SUPPLEMENTARY INFORMATION

Destabilization of the Tumor-Inducing Plasmid from an Octopine-Type *Agrobacterium tumefaciens* Lineage Drives a Large Deletion in the Co-Resident At Megaplasmid

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Running Head: Megaplasmid deletion in *Agrobacterium*

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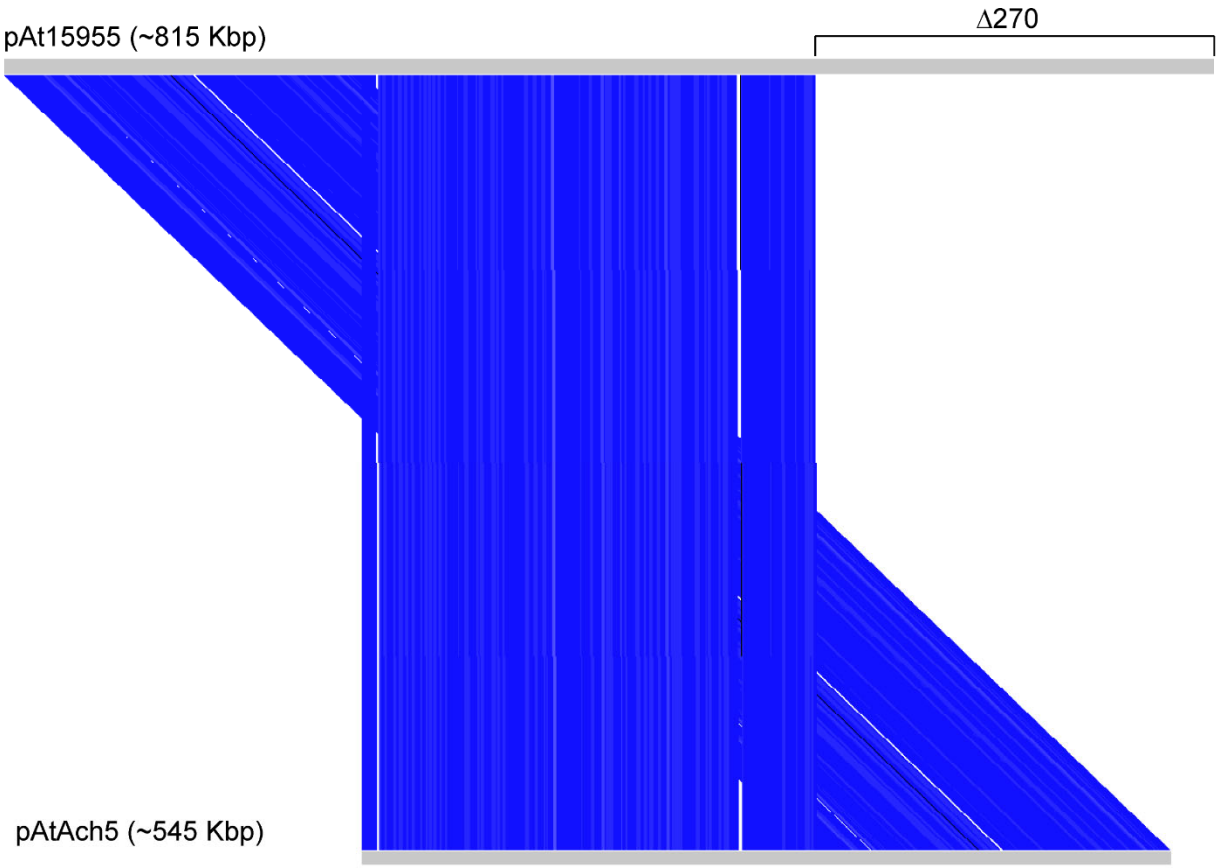

Figure S1: Analogous At plasmid deletion present in natural isolate, *A. tumefaciens* Ach5. Modified Artemis Comparison Tool (ACT) visualization of TBLASTX alignment between At plasmid sequences of *A. tumefaciens* 15955 and *A. tumefaciens* Ach5 indicates deletion in the *A. tumefaciens* Ach5 At plasmid (pAtAch5) at the precise location of the observed deletion in pAt15955. Gray bar depicts plasmid sequence. Blue connecting lines indicate stretches of homology >70% identity. Bracket indicates ~270 Kbp deletion segment.

