Supplemental Table 1. Bacterial strains and plasmids

Strain or Plasmid	Relevant Characteristics[a]	Source or Reference
Agrobacterium spp.		
C58C1RS	Sm^r, Rif^r; Ti-plasmidless derivative of C58; pAtC58	Our collection
NTL4	Ti-plasmidless derivative of C58; pAtC58; ΔtetAR	(Luo *et al.*, 2001)
Escherichia coli		
DH5α	*λ*$^-$ ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *recA1 endA1 hsdR17*(r_k^-, m_k^-) *supE44 thi-1 gyrA relA1*	Invitrogen
S17-1	Tp^r, Sm^r, *recA*, *thi*, *pro*, *hsdR-M*+ RP4::2-Tc::Mu::Km Tn7 λpir	(Simon *et al.*, 1983)
S17-1/λpir	λpir lysogen of S17-1	(Simon *et al.*, 1983)
Su202	Cm^r, Kan^r; *lexA71*::Tn5(Def) *sulA211* Δ*(lacIPOZYA)169/F' lacI*^{q}*lacZΔM15*::Tn9	(Dmitrova *et al.*, 1998)
Plasmids		
pAoF64/95	Wild type mannopine catabolism plasmid	(Wetzel *et al.*, 2014)
pAoF64/95::*mrtR*pVik107	Kan^r; *mrtR*::*lacZ* translational fusion; Δ*mrtR*	This work
pAoF64/95::*mrtR*pVik112	*Kan^r; mrtR*::*lacZ* transcriptional fusion; *mrtR*$^+$	This work
pAoF64/95::*tmsP*pVik107	*Kan^r; tmsP*::*lacZ* translational fusion; Δ*tmsP*	This work
pAoF64/95::*tmsP*pVik112	*Kan^r; tmsP*::*lacZ* transcriptional fusion, *tmsP*$^+$	This work
pAoF64/95::*traM*pVik107	*Kan^r; traM*::*lacZ* translational fusion; Δ*traM*	(Wetzel *et al.*, 2014)
pAoF64/95::*traR*pVik107	*Kan^r; traR*::*lacZ* translational fusion; Δ*traR*	This work
pAoF64/95::*traR*pVik112	Kan^r; *traR*::*lacZ* transcriptional fusion; traR$^+$	This work
pAoF64/95Δ*mocC*	Kan^r; Δ*mocC*	This work
pAoF64/95Δ*mocD*	Kan^r; Δ*mocD*	This work
pAoF64/95Δ*mrtR*	Kan^r; Δ*mrtR*	(Wetzel *et al.*, 2015)
pAoF64/95Δ*mrtRtmsP*::*lacZ*	Kan^r, Tet^r; Δ*mrtR*::*tetA* mutation of pAoF64/95::*tmsP*pVik112	This work
pAoF64/95ΔmtrRΔ*tmsP*::*lacZ*	Kan^r, Tet^r; Δ*mrtR*::*tetA* mutation of pAoF64/95::*tmsP*pVik107	This work
pAoF64/95Δ*tmsP*	Kan^r; Δ*tmsP*	This work
pAoF64/95Δ*tmsP*Δ*mrtR*	Kan^r, Tet^r; Δ*mrtR*::*tetA* mutation of pAoF64/95Δ*tmsP*	This work
pAoF64/95Δ*traM*	Kan^r; Δ*traM*	(Wetzel *et al.*, 2015)
pAoF64/95Δ*traR*	Kan^r; Δ*traR*	(Wetzel *et al.*, 2014)
pAW19	Amp^r, Kan^r; *sacB*	(Metcalf *et al.*, 1996)
pDP804	Amp^r; Expression plasmid carrying a *LexA$_{1-87}$408::Jun*	(Dmitrova *et al.*, 1998)
pJW106	Sp^r/Sm^r; λ Red helper plasmid	(Quick *et al.*, 2010)
pKD4	Amp^r; Frt flanked Kan^r cassette	(Datsenko & Wanner, 2000)
pKD46	Amp^r; λ Red helper plasmid	(Datsenko & Wanner, 2000)
pL6480p*traR*::*lacZ*	Tet^r, *traR*::lacZY of pLKC480::*traR* subcloned into pLAFR6	This work
pLAFR6	Tet^r; IncP; multilinker of pUC18 flanked by synthetic *trp* terminators	(Huynh *et al.*, 1989)
pLKC480	Kan^r; plasmid carrying *lacZY* cassette	(Tiedeman & Smith, 1988)
pLKC480::*traR*	Kan^r; 5' intergenic region and 5' portion of *traR* cloned into pLKC480	This work

