Homologues of genetic transformation DNA import genes are required for *Rhodobacter capsulatus* gene transfer agent recipient capability regulated by the CtrA response regulator

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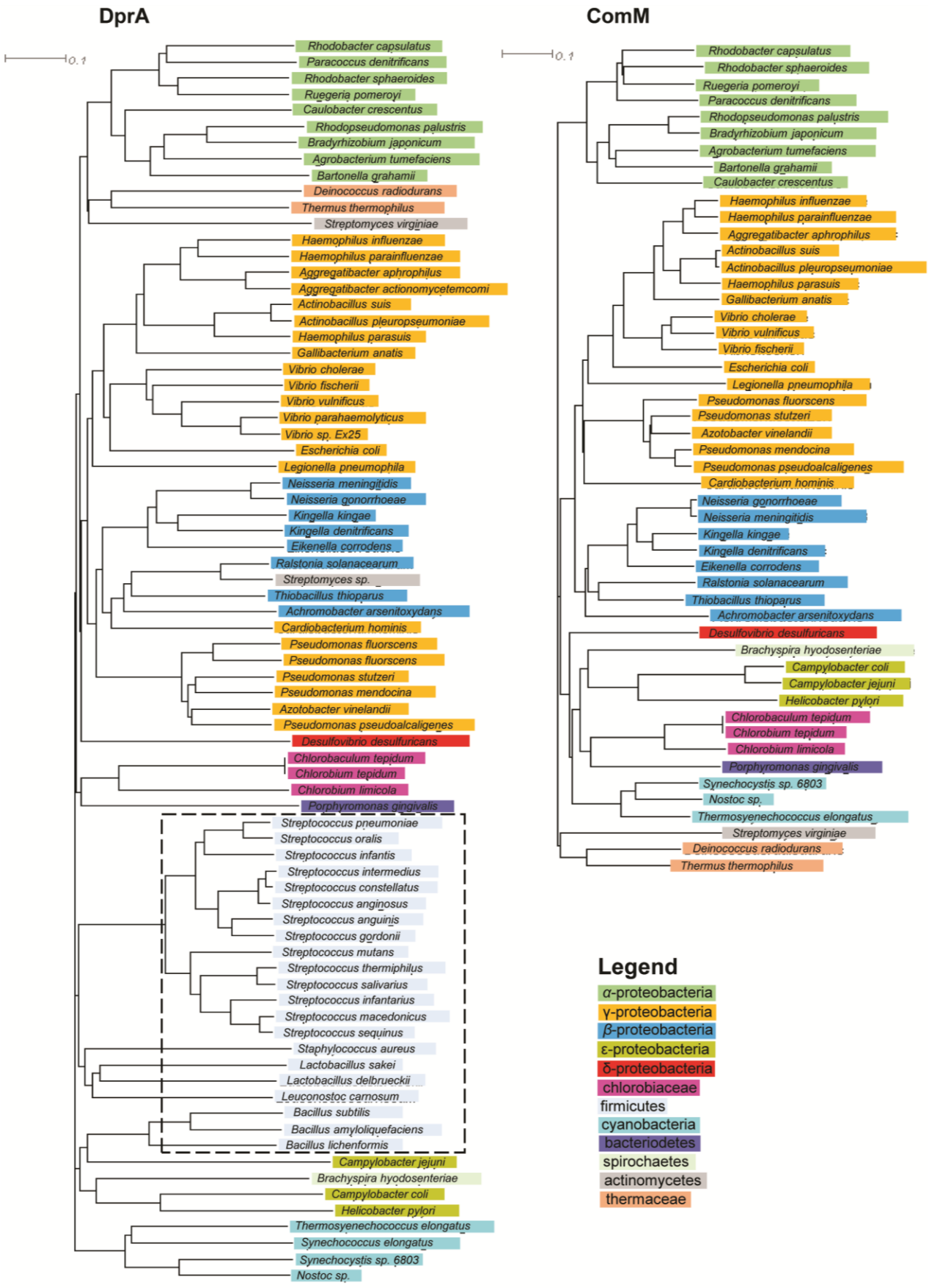
Supplemental Material