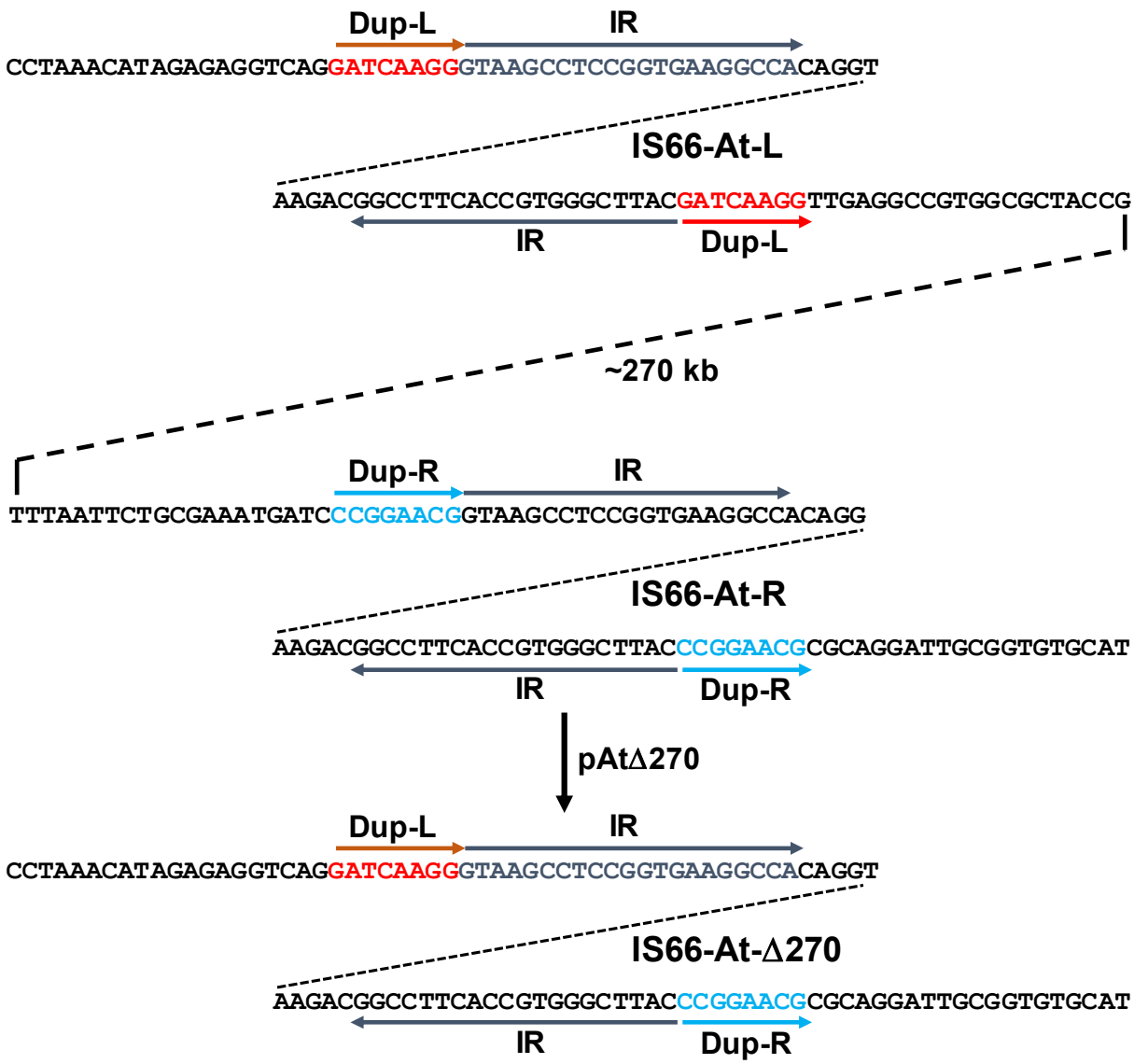

Figure S2. Flanking sequences from pAt15955 IS66 elements. IS66_At_L and IS66_At_R flank an ~270kb segment of the At plasmid which is deleted upon pTi15955 plasmid curing. Light blue and red arrows labeled as Dup indicate the 8 bp duplication generated by IS66 insertion and arrows labeled IR indicate the inverted repeats that define the end of each IS66 element.