pMS604	Tet^r; Expression plasmid carrying a *LexA*$_{1-87}$WT::*Fos*	(Dmitrova *et al.*, 1998)
pMWS109	Tet^r; Cosmid clone of pAoF64/94 containing the *tra* operons through part of the *trb* operon	(Wetzel *et al.*, 2014)
pMWS109*traA*::*lacZ*	Tet^r, Amp^r/Cb^r; *traA*::*lacZ*; Tn3-HoHo1 transposed into pMWS109	This work
pMWS112	Tet^r; Cosmid clone of pAoF64/9 containing mannopine uptake and utilization genes, *mrtR, tmsP*	(Wetzel *et al.*, 2014)
pRG970::*traC*	Sp^r, Amp^r/Cb^r; *traC-traA* intergenic region of pAoF64/95 cloned into pRG970b; *traC*::*lacZ*	This work
pRG970b	Sp^r, Amp^r/Cb^r; Bidirectionall *lacZY* and *uidA* fusion vector	(Van den Eede *et al.*, 1992)
pRG970b::*pmrtRpmocC*	Sp^r, Amp^r/Cb^r; *mrtR-mocC* intergenic region cloned into pRG970b; mrtR::*lacZ; mocC*::*uidA*	This work
pRG970b::*traA*	Sp^r, Amp^r/Cb^r; *traA-traC* intergenic region cloned into pRG970b; *traA*::*lacZ*	(Wetzel *et al.*, 2015)
pRG970b::*traI*	Sp^r, Amp^r/Cb^r; *traI-repA* intergenic region cloned into pRG970b; *traI*::*lacZ*	This work
pSa152	Sp^r, Kan^r, Cm^r; IncW cloning vector	(Tait *et al.*, 1983)
pSa152::*mot*15955	Sp^r, Kan^r; mannopine transport region (*mot*) of 15955 cloned into pSa152	This work
pSR658	Tet^r; ColE1 expression plasmid for LexA-WT dimerization system	(Daines *et al.*, 2002)
pSR658::*tmsPs*	*lexA*-WT::*tmsPs*	This work
pSR658::*traM*	*lexA*-WT::*traM*	This work
pSR659	Amp^r; p15A expression plasmid for LexA-MUT dimerization system	(Daines *et al.*, 2002)
pSR659::*tmsPs*	*lexA*-MUT::*tmsPs*	This work
pSR659::*traM*	*lexA*-MUT::*traM*	This work
pSRKGm	Gm^r; IPTG-inducible expression vector	(Khan *et al.*, 2008)
pSRKGm::*mrtR*	Gm^r; *mrtR* cloned into pSRKGm	This work
pSRKGm::*tmsPl*	Gm^r; longest version of *tmsP* cloned into pSRKGm	This work
pSRKGm::*tmsPm*	Gm^r; medium version of *tmsP* cloned into pSRKGm	This work
pSRKGm::*tmsPs*	Gm^r; shortest version of *tmsP* cloned into pSRKGm	This work
pSRKGm::*traM*	Gm^r; *traM* cloned into pSRKGm	(Wetzel *et al.*, 2014)
pSRKGm::*traR*	Gm^r; *traR* cloned into pSRKGm	(Wetzel *et al.*, 2014)
pSShe	Cm^r; transposase helper plasmid, *tnpA*$^+$	(Stachel *et al.*, 1985)
pTnHoHo1	Amp^r/Cb^r; Tn3-HoHo1, transposable *lacZYA* sequence, *tnpA*$^-$, *tnpR*$^+$	(Stachel *et al.*, 1985)
pVik107	Kan^r; promoterless *lacZY*; *pir* dependent; used for translational fusions	(Kalogeraki & Winans, 1997)
pVik112	Kan^r; promoterless *lacZY*; *pir* dependent; used for transcriptional fusions	(Kalogeraki & Winans, 1997)
pWM91	Amp^r; *sacB*	(Metcalf *et al.*, 1996)

[a] Sm^r, streptomycin resistance; Rif^r, rifampicin resistance; Tp^r, trimethoprim resistance; Cm^r, chloramphenicol resistance; Kan^r, kanamycin resistance; Tet^r, tetracycline resistance; Sp^r, spectinomycin resistance; Amp^r, ampicillin resistance; Cb^r, carbenecillin resistance; Gm^r, gentamicin resistance.

