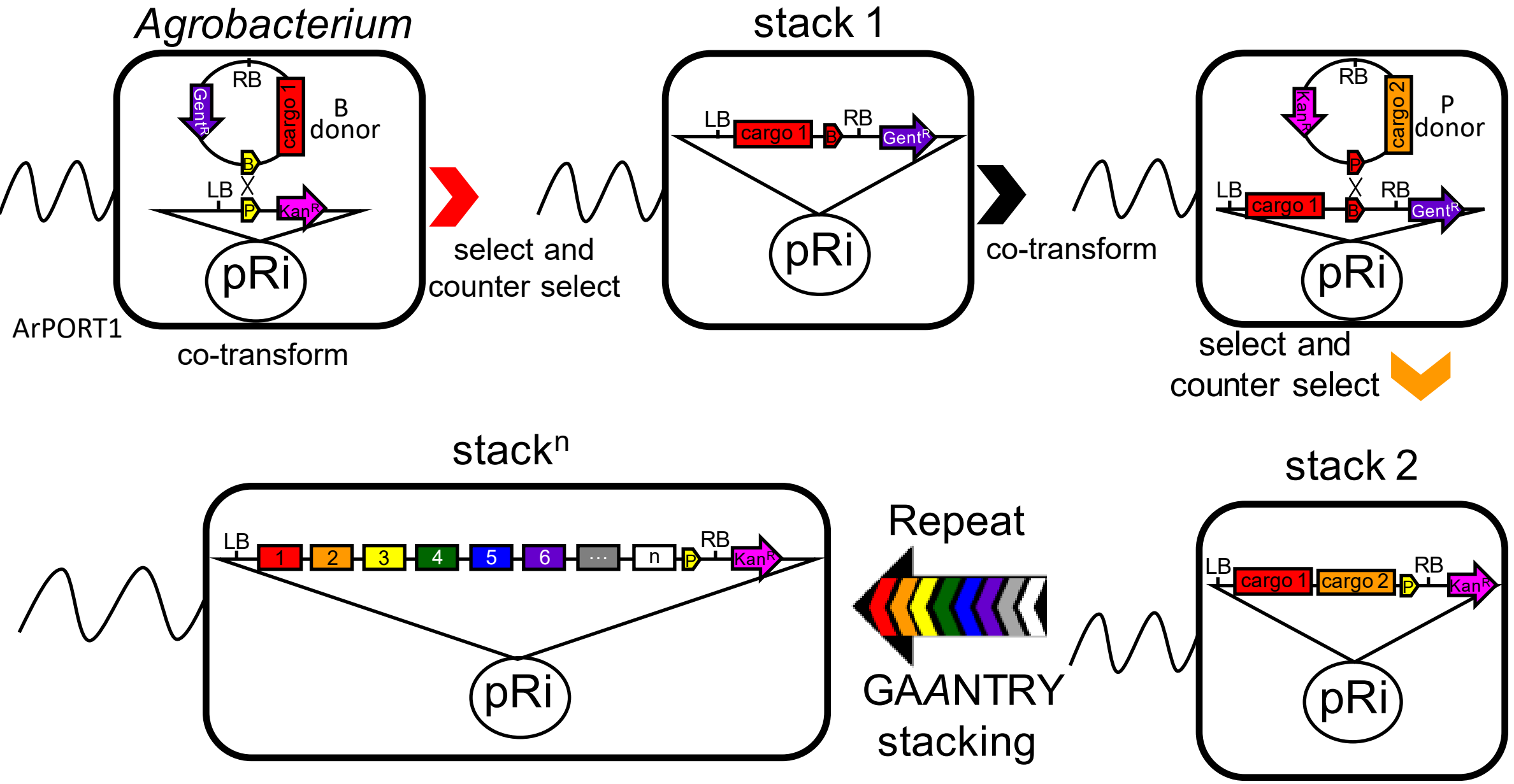


ParA performs unidirectional and irreversible excision of DNA flanked by directly oriented **MRS** recognition sites



TP901 and **A118** recombinases perform unidirectional and irreversible integration of DNA utilizing **attP** and **attB** sites

