Supplementary Material

Synthetic control of plasmid replication enables target- and self-curing of vectors and expedites genome engineering of *Pseudomonas putida*

by

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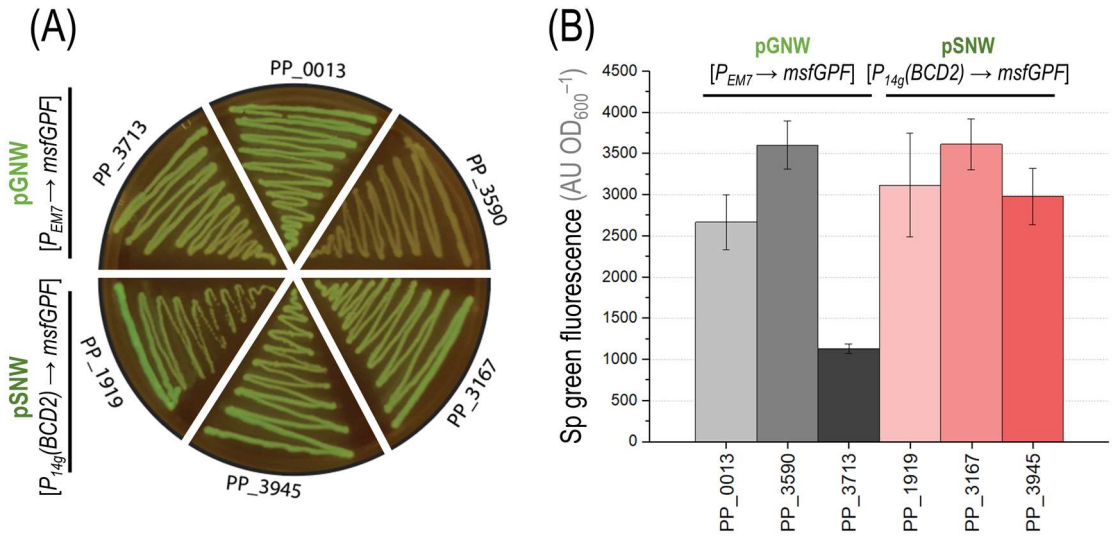
Table S1. Oligonucleotides used in this study.[a]

Oligonucleotide	Sequence (5'→3')	Use
RED-low-F†	ATC CTT GUC CAT AAA ACC GCC CAG TC	Amplification of genes encoding fluorescent markers
RED-low-R†	AGG ACT AGU TTA CTT GTA CAG CTC GTC C	
RED-mid-F†	ATC CTT GUG AAG CGA GAG CTT CAG ACC	
RED-mid-R†	AGG ACT AGU GTA GCG ATC TAC ACT AGC ACT ATC A	
RED-high-F†	ATC CTT GUG AAG CGA GAG CTT CAG ACC	
RED-high-R†	AGG ACT AGU GTA GCG ATC TAC ACT AGC ACT ATC A	
Ins-RED-F†	ACT AGT CCU AGA GTC GAC CTG CAG GCA TGC A	Reverse amplification of pS*x*28S vectors
Ins-RED-R†	ACA AGG AUC CCC GGG TAC CGA GCT CGA AT	
pQURE-F†	AGA CCG GAU CAG CTT AGT AAA GCC CTC	Construction of vector pQURE1
pQURE-R†	ATG CTG GTU GGA TGA CCT TTT GAA TGA CC	
Ins-pQURE-F†	AAC CAG CAU AAA GTG CAG TGT CCG GTT	Reverse amplification of vector pJBSD1
Ins-pQURE-R†	ATC CGG TCU GGA TTC TCA CCA ATA AAA AAC GCC C	
Ins-pQURE-Ab-F†	ATC CAT CCU CAG TGG AAC GAA AAC TCA CGT TAA GG	Swapping antibiotic resistance markers
Ins-pQURE-Ab-R†	ACC GAG CGU AGC GTC AGA CCC CGT AG AAA AG	
Ab-F†	ACG CTC GGU TGC CGC C	Amplification of antibiotic resistance markers
Ab-R†	AGG ATG GAU ATA CCG AAA AAA TCG CTA TAA	
P_{EM7}-Ins-F	TAT ATC GGC ATA GTA TAA TAC GAC AAA AGC TTA GGA GGA AAA ACA TAT GCG TAA	Construction of plasmid pS6313·GFP
P_{EM7}-Ins-R	CTA TGC CGA TGA TTA ATT GTC AAC ATT AAT TAA AGG CAT CAA ATA AAA CGA AAG GCT CA	
I-*Sce*I-Ins-F	AGG GTA ATC TTT TCC GCT GCA TAA CCC TGC	Engineering I-*Sce*I sites in SEVA plasmids
I-*Sce*I-Ins-R	GTT ATC CCT AGA CAA CGC GCG GAC CG	
P14g-F†	ATC GCC AUG CCC ATT GAC AAG GCT CTC	Amplification of a P_{14g}*(BCD2)* element
BCD2-R†	AGA AAA CCU CCT TAG CAT GAT TAA G	
GFP4BCD2-F†	ATC GCC AUG CCC ATT GAC AAG GCT CTC	Reverse amplification of pGNW vectors
EMG4GFP-R†	ATG GCG AUA GCT AGA CTG GG	
*PP_2045*_US-F†	AGA TCC UAC CCT GCA GAT CGC GCT GCG	Deletion of *PP_2045*
*PP_2045*_US-R†	ATT TCC UTG TTG TTC TTG AGG CAG AAC ACT C	
*PP_2045*_DS-F†	AGG AAA UCC CGC GGC AGC GGG CGG CGC C	
*PP_2045*_DS-R†	AGG TCG ACU GCT GGC CTG ATG CTG CTG AG	
*pvd*_US-F†	AGA TCC UCC CAC CAC CGC CAG TCG CTG	Deletion of *pvdD* (*PP_4219*)
*pvd*_US-R†	AGG GCG GCC CCU CTG GAG AAT CGA ACG	
*pvd*_DS-F†	AGG GGC CGC CCU ACC AGG GCA CCG GTC TTT	
*pvd*_DS-R†	AGG TCG ACU GCC GGC ACC GGA CCC GAG CA	
*PP_0052*_US-F†	AGA TCC UGG TGT GTG ATG AAT GTG CAA TAC CCG	Deletion of *PP_0052*
*PP_0052*_US-R†	AGG GCG CCA CGU TCT CCA ATT TAT CCG CCC	
*PP_0052*_DS-F†	ACG TGG CGC CCU GCC CCC ATA ATC AGA TTT CCA	
*PP_0052*_DS-R†	AGG TCG ACU GCT TTT GCA CCA GCT CCC AGG	

