Supplementary material

Rapid generation of recombinant *Pseudomonas putida* secondary metabolite producers using yTREX

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Tab. S1: Oligonucleotides used in this study

No.	Name	Sequence 5'-3'	Size [bp]
AD9	AD9AphIIfwd	GGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATCGATCGCCCGGATGAATGTCAGCTAC	66
AD10	AD10AphIIrev	AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGGAGCTCGCTCAGAAGAACTCGTCAAG	66
AD39	AD39seqAphIIfwd	TCTGACGCTCAGTGGAAC	18
AD40	AD40seqAphIIrev	GCGCATTCACAGTTCTCC	18
AD41	AD41seqTREXfwd	GATGAACAGGCAGACATC	18
AD42	AD42seqTREXrev	GGCGAATCATGGACATAC	18
AD65	AD65GAvioALTREX	AGAAATATTAGCTAATTTAATCTCTCAACCCGCGATGAAGCATTCTTCCG	50
AD66	AD66GAvioERTREX	GAAACAGCTATGACCATGATTACGCCAACGATCCAGGCCCTAGCGCTTG	49
AD89	AD89phzA	AGAAATATTAGCTAATTTAATCTCTCAACCCCGGCATACCTGGAGAGCCCTCTCG	50
AD90	AD90HR-L-F	GGCCGTTGAATCGGGATATG	20
AD93	AD93HR-R-R	TAGCAGCACGCCATAGTGAC	20
AD123	AD123phzG	GTAATCATGGTCATAGCTGTTTCCTGTGTGTCACGGTTGCAGGTAGCGGTGCTTC	55
AD124	AD124LacZstart	CACACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTAC	53
AD125	AD125LacZend	GAAACAGCTATGACCATGATTACGCCAAGCTAGCGCTTATTTTTGACACCAGACC	55
AD128	AD128vioCr	GCGGTTCCCGGTTTTCCATGCGGCCTCCTCAGTTGACCCTCCCTATCTTG	50
AD129	AD129vioEf	GGAGGCCGCATGGAAAACCGGGAACC	26
AD130	AD130vioBr	GCGGTTCCCGGTTTTCCATGCGGCCTCCTCAGGCCTCTCTAGAAAGCTTTCCAC	54

Tab. S2: PCR fragments generated in this study

Oligonucleotide pair	Template	Size [bp]	Genes
AD9/AD10	pK18	1155	*aphII* (KmR)
AD65/AD66	pAra-vio	7398	*vioABCDE*
AD65/AD128	pAra-vio	5681	*vioABC*
AD65/AD130	pAra-vio	4390	*vioAB*
AD129/AD66	pAra-vio	624	*vioE*
AD89/AD123	pUC18-pyo	6363	*phzABCDEFG*
AD124/AD125	pRcExpII2-YF1-FixJ-PFixK2lacZ	3127	*lacZ*

Tab. S3: Plasmids used in this study

Plasmid	Relevant characteristics	Reference
pAra-vio	KmR, *C. violaceum* ATCC12472 *vioABCDE*	[1]
pK18	KmR	[2]
pPIG	AmpR, *S. marcescens* W838 *pigABCDEFGHIJKLMN*	[3]
pRcExpII2-YF1-FixJ-PFixK2lacZ	KmR, CmR, *lacZ*	Loeschcke, unpublished
pUC18-pyo	AmpR, *P. aeruginosa* PA01 *phzABCDEFG*	Thies, unpublished
YCp50-poly	*URA3*, *CEN4*/*ARS1*, AmpR, pMB1 ori	ATCC® 87555™ [4]
YCp50-poly-KmR	*URA3*, *CEN4*/*ARS1*, KmR, pMB1 ori	this study
yTREX	YCp50-poly-KmR + L- & R-yTREX	this study
yTREX-phzA-GlacZ	*P. aeruginosa* PA01 *phzABCDEFG*, *lacZ*	this study
yTREX-pig	*S. marcescens* W838 *pigABCDEFGHIJKLMN*	this study
yTREX-vio	*C. violaceum* ATCC 12472 *vioABCDE*	this study
yTREX-vioABCE	*C. violaceum* ATCC 12472 *vioABCE*	this study
yTREX-vioABE	*C. violaceum* ATCC 12472 *vioABE*	this study