GAANTRY Assembly Process

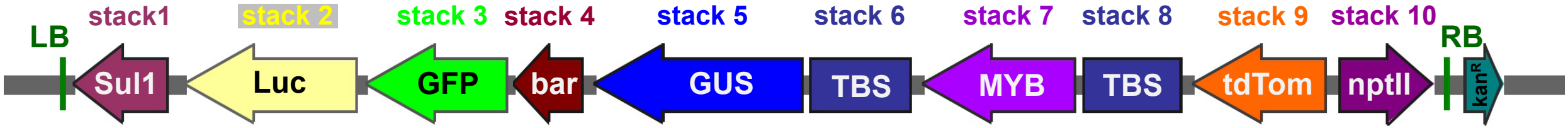


GAANTRY 10-stack T-DNA

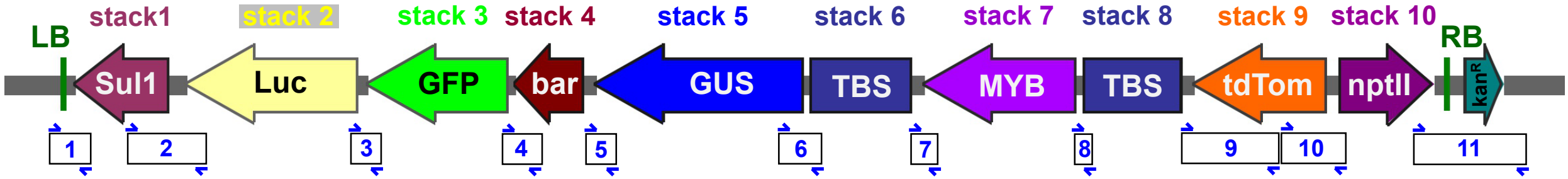
- We performed 10 sequential stacking events to build a 28.5 kb T-DNA containing 8 transgene cargoes

The 10 stacked cargos are:

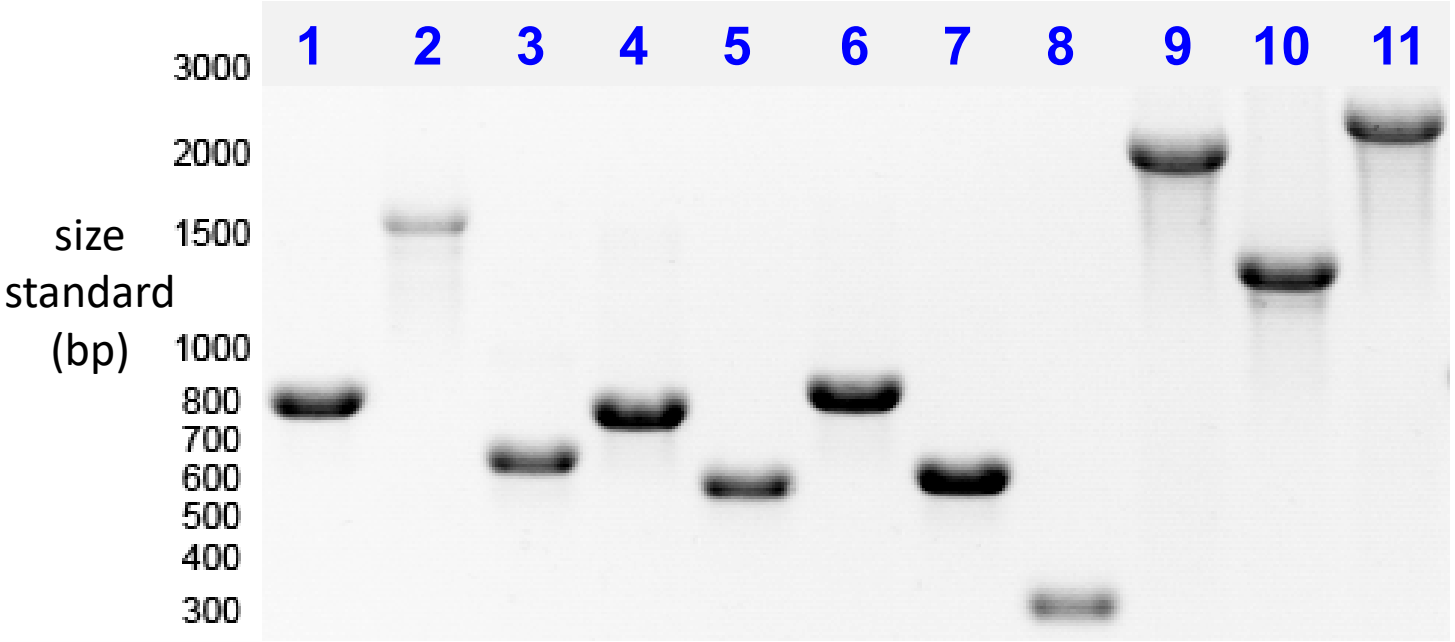
Expression Cassette	Vector	Plant Phenotype	Size (kb)
1. pCaMV35S-sul1-35Sterm	(B Donor)	sulfadiazine resistance	1.9
2. pSt409S-Fluc-nosTerm	(P Donor)	luciferase activity	3.6
3. pStUbi7-GFP-nosTerm	(B Donor)	green fluorescence	2.9
4. pNos-bar-nosTerm	(P Donor)	Finale herbicide resistance	1.4
5. pPrSuperUbi-GUS-nosTerm	(B Donor)	β-glucuronidase activity	4.3
6. TBS insulator	(P Donor)	insulation of pHTH from 35S enhancer	2.0
7. pHTH-CsMybA-nosTerm	(B Donor)	anthocyanin accumulation	3.2
8. TBS insulator	(P Donor)	insulation of pHTH from 35S enhancer	2.0
9. pCaMV35S-tdTom-nosT35St	(B Donor)	red fluorescence	2.8
10. pAtUBQ10-nptII-UBQ10Term	(P Donor)	kanamycin resistance	1.9
		Total:	28.5 kb