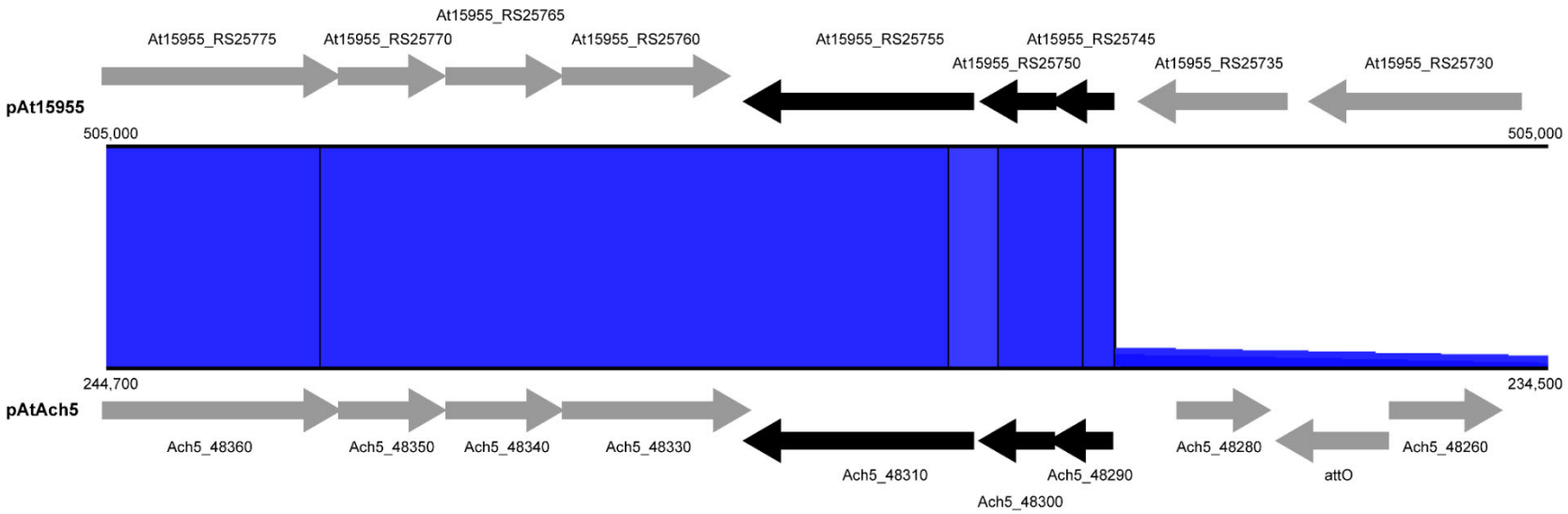

Figure S3: IS-element occurs at site of deletion in *A. tumefaciens* 15955 and *A. tumefaciens* Ach5. Modified ACT visualization of TBLASTX alignment between pAt15955 and pAtAch5 at deletion site identifies homologous IS-element at junction. Shown is alignment of junction site in pAtAch5 with one side of deletion interval in pAt15955 that still carries the ∆270 segment. Blue connecting lines indicate stretches of >90% identity