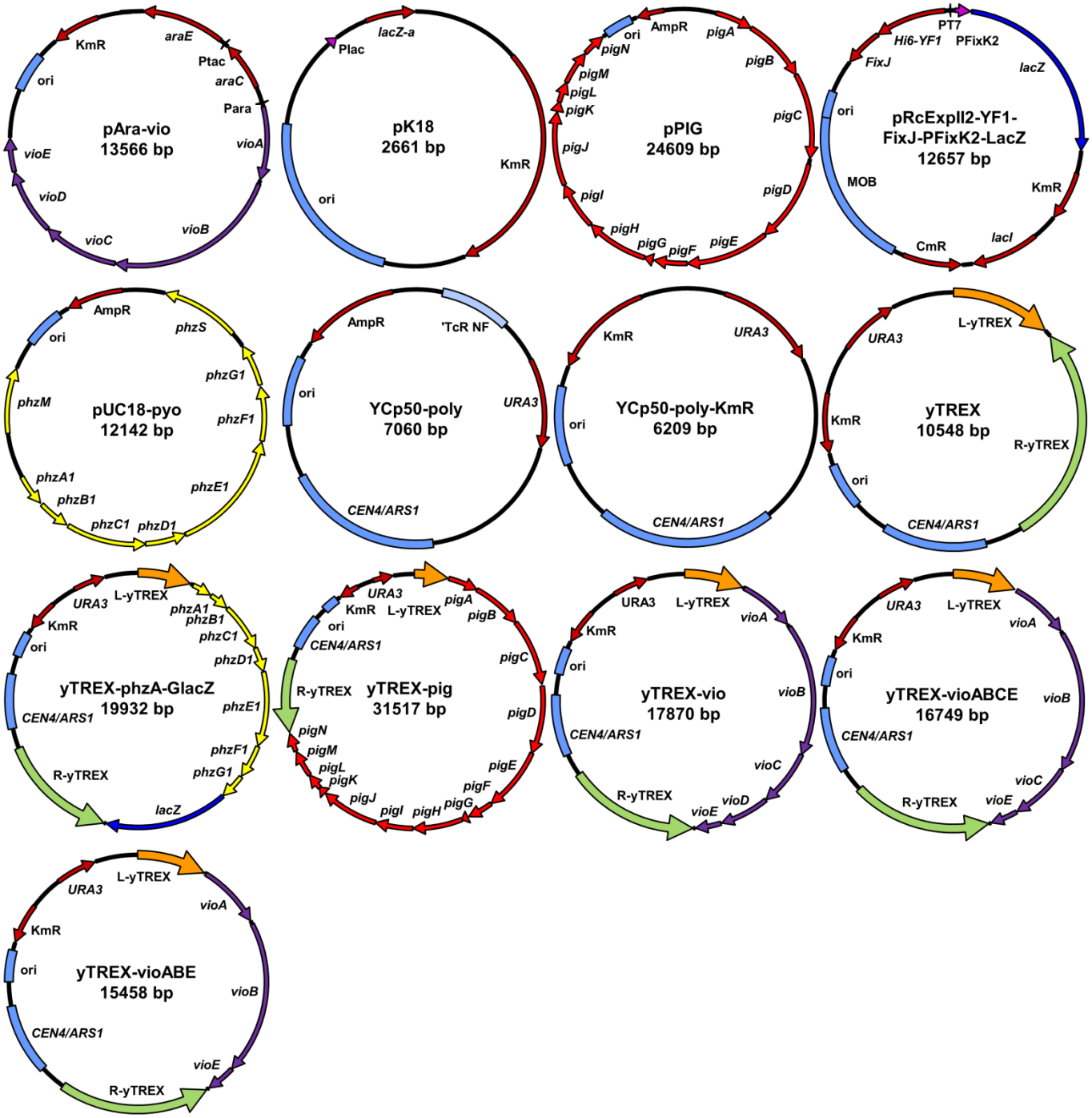

Fig. S1: All plasmids used in this study, including yTREX constructs, shown as plasmid maps. Relevant features: AmpR, ampicillin resistance gene; KmR, kanamycin resistance gene; ori, bacterial origin of replication; *URA3*, pyrimidine ribonucleotide biosynthetic gene; *CEN4*/*ARS1*, *S. cerevisiae* origin of replication; L-yTREX & R-yTREX, yTREX cassettes; TcR, tetracycline resistance gene; *lacZ*, β-galactosidase encoding gene; *phz*, phenazine biosynthetic genes; *pig*, prodigiosin biosynthetic genes; *vio*, violacein biosynthetic genes.