Supplemental Table 2. PCR primers used and their purposes

Plasmid	Purpose of resulting construct	Primer names	Primer sequences or citation
pRG970b::*traC*$_{pAoF64/95}$	*ptraC*::*lacZ*	traClacZBamHI	GCTAGAGGATCCTGGGTGAGAAAGCTTGTC
		traAuidAXmaI	GCTAGACCCGGGAAATCTTTTCCCGGTTGC
pRG970b::*traI*$_{pAoF64/95}$	*ptraI*::*lacZ*	traIlacZBamHI	GGTATAGGATCCTGTGGGTCGGCGGGGAAG
		repAuidAXmaI	GGTATACCCGGGGCAAACTATATCCTGGGAGGCTAAAC
pRG970b::*mrtRmocC*	*pmocC*::*uidA*	mocCuidAXmaI	GCTAGACCCGGGTCTTCACTTTCTAACGAAGC
		mrtRlacZBamHI	GCTAGAGGATCCCAATTGGTATGCTTGC
pLKC480::*traR*	*traR*::*lacZ*	traRintersenseSalI	GAATTCTATGTCGACAGAGCTATTGC
		traRinterantisenseHindIII	TTTGATCATCCGTTCGTCATGCGAAGCTTCTGTCATGTCG
pVik112::3'*traR*	*traR*::*lacZ*	midtraRpVIk107EcoRI	GAGTACGAATTCCCGCATCGACCCGGTG
		midtraRpVIk107SalI	GAGTACGTCGACGGGTATAGTCACCCCAC
pVik107::mid*traR*	Δ*traR*::*lacZ*	3'traRpVik112EcoRI	GTCGACGAATTCGGTTCTGGCAATCCATTAC
		3'traRpVik112KpnI	GGATCCGGTACCCTACAGCAGGCCGTGGTC
pVik112::3'*mrtR*	*mrtR*::*lacZ*	3'mrtRpVik112EcoRI	CAGCTGGAATTCTGAATCTCTCAGCGGGCGCT
		3'mrtRpVik112KpnI	CAGCTGGGTACCTAGCCACCTTTGTGTGGTGT
pVik107::mid*mrtR*	Δ*mrtR*::*lacZ*	midmrtRpVik107XmaI	CAGCTGCCCGGGCAAGATCTCCTGCGGTCTATT
		midmrtRpVik107SalI	CAGCTGGTCGACTTCACTTATGGGCCGGTCGAA
pVik112::3'*tmsP*	*tmsP*::*lacZ*	3'tmsPEcoRIpVik112	GCATGACTGAATTCCGGTGGATTTCTCAAGGCAGG
		3'tmsPXbaIpVik112	GCATGACTTCTAGATTATTCAACCGGCTTTTGAAGCTC
pVik107::mid*tmsP*	Δ*tmsP*::*lacZ*	midtsmPEcoRIpVik107	GCATGACTGAATTCGAGTGCGAGTTTGTCATGAAG
		midtmsPSalIpVik107	GCATGACTGTCGACCGCGCGCTCAAGGCAGAGCTC
pSa152::*mot*19555	Expression of the mannopine transporter	motA15955XmaI	GGTATACCCGGGACGACGCTGATTTCGCA
		motD15955XmaI	GGTATACCCGGGTCGTTTTCCCCTCTAGGGG
pSRKGm::*mrtR*	Expression of *mrtR*	mrtRsenseNdeI	GCCCGAGCTCATATGGACACCGAGCCAATGTAC
		mrtRantisenseBamHI	GCGGAGCTCGGATCCTAGCCACCTTTGTGTGGTG
pSRKGm::*tmsPI*	Expression of *tmsPI*	tmsPstart1NdeI	CTAGCTAGCATATGGCTGCCGTCGCACAG
		tmsPstopBamHI	CTAGCTAGGGATCCTTATTCAACCGGCTTTTGAAGC
pSRKGm::*tmsPm*	Expression of *tmsPm*	tmsPstart2NdeI	CTAGCTAGCATATGGCTTCAACCGACACTTCAATGC
		tmsPstopBamHI	See above
pSRKGm::*tmsPs*	Expression of *tmsPs*	tmsPstart3NdeI	CTAGCTAGCATATGCTGGCGTCCGGTACCAA
		tmsPstopBamHI	See above
pSR658::*traM*	Expression of *lexA*(WT)::*traM*	traMstartATGSacI	TCGATCGAGCTCATGAGCGACGTGAACTCG
		traMendKpnI	TCGATCGGTACCTCAATCACCGACTTCGGG
pSR659::*traM*	Expression of *lexA*(Mut)::*traM*	traMstartATGSacI	See above
		traMendKpnI	See above
pSR658::*tmsPs*	Expression of *lexA*(WT)::*tmsPs*	tmsPstart3XhoI	TCGATCCTCGAGATGCTGGCGTCCGGTAC
		tmsPendPstI	TCGATCCTGCAGTTATTCAACCGGCTTTTGAAGCTC
pSR659::*tmsPs*	Expression of *lexA*(Mut)::*tmsPs*	tmsPstart3XhoI	See above
		tmsPendHindIII	TCGATCAAGCTTATTCAACCGGCTTTTGAAGCTC
pMWS112Δ*mocC*	Δ*mocC*	mocCforwardKan	CTCGGGCGACAACTGCCCGCAGACAGCTTCGTTAGAAAGTGAAGAACCTGTGTAGGCTGGAGCTGCTTCG
		mocCreverseKan	CAGACTTGCGCACAGACATCTAAATGCTCCCACGGCACCCATTGATCACATATGAATATCCTCCTTAGT
pMWS112Δ*mocD*	Δ*mocD*	mocDforwardKan	AAACCTGAAGCTTATACCCAACGCCTGACTAGATAAAGGAATGATCAATGTGTAGGCTGGAGCTGCTTCG
		mocDreverseKan	GCATTTGTTTAGGGAGGCGAAACGAACTGCGAACCGCCTCCCGGACTACATATGAATATCCTCCTTAGT
pMWS112Δ*tmsP*	Δ*tmsP*	tmsPforwardKan	CGATACCGCTCGAACTCCCAGCCTCCTCCCGGGGCACCTGTGTAGGCTGGAGCTGCTTCG
		tmsPreverseKan	GGCGCGGTGTCAGGGGATAGAGCGTTCAGGGCGCGATCGCATATGAATATCCTCCTTAGT
pAW19::112Δ*mrtR*(*tetA*)	Δ*mrtR*(*tetA*)	mrtRforwardTet	AGTGACAGTCGCAAGCATACCAATTGGAATGGAGAGCCCATCCCTCTTGACTTATCATTG
		mrtRreverseTet	GTGGAAAATCTGTTCGGACTTAAAGCGGTCTCGAAGAGGGGCGCATGACGTCCGGAGTCT
NA[a]	confirmation of the *mocC* mutation	mocCforwardwannercheck	CTCCAGCCGCTCTGAAGAGC
		mocCreversewannercheck	GCCGCATGACAACGCCGAACG
NA	confirmation of the *mocD* mutation	mocDforwardwannercheck	GCTCGGTCAAGTCTTCAAGC
		mocDreversewannercheck	CGTGAAGCTGGACTAGGGTG
NA	confirmation of the *tmsP* mutation	KT	(Datsenko & Wanner, 2000)
		mocDforwardNdeI	GATCGATCCATATGACAACCCAAAAGACTGATG
NA	confirmation of the *mrtR*(*tetA*) mutation	mrtRcheckup	(Wetzel, *et. al*., 2015)
		mrtRcheckdwn	(Wetzel, *et. al*., 2015)