DprA
0.1
Rhodobacter capsulatus
Paracoccus denitrificans
Rhodobacter sphaeroides
Ruegeria pomeroyi
Caulobacter crescentus
Rhodopseudomonas palustris
Bradyrhizobium japonicum
Agrobacterium tumefaciens
Bartonella grahamii
Deinococcus radiodurans
Thermus thermophilus
Streptomyces virginiae
Haemophilus influenzae
Haemophilus parainfluenzae
Aggregatibacter aphrophilus
Aggregatibacter actinomycetemcomi
Actinobacillus suis
Actinobacillus pleuropseumoniae
Haemophilus parasuis
Gallibacterium anatis
Vibrio cholerae
Vibrio fischerii
Vibrio vulnificus
Vibrio parahaemolyticus
Vibrio sp. Ex25
Escherichia coli
Legionella pneumophila
Neisseria meningitidis
Neisseria gonorrhoeae
Kingella kingae
Kingella denitrificans
Eikenella corrodens
Ralstonia solanacearum
Streptomyces sp.
Thiobacillus thioparus
Achromobacter arsenitoxydans
Cardiobacterium hominis
Pseudomonas fluorscens
Pseudomonas fluorscens
Pseudomonas stutzeri
Pseudomonas mendocina
Azotobacter vinelandii
Pseudomonas pseudoalcaligenes
Desulfovibrio desulfuricans
Chlorobaculum tepidum
Chlorobium tepidum
Chlorobium limicola
Porphyromonas gingivalis
Streptococcus pneumoniae
Streptococcus oralis
Streptococcus infantis
Streptococcus intermedius
Streptococcus constellatus
Streptococcus anginosus
Streptococcus anguinis
Streptococcus gordonii
Streptococcus mutans
Streptococcus thermiphilus
Streptococcus salivarius
Streptococcus infantarius
Streptococcus macedonicus
Streptococcus sequinus
Staphylococcus aureus
Lactobacillus sakei
Lactobacillus delbrueckii
Leuconostoc carnosum
Bacillus subtilis
Bacillus amyloliquefaciens
Bacillus lichenformis
Campylobacter jejuni
Brachyspira hyodosenteriae
Campylobacter coli
Helicobacter pylori
Thermosynechococcus elongatus
Synechococcus elongatus
Synechocystis sp. 6803
Nostoc sp.
ComM
0.1
Rhodobacter capsulatus
Rhodobacter sphaeroides
Ruegeria pomeroyi
Paracoccus denitrificans
Rhodopseudomonas palustris
Bradyrhizobium japonicum
Agrobacterium tumefaciens
Bartonella grahamii
Caulobacter crescentus
Haemophilus influenzae
Haemophilus parainfluenzae
Aggregatibacter aphrophilus
Actinobacillus suis
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Neisseria gonorrhoeae
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Chlorobaculum tepidum
Chlorobium tepidum
Chlorobium limicola
Porphyromonas gingivalis
Synechocystis sp. 6803
Nostoc sp.
Thermosynechococcus elongatus
Streptomyces virginiae
Deinococcus radiodurans
Thermus thermophilus
Legend
α-proteobacteria
γ-proteobacteria
β-proteobacteria
ε-proteobacteria
δ-proteobacteria
chlorobiaceae
firmicutes
cyanobacteria
bacteriodetes
spirochaetes
actinomycetes
thermaceae

Figure S1. Neighbour joining phylogenetic tree representation of DprA (left) and ComM (right) homologues from all naturally competent and gene transfer agent containing bacterial genomes. Shown in the dashed box are all DprA homologues which lack a DprA domain 3; all genomes in which the DprA protein encodes a predicted DD3 also encode a ComM homologue. Multiple sequence alignment was done using the MUSCLE algorithm.

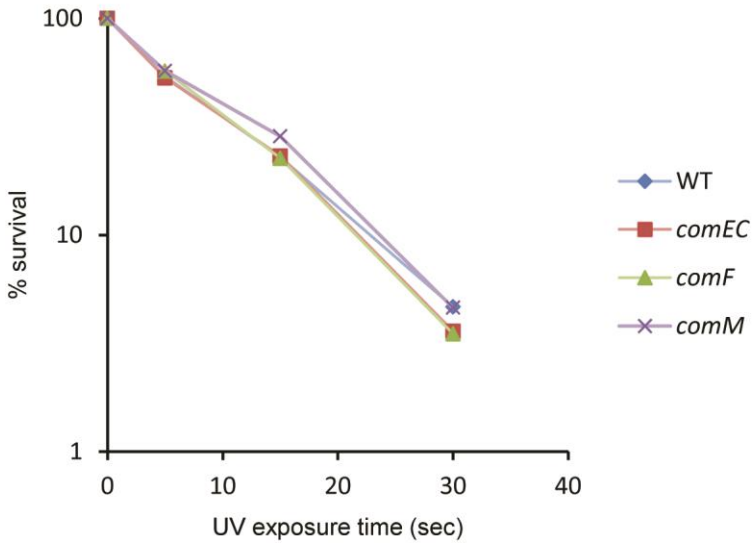

Figure S2. UV-sensitivity of WT, *ΔcomEC*, *ΔcomF*, and *ΔcomM* strains expressed as % survival.