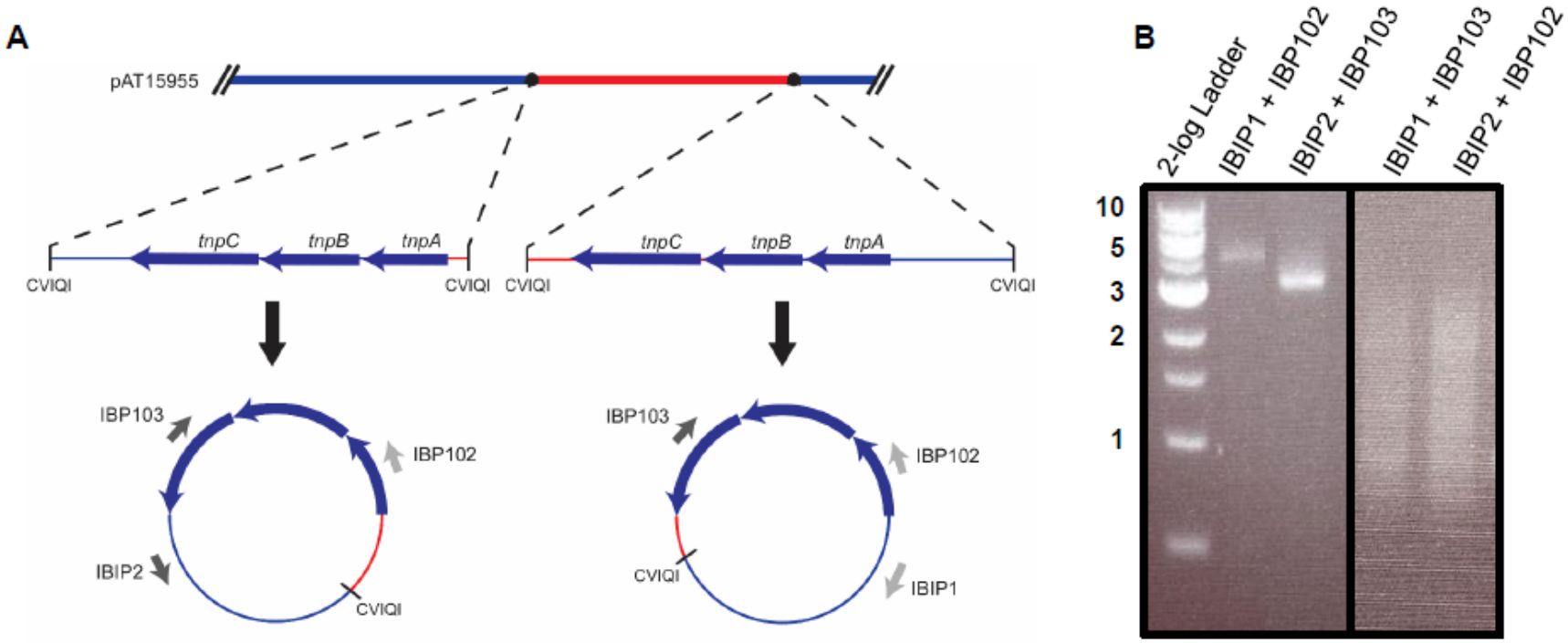

Figure S4: Inverse PCR approach confirms IS66 elements flank pAt15955 segment in a direct orientation. A) Schematic depicting inverse PCR design. gDNA from WT *A. tumefaciens* 15955 was digested with the restriction enzyme, CVQI, and subsequently ligated with T4 ligase. PCR analysis was then conducted on ligation products with sets of primers specific for either the IS66 element or flanking regions. Black circles or blue arrows represent IS66 elements. Red or blue lines indicate sequence within or without pAt15955 deletion segment, respectively. Larger, black arrows represent ligation step. Smaller, gray arrows represent PCR primers. Hash marks at end of replicon indicate continuation of sequence. Hashed lines indicate zoomed in depiction of region. B) Inverse PCR reactions performed on ligation products as depicted in A. Primer pairs used in each reaction are indicated. Ladder sizes shown in Kbp.