[a]. NA: not applicable

Supplemental Figure 1. Opines induce transfer of Ti plasmids because they induce transcription of traR. The operon colored black is an operon that is inducible by the conjugative opine. The *tra* regulon is light grey and the plasmid replication and partitioning genes are dark grey. The genes and proteins involved in regulation of transfer are as follows: *traR*/TraR, black dots on a white background; *traM*/TraM, diagonal stripes; *traI*/TraI, white; and a transcriptional regulator that also senses the conjugative opine, horizontal stripes; Agrobacterium autoinducer (AAI) is indicated by σ, while the conjugative opine is indicated by asterisks (*). Promoters are indicated by "P" and transcriptional direction is indicated by the arrows. (A) Under non-inducing conditions TraM binds to the low levels of TraR that are produced thereby inactivating the transcriptional regulator. (B) Under inducing condition the presence of the conjugative opine induces transcription of an opine-regulated operon, including *traR*. In this manner TraR titrates the effects of TraM and, as long as the population has reached its critical threshold and enough AAI is available, TraR can form active dimers and activate transcription of the *tra* regulon.

Supplemental Figure 2. TmsPs aligns with the C terminal domain of TraR proteins. The drawing represents the N terminal AAI binding domain and C terminal DNA binding domain of TraR proteins. The region of TraR that interacts with TraM is also located at the C terminal region. TmsPs and the C terminal region of three TraR proteins were aligned using Clustal W. Invariant residues are purple, conserved resides are blue, while residues that are similar are pink. Above the aligned sequences, the residues of $TraR_{pTiC58}$ important for TraR-TraM interaction (Luo *et al.*, 2000, Qin *et al.*, 2007) are indicated with a black five-

pointed star, while the residues of $TraR_{pNGR234a}$ that are sequence-specific for the TraR-TraM interaction (Chen *et al.*, 2007) are indicated by a white 4-pointed star.

Supplemental Figure 3. TmsP has three potential start sites. The nucleotide sequence of *tmsP* and 100 nucleotides upstream of the gene are shown. The three potential start sites are labeled and colored in green, while the stop codon is colored red.

Supplemental Figure 4. A putative MrtR-binding box is encoded in the intergenic sequence between mrtR and mocC. (A) *mrtR* and *mocC* are divergently transcribed. The sequence of the nucleotides between the two genes is shown with the start codons for each gene colored in green. The putative MrtR-binding box is a 14 base pair palindromic sequence and is colored red. (B) An alignment of the DNA binding sites for the GntR regulator ExuR from *Escherichia coli* (Rigali *et al.*, 2002), and the putative MrtR box of pAoF64/95.