GAANTRY 10-stack T-DNA



PCR amplicons spanning the junctions between stacks of cargo



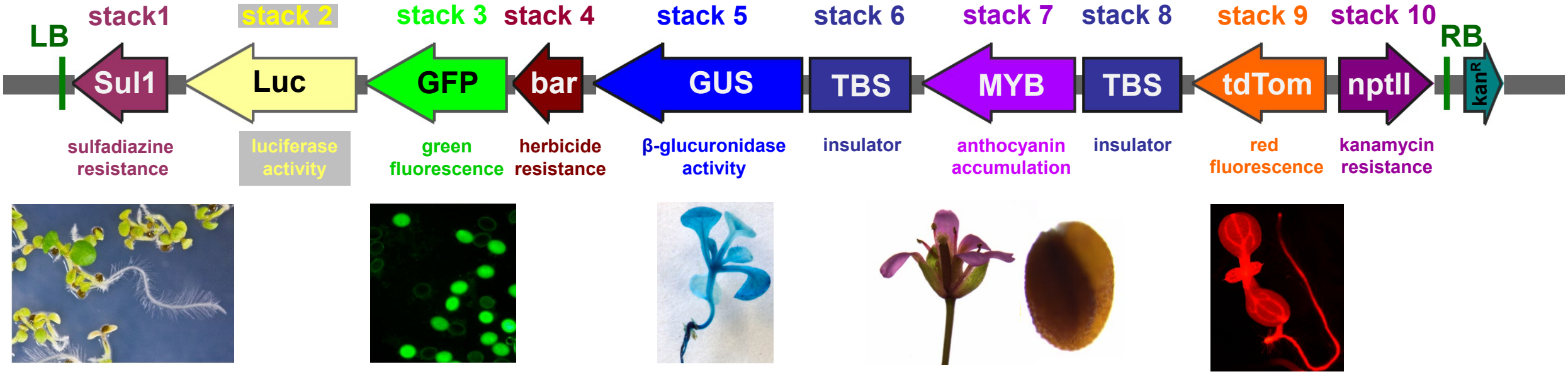
- Following each stacking event, the new *Agrobacterium* strain was validated using microbiological and molecular techniques

  No incorrect assemblies were found

- The stability of the 10-stack *Agrobacterium* GAANTRY strain was also assessed following 3 days of growth without antibiotic selection

  No instability was detected

GAANTRY 10-stack T-DNA



8 functional phenotypes

Arabidopsis transformation efficiency: 1.0%

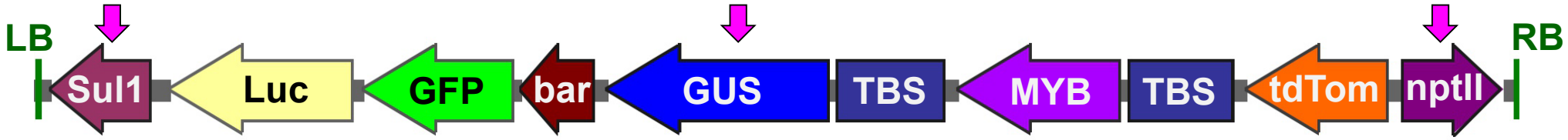
93% carry only the T-DNA (i.e. they are vector backbone free)