*PP_1775*_US-F†	AGA TCC UCC CGA AGA CCG GCC AGT CG	Deletion of *PP_1775*
PP_1775-US-R†	ACC CGC TGC UGC GCA CAC TCG CCT GGA CCG AAA	
PP_1775-DS-F†	AGC AGC GGG UTG GCC ATT GAA CGT TCA C	
*PP_1775*_DS-R†	AGG TCG ACU GTG ACC GTA AGG ATT TCA ACC ACA CC	
*PP_3291*_US-F†	AGG TCG ACU AGC CAT TTC GTG CCG GCG	Deletion of *PP_3291*
*PP_3291*_US-R†	AGC CTC TAC AUG GCC AAG GCT CCC AAC TTT CG	
*PP_3291*_DS-F†	ATG TAG AGG CUG TTT TCC ACG CAA CAT CA	
*PP_3291*_DS-R†	AGA TCC UAT ATC CAC TTG AGC CTG CAG GTT	
*PP_1952*_US-F†	AGA TCC UGT CAG CCT AGA TGC TGT GTT CTA T	Deletion of *PP_1952*
*PP_1952*_US-R†	AGG GGT TTU ACA CGT GGC GAA CGT CTG C	
*PP_1952*_DS-F†	AAA ACC CCU TAA AAA TAG TGC GAA TTT GTA ATT	
*PP_1952*_DS-R†	AGG TCG ACU TCC GAG GTA TCG GCC AGG	
*PP_0772*_US-F†	AGA TCC UAC GAT GGT GCT CAA ACC C	Deletion of *PP_0772*
*PP_0772*_US-R†	ATC ACA TUG TGA ATA AGG GAA CCG GTT A	
*PP_0772*_DS-F†	AAT GTG AUG GCA CAT GAA GGC ACT TTC ATG	
*PP_0772*_DS-R†	AGG TCG ACU TTG GGG CGA TGT TGG CCG	
*PP_1239*_DS-F†	ACC TGG GCU AAG TGT AGC CCA ACA CCT CAA GCA	Deletion of *PP_1239*
*PP_1239*_US-R†	AGC CCA GGU GGT CCT GAA TAG CGC TCA	
*PP_1239*_US-F†	AGA TCC UTC AAT ACC GGC ACC GAT G	
*PP_1239*_DS-R†	AGG TCG ACU TTC AAC GAC AGC TTC TTC AT	
*PP_2876*_US-F†	AGA TCC UGC ACG TTA CCG CGG GCC	Deletion of *PP_2876*
*PP_2876*_US-R†	ACA TGG UGA GTC CTT TTG TGG AGC GTG TCC	
*PP_2876*_DS-F†	ACC ATG UAA CGC TCG CGT TGA TTG T	
*PP_2876*_DS-R†	AGG TCG ACU CGG CGG TAG ACC TTG AAG CGG	
*benABCD*_US-F†	AGG CGG UTT ATT TAC CAA GCG ATG GGG A	Deletion of the *benABCD* gene cluster (*PP_3161-PP_3164*)
*benABCD*_US-R†	ATG GCC AGG GUC TCC CTT GTT ATT GTT TAG	
*benABCD*_DS-F†	ACC CTG GCC AUG TGA ACC GCA ACC TCA AGG CAA A	
*benABCD*_DS-R†	AGG TCG ACU ACT TAT TCG TTC ATC AGT GCC ACG GC	