Supplemental Figure 5. MocR does not regulate transfer of pAoF64/95. NTL4 harboring pAoF64/95 and the *mocR* mutant, NTL4(pAoF64/95Δ*mocR*) were assessed for conjugative transfer when grown in AB minimal medium with mannitol (light grey) or mannopine (dark grey) as the primary source of carbon.

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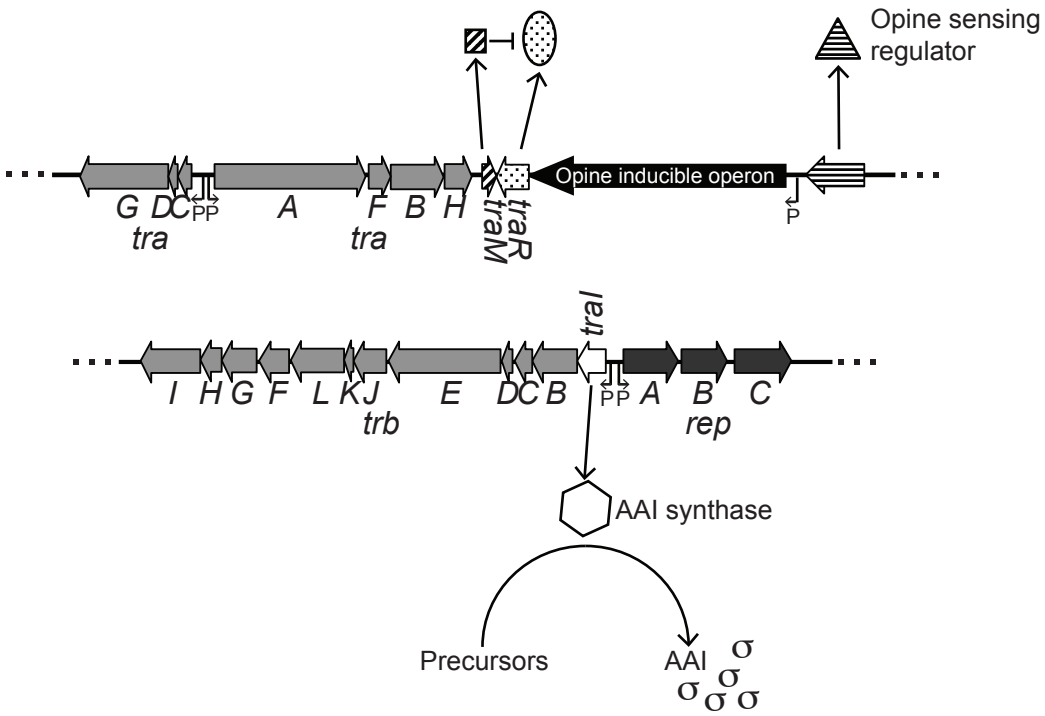
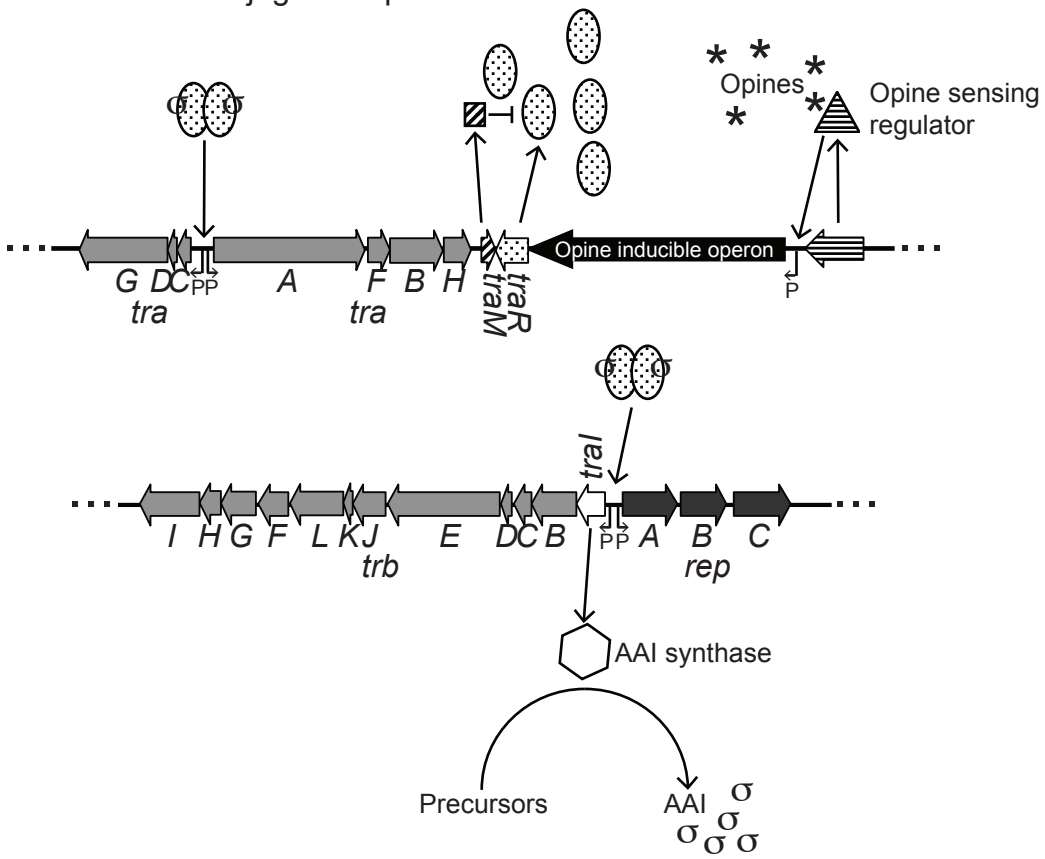
Supplemental Figure 1