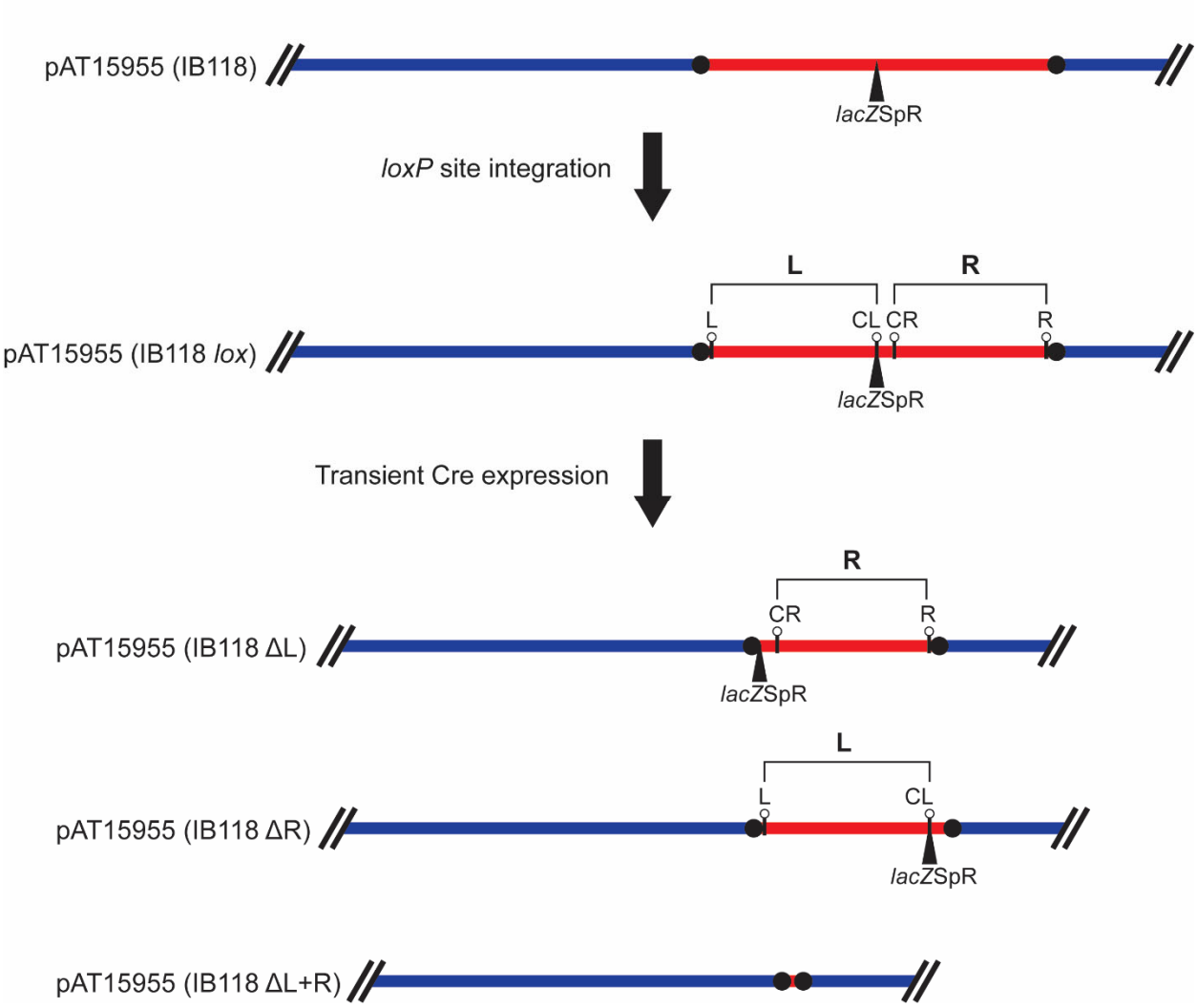

Figure S5: Schematic depicting Cre/*lox* approach for pAt15955 segment truncation. Cre/*lox* was utilized to truncated the pAt15955 segment within the reporter strain IB118. *loxP* sites were introduced sequentially into four locations within pAt15955 (denoted L, CL, CR, and R). All four sites are shown here (IB118 *lox*), but only two *loxP* sites were introduced in combination into three independent strains (see Methods, Table S1) to generate three variants upon transient Cre expression (IB118 ΔL, IB118 ΔR, and IB118 ΔL+R) that have been truncated for either the left (**L**) or right (**R**) half, or entire pAt15955 segment. Red or blue lines represent pAt15955 sequences within or without the pAt15955 deletion segment, respectively. Black circles indicate IS66 elements. *lacZ*Sp^R marker position is shown. Positions of *loxP* integration sites are shown