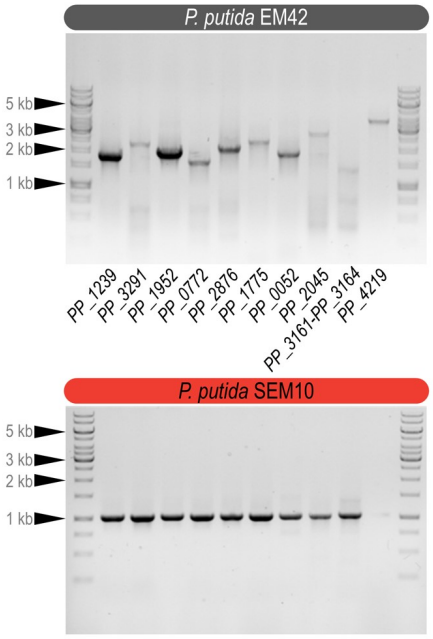
[a] Oligonucleotides designed for *USER* cloning are indicated with a † symbol.

Fig. S1. Comparison of msfGFP fluorescence in *P. putida* co-integrants carrying pGNW and pSNW vectors.



(A) *P. putida* KT2440 was co-integrated with plasmid pGNW (carrying homology regions for deletion of *PP_0013*, *PP_3590*, or *PP_3713*) or pSNW (carrying homology regions for deletion of *PP_1919*, *PP_3167*, or *PP_3945*). The only difference among plasmids of the same type is the insertion locus. An individual colony of each co-integrate was streaked onto and LB medium plate containing Km, and incubated overnight (18 h) at 30°C. The plates were photographed under blue light. **(B)** Specific (Sp) green fluorescence levels in *P. putida* KT2440 carrying the vectors indicated in **(A)** co-integrated in the chromosome. Cells were cultured in minimal de Bont medium in the presence of 30 mM glucose in microtiter plates. The Sp green fluorescence and the optical density measured at 600 nm (OD_{600}) were calculated as indicated in **Fig. 1** in the main text. Data represent the mean value of each parameter ± standard deviation of triplicate measurements from at least two independent experiments.

Fig. S2. Genetic characterization of engineered *P. putida* strain SEM10.



Gene/locus target	Forward primer	Reverse primer	Expected amplicon size (kb) in strain	
			EM42	SEM10
PP_1239	*PP_1239*_US-F	*PP_1239*_DS-R	1.8	1
PP_3291	*PP_3291*_US-F	*PP_3291*_DS-R	2.4	1
PP_1952	*PP_1952*_US-F	*PP_1952*_DS-R	1.9	1
PP_0772	*PP_0772*_US-F	*PP_0772*_DS-R	1.6	1
PP_2876	*PP_2876*_US-F	*PP_2876*_DS-R	2.1	1
PP_1775	*PP_1775*_US-F	*PP_1775*_DS-R	2.4	1
PP_0052	*PP_0052*_US-F	*PP_0052*_DS-R	1.9	1
PP_2045	*PP_2045*_US-F	*PP_2045*_DS-R	3.0	1
PP_3161-PP_3164	*benABCD*_US-F	*benABCD*_DS-R	3.8	1
PP_4219	*pvd*_US-F	*pvd*_DS-R	1.4	1

Locus-specific PCR amplification was used to characterize deletions introduced in *P. putida* EM42 to constructed strain SEM10. Note that there is a significant difference in amplicon size between the two strains that allow for rapid identification of successful recombination events leading to the target deletion. Sequencing of these amplicons indicated that no secondary, unexpected mutations were introduced because of the genome engineering adopted in this study. The sequences of the oligonucleotides used for this analysis are indicated in **Table S1**.