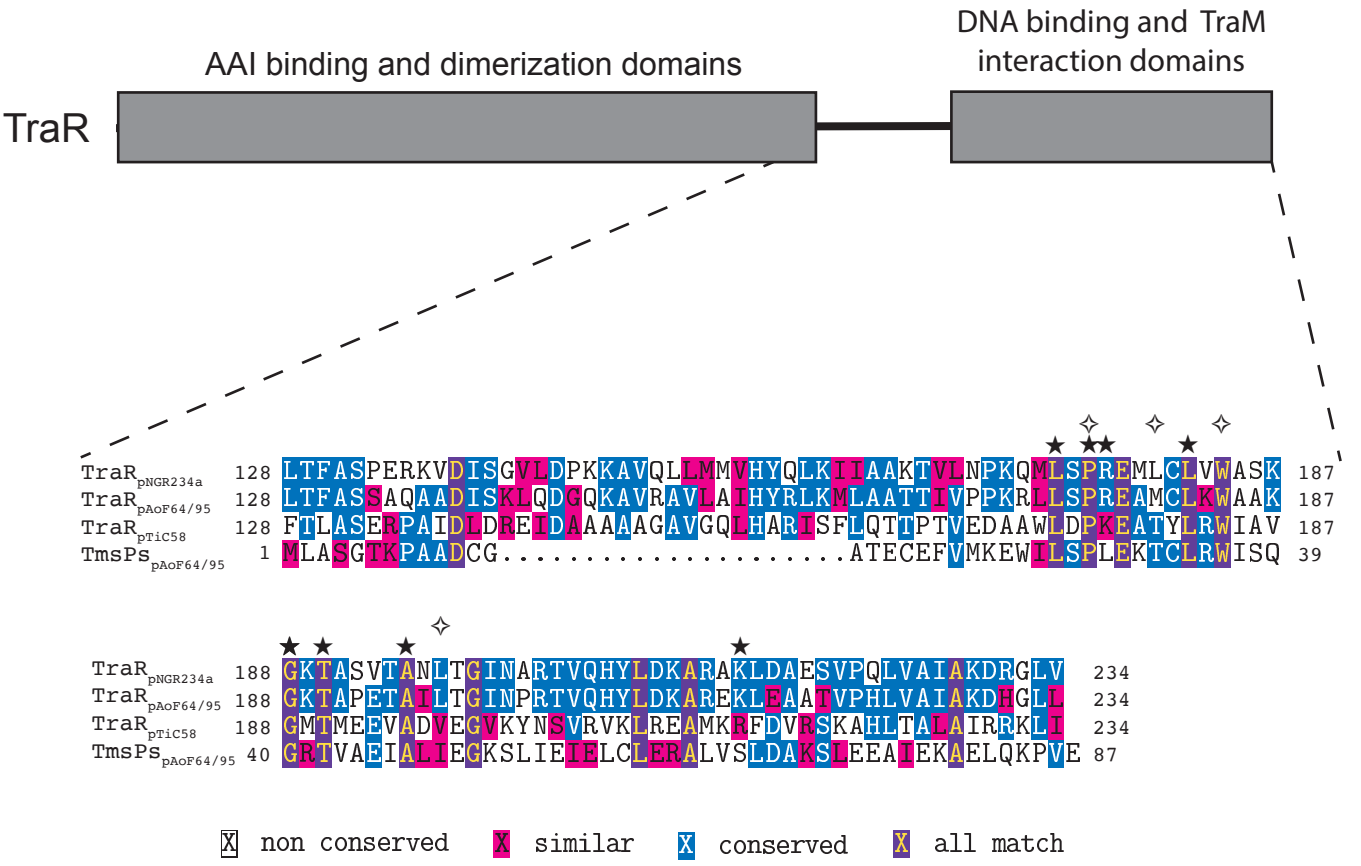
A Uniduced conditions: no conjugative opine