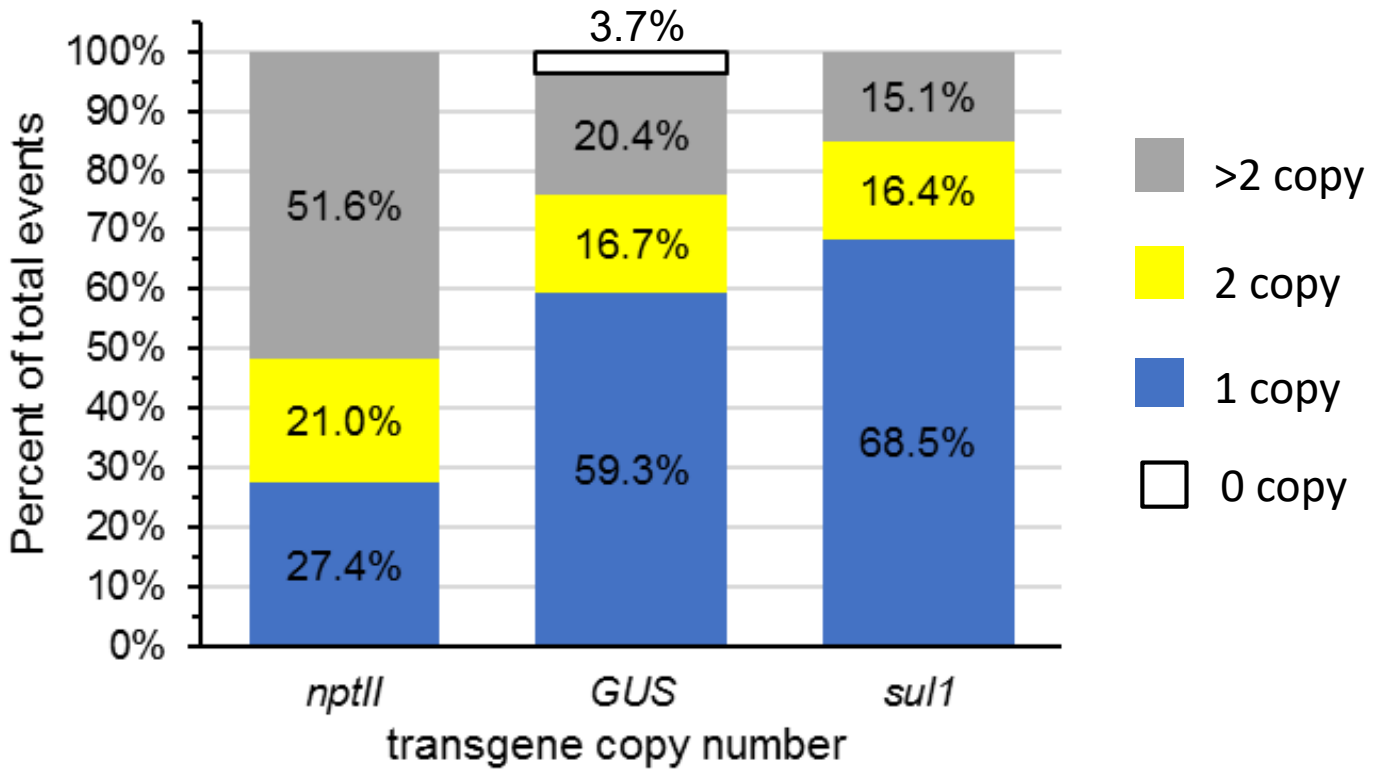
	Phenotype Positive
sulfadiazine resistance	100.0%
luciferase activity	98.6%
green fluorescence	97.5%
herbicide resistance	94.9%
β-glucuronidase activity	98.9%
anthocyanin accumulation	96.7%
red fluorescence	98.9%
kanamycin resistance	100.0%

➢ **390 of the 434 lines (90%) are positive for all 8 phenotypes!**

GAANTRY 10-stack Transgene Copy Number

- **Used droplet digital PCR to measure transgene copy number in the 10-stack transgenic plants**