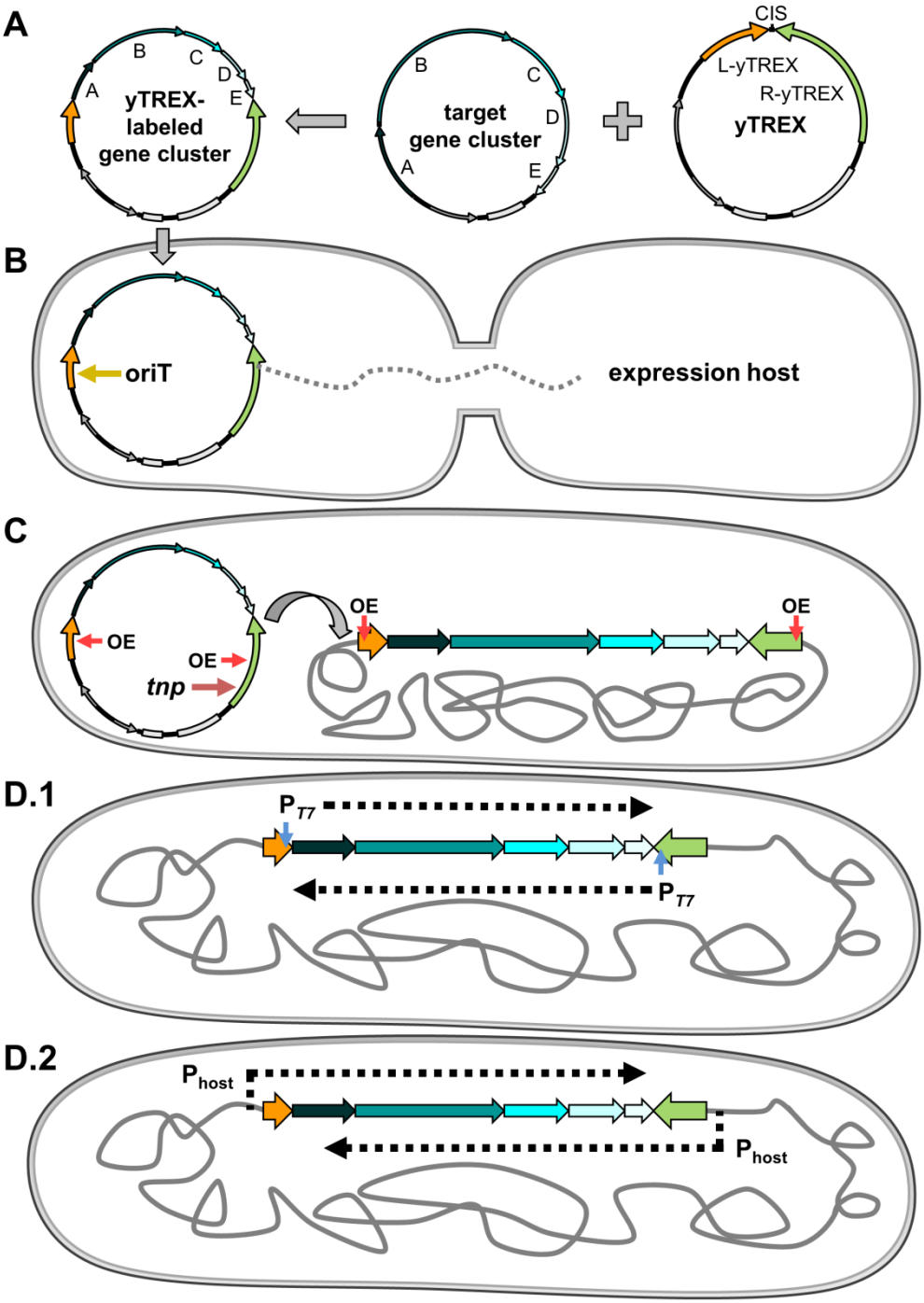

Fig. S2: yTREX application scheme. The yTREX application consists of four steps, *i.e.* labelling, transfer, integration and expression. **(A)** yTREX labeling: Cloning of a gene cluster of interest in the CIS between the two yTREX cassettes in the yTREX vector. The respective gene cluster may be present on a vector such as a plasmid or cosmid (as depicted here) or may be obtained by PCR from chromosomal DNA. As opposed to the initial TREX system [3], this step can be accomplished *via* highly efficient yeast recombinational cloning. **(B)** Conjugational transfer of yTREX-labeled genes into a desired bacterial expression host. **(C)** Transposon Tn5-mediated randomized integration of the yTREX-transposon into the bacterial chromosome. An antibiotic resistance marker for tetracycline enables selection of clones with integrated transposon. **(D)** Expression of all clustered genes can either be implemented by T7 RNA polymerase in a bidirectional manner, using T7 promotors in the yTREX cassettes, as previously shown with the initial TREX system [3] **(D.1)**. Alternatively, genes may be expressed by native chromosomal host promotors. Here, random insertion of the yTREX-transposon next to one strong promotor is sufficient for the expression of unidirectional gene clusters, as shown before with the initial TREX system [5] and further established in the present study. Note that for the expression of complex gene clusters with genes encoded in both directions, more unlikely insertion between two convergently oriented promotors is necessary **(D.2)**. CIS, cluster integration site; OE, outside ends of transposon Tn5; oriT, origin of transfer; *tnp*, transposase gene; P_{T7}, T7 bacteriophage promoter; P_{host}, native chromosomal promoter of the expression host.