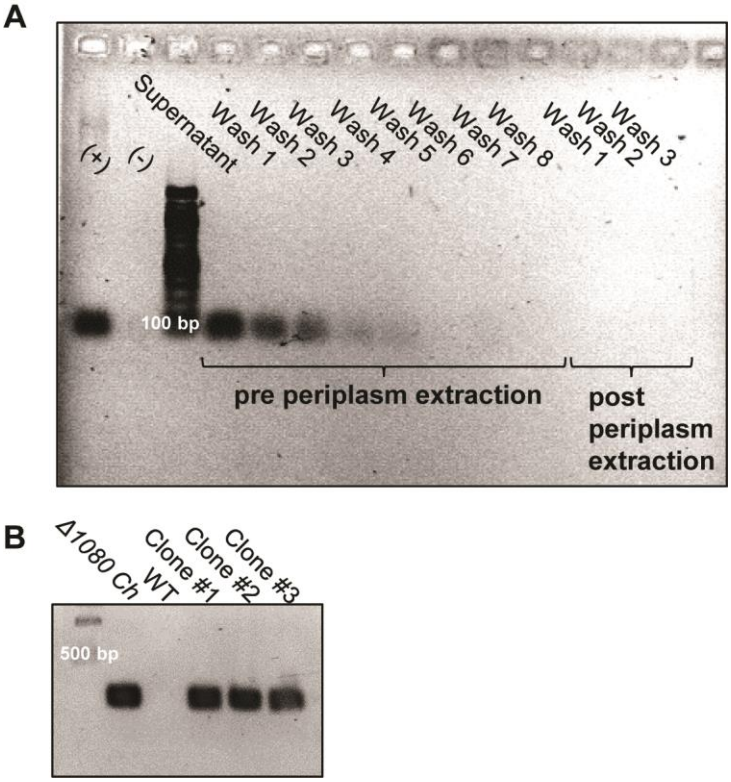

Figure S3. RcGTA-borne DNA tracking assay control experiments**. A,** Evaluation of residual RcGTA-borne DNA release from cells after the consecutive wash steps described in the RcGTA tracking assay. 2.5 µl of each fraction were used as a PCR template with the Gm_qPCR primers to detect RcGTA-borne DNA. (+) indicates a positive control reaction where *Δ1080* mutant chromosomal DNA was used as a PCR template, and (-) indicates a negative control where WT B10 DNA was used as a PCR template. The PCR program used was: 95 °C 2:00 min, [95 °C 15 sec, 55 °C 15 sec, 72 °C 30 sec]x30, 72 °C 7:00 min. **B,** PCR products obtained when using *Δ1080* mutant chromosome DNA, WT B10 chromosomal DNA, or three gentamycin-resistant colonies obtained from the RcGTA tracking assay as a PCR template using the Gm_for and Gm_rev primer set.