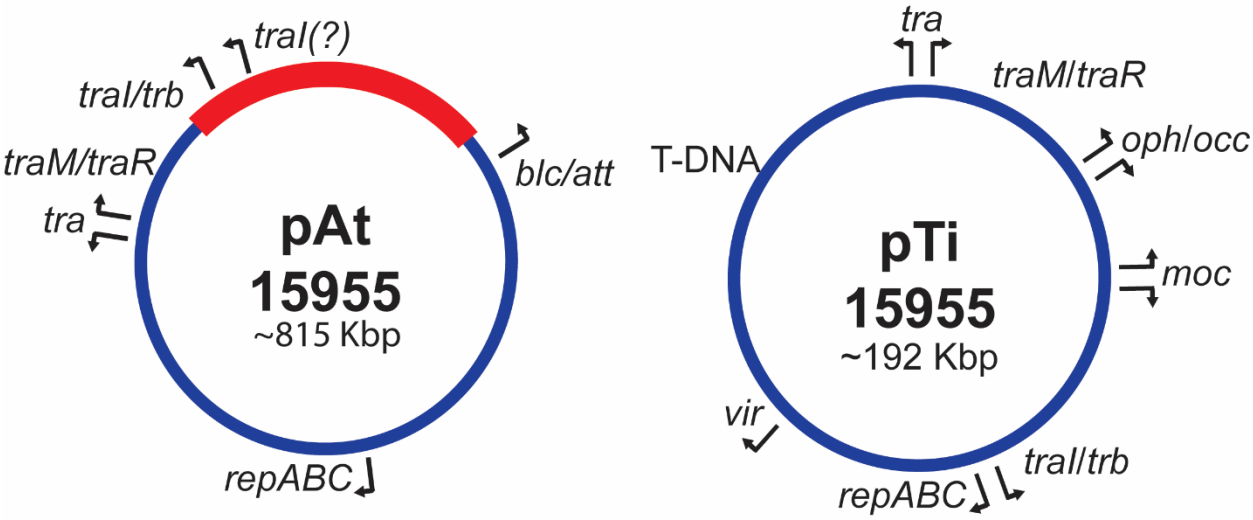

Figure S6: Organization of select genes within pAt15955 and pTi15955. Schematic showing placement of subset of operon locations within pAt15955 and pTi15955. The red segment in pAt15955 is the ∆270 region