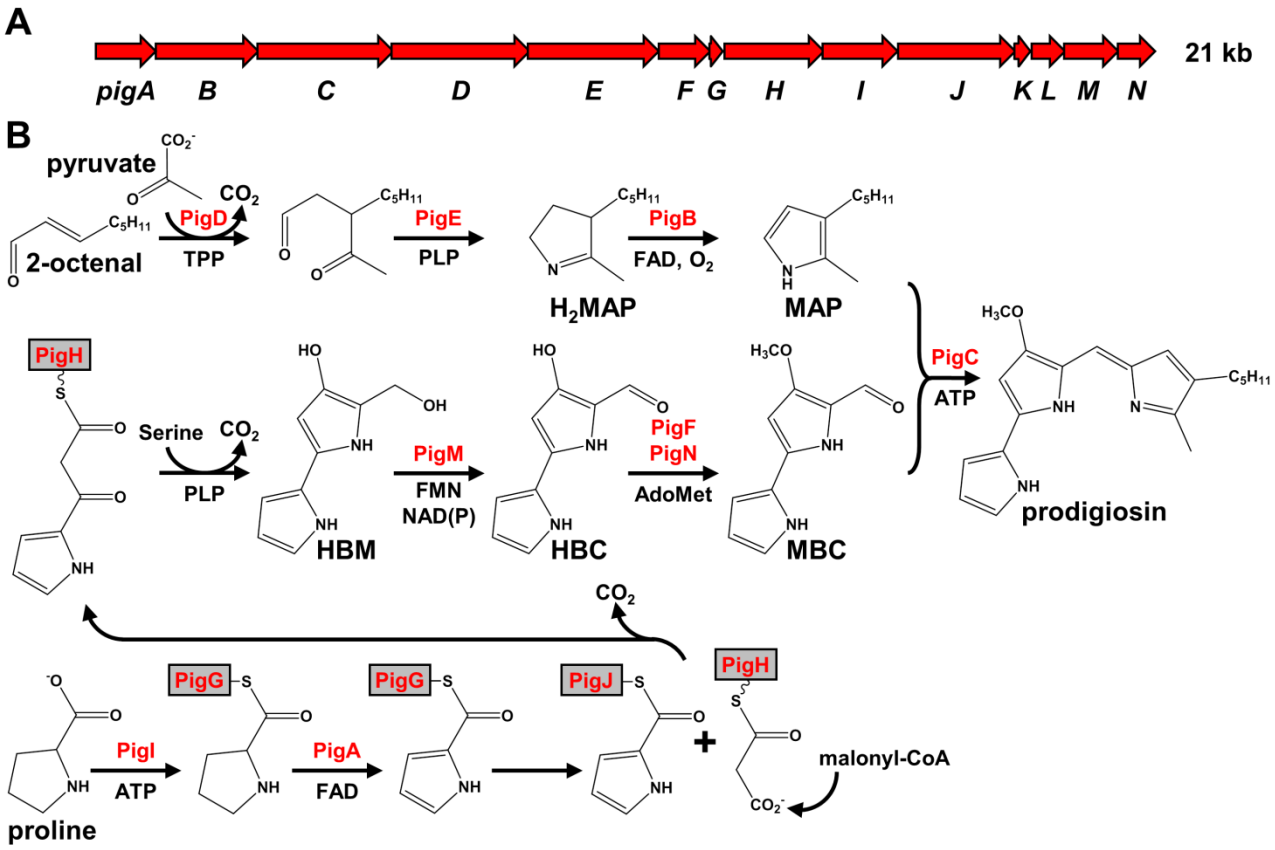

Fig. S3: Prodigiosin biosynthetic gene cluster and pathway from *Serratia marcescens* W838. Biosynthetic genes **(A)** and biosynthetic pathway **(B)** as elucidated previously [6,7]. AdoMet, S-adenosylmethionine; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; HBC, 4-hydroxy-2,2'-bipyrrole-5-carbaldehyde; HBM, 4-hydroxy-2,2'-bipyrrole-5-methanol; MAP, 2-methyl-3-n-amyl-pyrrole; MBC, 4-methoxy-2,2'-bipyrrole-5-carbaldehyde; NAD(P), nicotinamide adenine dinucleotide (phosphate); PLP, pyridoxal phosphate; TPP, thiamine pyrophosphate; ATP, adenosine triphosphate.