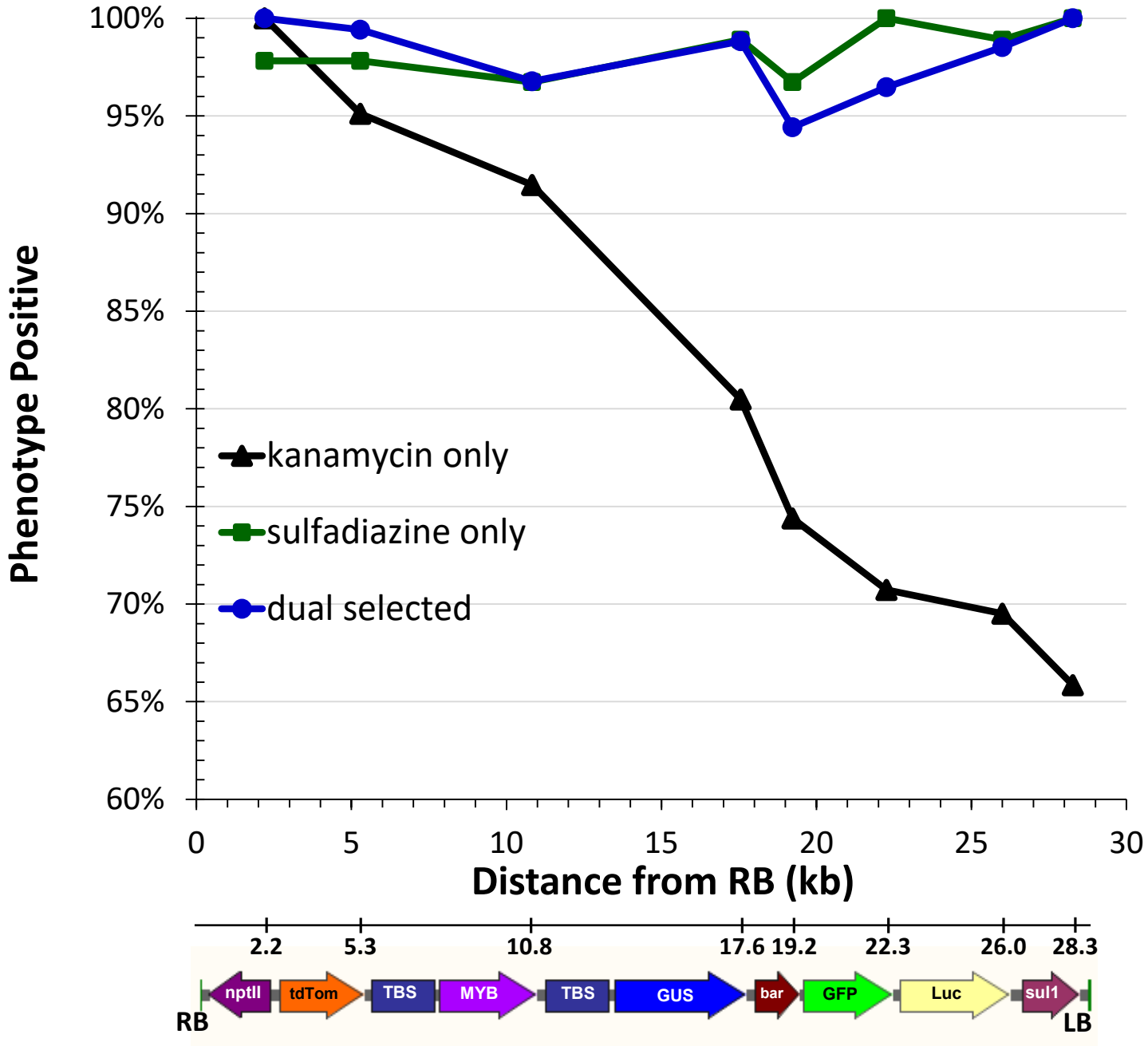




see Collier et al. Plant Journal 90: 1014-1025, 2017 for the transgene copy number measurement method

GAANTRY 10-stack

T-DNA Transfer and Integration



Acknowledgments

Lab Members:

- **Ray Collier**
- **Leyla Hathwaik**

Collaborators:

- **James Thomson**

Funding:

USDA ARS

GA*A*NTRY Publication:

R. Collier, J. Thomson and R. Thilmony

A versatile and robust *Agrobacterium*-based gene stacking system generated high-quality transgenic Arabidopsis plants.

***The Plant Journal* 95 (4): 573-583**

August 2018 doi: 10.1111/tpj.13992