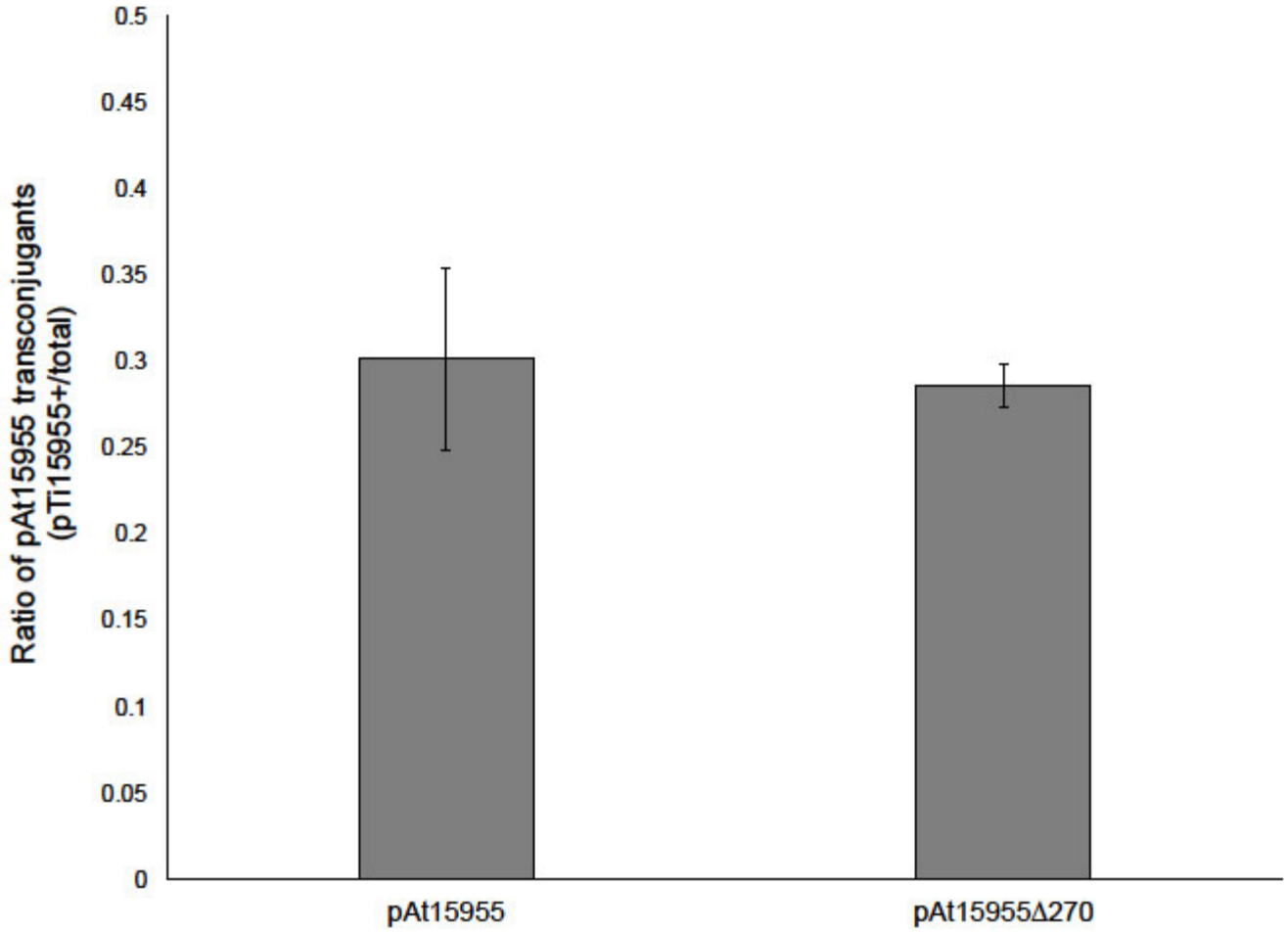

Figure S7: pAt15955 segment does not affect At and Ti plasmid co-transfer. Frequency of co-transfer between pAt15955 or pAt15955Δ270 and pTi15955 were determined by screening At plasmid transconjugants from IB125 or IB131 donor conjugations (Figure 4) for presence or absence of pTi15955 by octopine auxotrophy screening on ATO media (Methods). Co-transfer frequencies reported as the ratio of pTi15955 (+) to total transconjugants.

Table S1: Strains used in this study

Strain/plasmid	Genotype/Markers	Notes	Reference
E.coli			
DH5α/λpir	λpir ; cloning strain		(Chiang et al. 2002)
S17-1/λpir	λpir ; Tra^+ , cloning host		(Kalogeraki et al. 1997)
***A. tumefaciens* 15955**			
WT	pAt15955; pTi15955		
SA122	pAt15955Δ270	Cured of pTi15955 via chemical methods	S.K. Farrand
IBE13A	pAt15955Δ270; pTi15955		This study
IB118	pAt15955::*lacZ/*Sp^R; pTi15955::*gusA/*Gm^R		This study
IB119	Circ::Km^R	Marked on circular chromosome	This study
IB120	Circ::Km^R; pAt15955Δ270; pTi15955	IBE13A marked on circular chromosome	This study
IB123	pAt15955; pTi15955::*gusA/*Gm^R		This study
IB118ΔIS66	pAt15955::*lacZ/*Sp^R; pTi15955::*gusA/*Gm^R; ΔIS66	IB118 that has been deleted flanking IS66 on pAt15955	This study
IB124	pAt15955Δ270	Cured of pTi15955 via incompatibility	This study
IBJUN138	pAt15955	pTi15955-cured derivative with intact pAt15955	This study
IB125	pAt15955::Km^R; pTi15955		This study
IB126	Circ::Sp^R; pAt15955; pTi15955::*gusA/*Gm^R	IB123 marked on circular chromosome	This study
IB127	Circ::Sp^R; pAt15955Δ270	IB124 marked on circular chromosome	This study

IB128	Circ::Sp^R; pAt15955	IBJUN138 marked on circular chromosome	This study
IB129	Circ::Sp^R; pAt15955∆270; pTi15955::*gusA*/Gm^R	IB127 with pTi15955 reintroduced through mating with IB123	This study
IB130	Circ::Sp^R; pAt15955; pTi15955::*gusA*/Gm^R	IB128 with pTi15955 reintroduced through mating with IB123	This study
IB118 *lox*L	pAt15955*lox*L::*lacZ*/Sp^R; pTi15955::*gus*/Gm^R	IB118 with two *loxP* sites introduced within left half (**L**) of pAt15955 deletion interval	This study
IB118 *lox*R	pAt15955*lox*R::*lacZ*/Sp^R; pTi15955::*gusA*/Gm^R	IB118 with two *loxP* sites introduced within right half (**R**) of pAt15955 deletion interval	This study
IB118 *lox*L+R	pAt15955*lox*L+R::*lacZ*/Sp^R; pTi15955::*gusA*/Gm^R	IB118 with two *loxP* sites introduced within pAt15955 deletion interval	This study
IB118 ∆L	pAt15955∆L::*lacZ*/Sp^R; pTi15955::*gusA*/Gm^R	IB118 *lox*L with truncation of left half of pAt15955 segment (~130Kbp) via Cre	This study
IB118 ∆R	pAt15955∆R::*lacZ*/Sp^R; pTi15955::*gusA*/Gm^R	IB118 *lox*R with truncation of right half of pAt15955 segment (~130Kbp) via Cre	This study
IB118 ∆L+R	pAt15955∆L+R; pTi15955::*gusA*/Gm^R	IB118 *lox*L+R with truncation of entire pAt15955 segment (~270Kbp) via Cre—removing 1234 *lacZ* Sp^R marker	This study
IB131	pAt15955∆270::Km^R pTi15955	IBE13A with Km^R marker on pAt15955, outside of deletion interval	This study