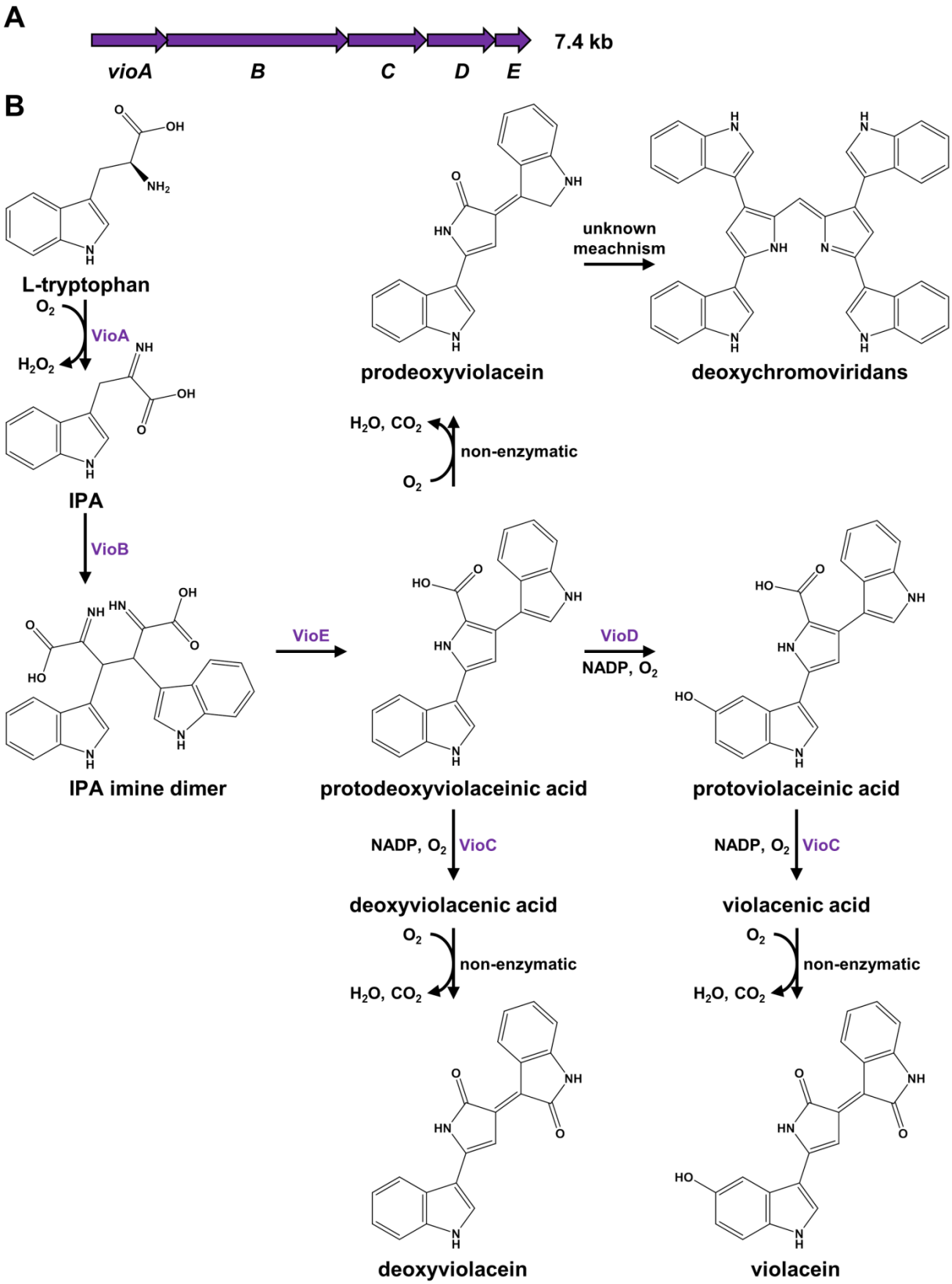

Fig. S4: Violacein biosynthetic gene cluster and pathway from *Chromobacterium violaceum* ATCC 12472. Biosynthetic genes **(A)** and biosynthetic pathway **(B)** as elucidated previously [8–10]. IPA, indole-3-pyruvic acid; NADP, nicotinamide adenine dinucleotide phosphate.