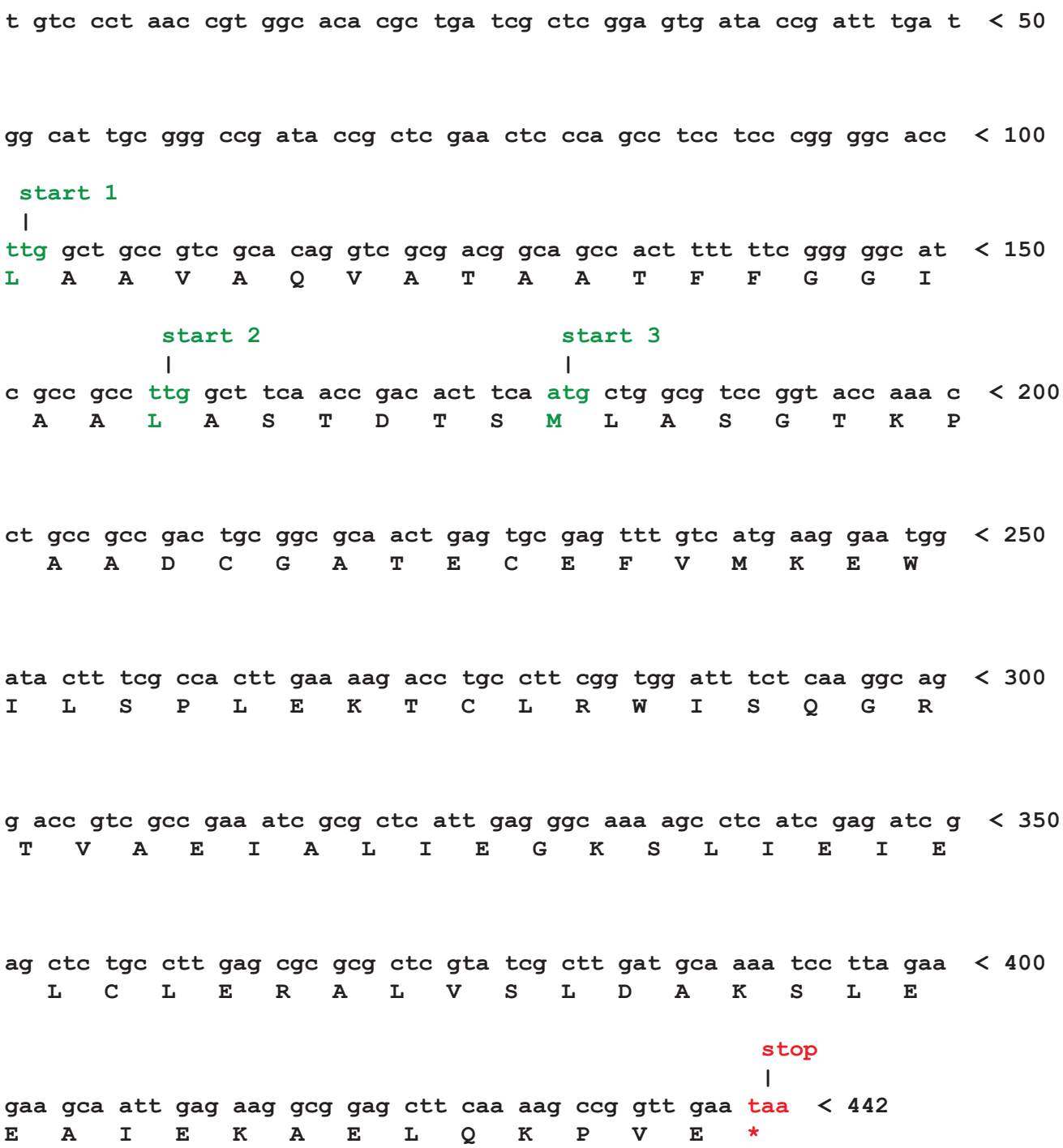
B Induced conditions: conjugative opine



Supplemental Figure 2

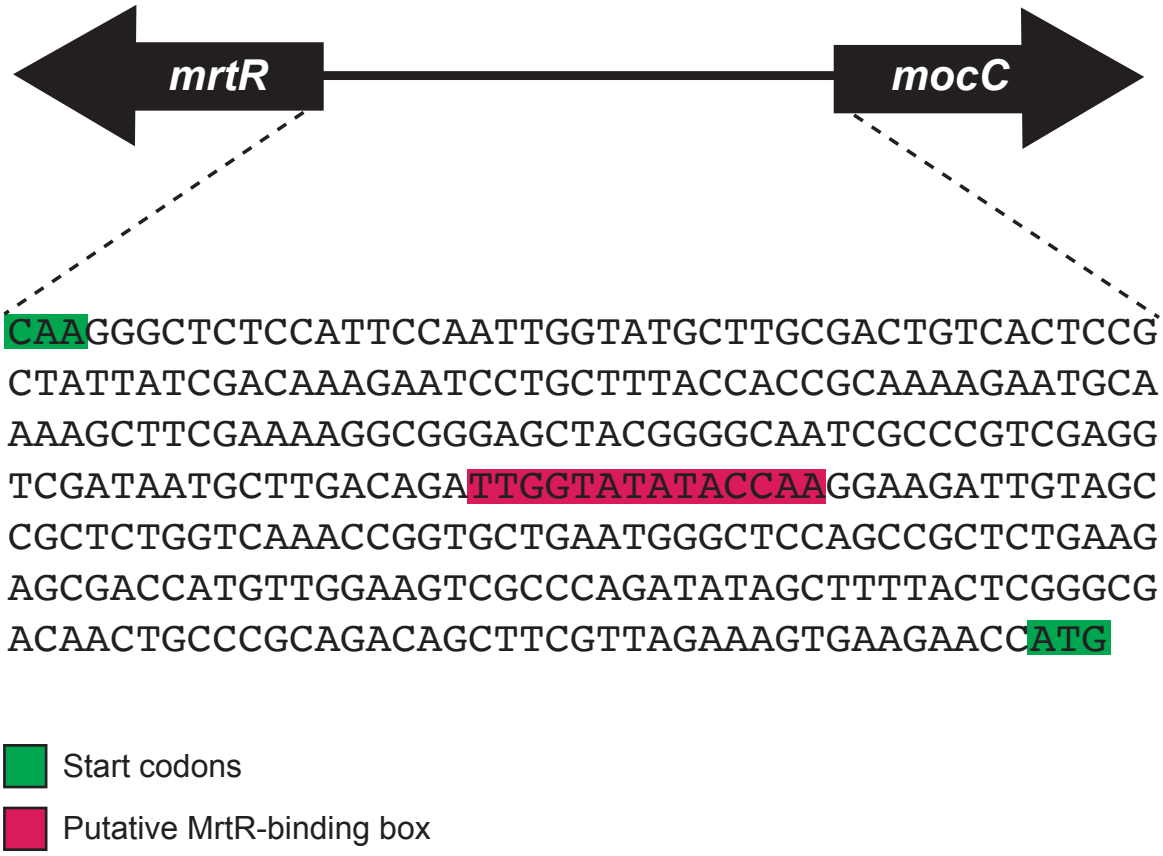


Supplemental Figure 3