IB132	pAt15955Δ270::Km^R	IB124 with Km^R marker on pAt15955, outside of deletion interval	This study
IB133	pAt15955::Km^R	IBJUN138 with Km^R marker on pAt15955, outside of deletion interval	This study
ERM52	Circ::Sp^R	*A. tumefaciens* C58 derivative, Plasmidless Ti- Ai-	(Morton et al. 2014)

Table S2: Plasmids used in this study

Plasmid	Features	Reference
pSRKKm	Broad host range *P*lac expression vector; lacIQ; Gm^R	(Khan et al. 2008)
pNPTS138	ColE1 suicide plasmid; sacB; Km^R	Gift of M. Alley
pGEM T-Easy	PCR cloning vector; Ap^R	Promega
pCRE1-Ap	Cre recombinase suicide vector	Gift from D. Rowe-Magnus; Bailey and Manoil, 2002, Nat. Biotech. 20:839-42
pIB301	pTi15955 curing vector; sacB; Km^R;	This study
pAt15955::*lacZ*/Sp^R	p*lac::lacZ;* Sp^R; marked within deletion segment	This study
pAt15955::Km^R	Km^R; marked outside deletion segment	This study
pAt15955*lox*L::*lacZ*/Sp^R	Two *loxP* sites integrated into left (**L**) side of pAt15955 segment	This study
pAt15955*lox*R::*lacZ*/Sp^R	Two *loxP* sites integrated into right (**R**) side of pAt15955 segment	This study
pAt15955*lox*L+R::*lacZ*/Sp^R	Two *loxP* sites integrated within pAt15955 segment, just inside flanking IS66	This study
pAt15955ΔL::*lacZ*Sp^R	Cre-mediated deletion of left (**L**) side of pAt15955 segment	This study
pAt15955ΔR::*lacZ*Sp^R	Cre-mediated deletion of right (**R**) side of pAt15955 segment	This study
pAt15955ΔL+R	Cre-mediated deletion of entire pAt15955 segment	This study
pAt15955Δ270::Km^R	Km^R	This study
pTi15955::*gusA*/Gm^R	p*Lac*::*gusA*; Gm^R	This study

Table S3: Primers used in this study*

Primer	Sequence	Description
IBIP1	TCACTCATTGGCGTCTCGTT	IS66 Inverse PCR
IBIP2	CGCCTATGCAAAGTGAAGCC	IS66 Inverse PCR
IBP102	GTGCAAAGGCACTCGTATCG	IS66 Inverse PCR/ Southern probe
IBP103	GGCGAACTTCTCGAACAGGA	IS66 Inverse PCR/ Southern probe
IBP200	TCCTGCAGCCCGGGGGTTGATCTCCGCAACAAGAAACGA	*repABC* NEBuild primer for curing vector
IBP201	ATGCCGATATGGTGGCCGAAGGCAT	*repABC* NEBuild primer for curing vector
IBP202	GCCACCATATCGGCATTTTCTTTTGCGTTTTTATTTGTTAAC	*sacB* NEBuild primer for curing vector
IBP203	CCGCTCTAGAACTAGTGCTATTATTTAGTGAAATGAGATATT ATGATATTTTCTGAATTGTGAT	*sacB* NEBuild primer for curing vector

*All other primer sequences used in strain creation or diagnostics are available upon request.

References

Chiang, SL,Rubin, EJ (2002). Construction of a mariner-based transposon for epitope-tagging and genomic targeting. Gene 296: 7.

Kalogeraki, VS,Winans, SC (1997). Suicide plasmids containing promoterless reporter genescan simultaneously disrupt and create fusions to target genes of diverse bacteria. Gene 188: 7.

Khan, SR, Gaines, J, Roop, RM, 2nd,Farrand, SK (2008). Broad-host-range expression vectors with tightly regulated promoters and their use to examine the influence of TraR and TraM expression on Ti plasmid quorum sensing. Applied and environmental microbiology 74(16): 5053-5062.

Morton, ER, Platt, TG, Fuqua, C,Bever, JD (2014). Non-additive costs and interactions alter the competitive dynamics of co-occurring ecologically distinct plasmids. Proc Biol Sci 281(1779): 20132173.