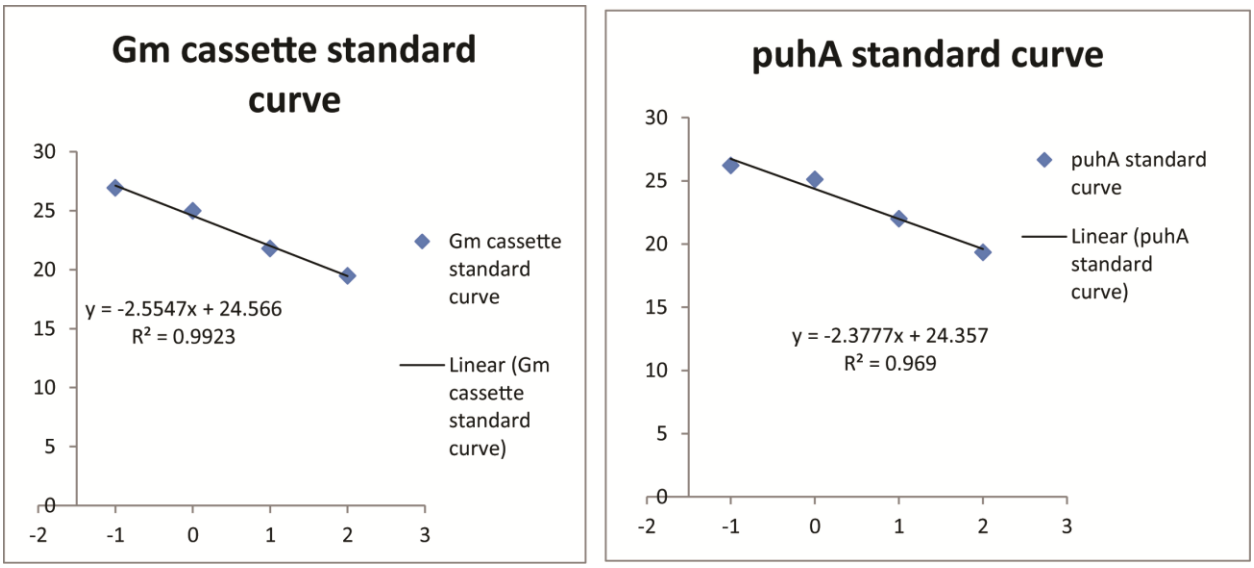

Figure S4. Calibration curves for Gm cassette and *puhA* qPCR primer sets with known concentrations of DNA and associated Ct values. These standards were used to calculate the concentration of DNA obtained from the RcGTA tracking assay.

Table S1. Presence of ComEC and ComF homologues in other bacterial species that contain function or putative GTAs, or are naturally competent.

Species	**ComEC**	**ComF**	**Verified funtional gene transfer agent**	**Putative gene transfer agent**	**Naturally competent**
Rhodobacter capsulatus	yes	yes	yes	N/A	no
Synechocystics PC 6803	yes	yes	no	no	yes
Helicobacter pylori	yes	yes	no	no	yes
Rhizobium elti	yes	yes	no	yes	no
Bartonella grahamii	yes	yes	yes	N/A	no
Brucella abortus	yes	yes	no	yes	no
Sphingomonas alaskensis	yes	no	no	yes	no
Caulobacter crescentus	yes	yes	no	yes	no
Rhodopseudomonas palustris	yes	yes	no	yes	no
Nitrobacter hamburgensis	yes	yes	no	yes	no
Rickettsia felis	yes	yes	no	yes	no
Ruegeria mobilis	yes	yes	yes	N/A	no
Paracoccus denitrificans	yes	yes	no	yes	no
Rhodobacter sphaeroides	yes	yes	no	yes	no
Ruegeria pomeroyi	yes	yes	yes	N/A	no
Roseovarious nubinhibens	yes	yes	yes	N/A	no
Wolbachia pipientis	yes	yes	no	yes	no
Haemophils influenzae	yes	yes	no	no	yes
Vibrio cholerae	yes	yes	no	no	yes
Desulfovibria desulfuricans	yes	yes	yes	N/A	no
Streptococcus pneumoniae	yes	yes	no	no	yes
Bacillis subtilis	yes	yes	no	no	yes
Neisseria meningitidis	yes	yes	no	no	yes

Table S2. One way ANOVA results comparing relative RcGTA-borne periplasmic DNA of WT, *ΔcomEC*, and *ΔcomF* strains.

Strain 1	**Strain 2**	**p-value**
WT	*ΔcomEC*	0.02029
WT	*ΔcomF*	0.0000181
ΔcomEC	*ΔcomF*	0.4518

Table S3. Raw data for RcGTA tracking assay qPCR analysis

log10(DNA conc.)	**GmR cassette target**	**Ct**	**Calculated DNA conc. (ng)**	**log10(DNA conc.)**	***puhA* target**	**Ct**	**Calculated DNA conc. (ng)**	
	0 ng	37.02			0 ng	36.93		
-1	0.1 ng	26.92		-1	0.1 ng	26.22		
0	1 ng	24.97		0	1 ng	25.11		
1	10 ng	21.79		1	10 ng	22.00		
2	100 ng	19.46		2	100 ng	19.33		
								Ratio Gm/puhA (x100)
	No GTA1	27.66	0.0612		No GTA1	15.81	2756.25	0.002223
	WT1	20.10	55.75		WT1	14.57	9224.23	0.604468
	*ΔcomEC*1	18.42	252.99		*ΔcomEC*1	15.12	5407.12	4.678850
	*ΔcomF*1	18.07	347.78		*ΔcomF*1	14.34	11547.12	3.011910
	No GTA2	29.72	0.0095		No GTA2	16.15	1997.92	0.000478
	WT2	20.69	32.85		WT2	15.33	4391.08	0.74828
	*ΔcomEC*2	16.98	925.67		*ΔcomEC*2	14.09	14676.18	6.30735
	*ΔcomF*2	18.00	371.01		*ΔcomF*2	14.83	7138.49	5.19734
	No GTA3	30.26	0.0058		No GTA3	17.13	771.90	0.000763
	WT3	23.62	2.34		WT3	17.99	335.43	0.698033
	*ΔcomEC*3	20.55	37.02		*ΔcomEC*3	16.60	1290.64	2.868773
	*ΔcomF*3	21.63	14.02		*ΔcomF*3	17.79	408.17	3.436477