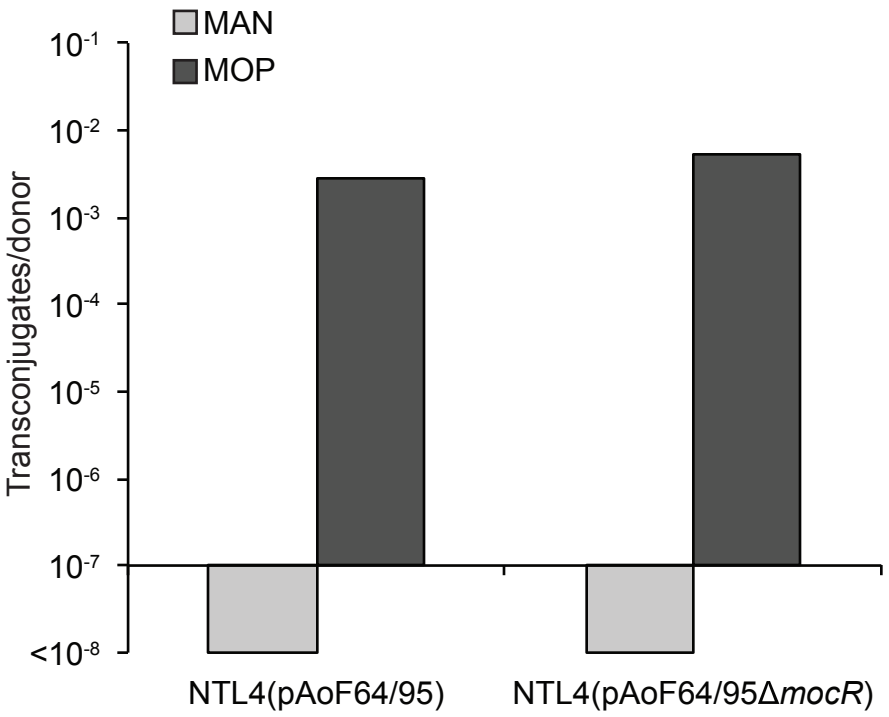


Supplemental Figure 4

A



B

Regulator	Operator site sequences
ExuR	AAATTGGTATA..CCAATTT
MrtR	...TTGGTATATACCAA...

Supplemental Figure 5