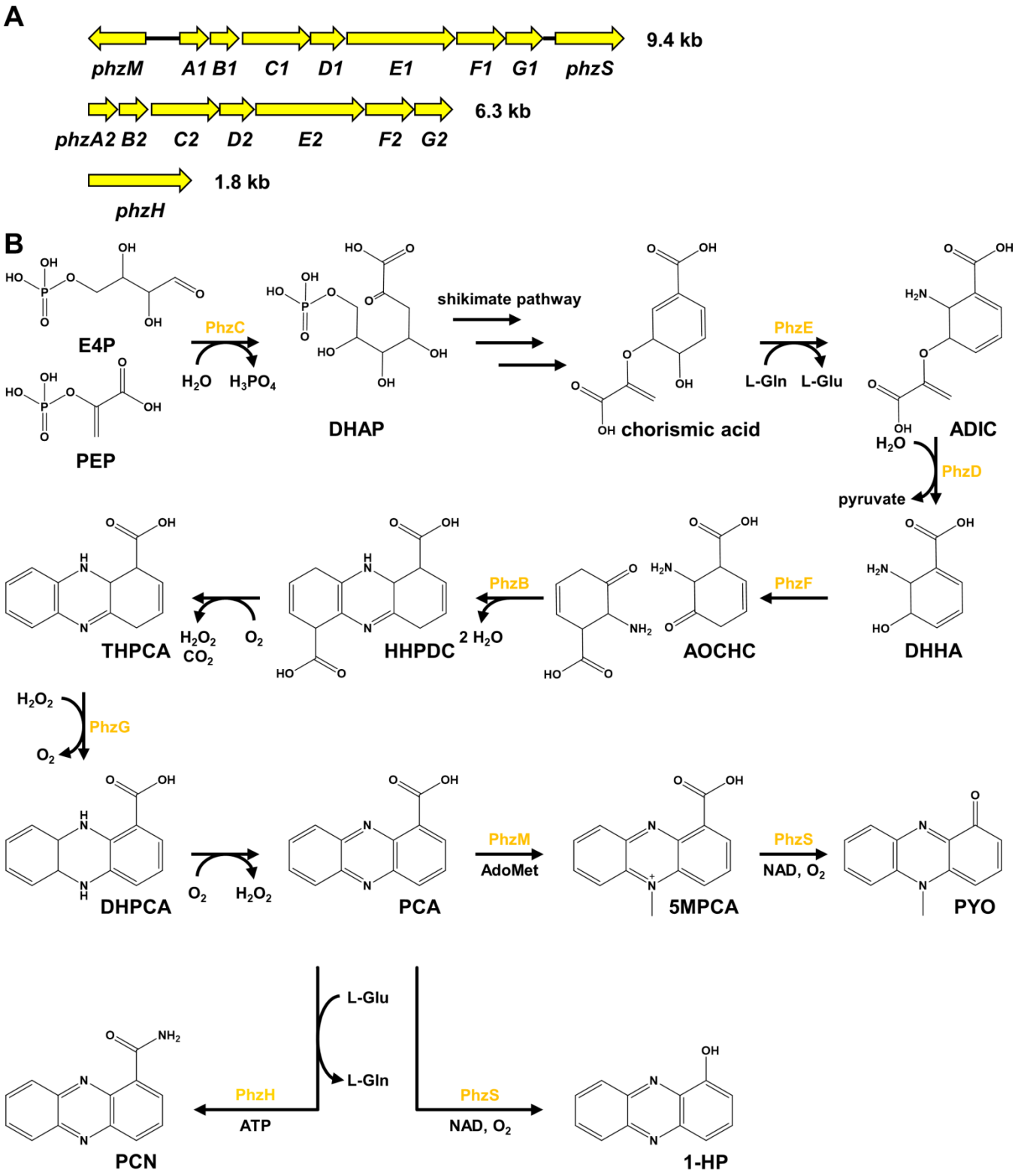

Fig. S5: Phenazine biosynthetic gene cluster and pathway from *Pseudomonas aeruginosa* PAO1. Biosynthetic genes **(A)** and biosynthetic pathway **(B)** as elucidated previously [11,12]. In *P. aeruginosa*, phenazine biosynthesis is encoded in two similar gene clusters, and an additional locus harboring *phzH*. Genes used in this study were *phzA1-G1*. E4P, erythrose-4-phosphate; PEP, phosphoenolpyruvate; DHAP, 3-deoxy-D-arabinoheptulosonic acid 7-phosphate; ADIC, 2-amino-2-deoxyisochorismic acid; DHHA, *trans*-2,3-dihydro-3-hydroxyanthranilic acid; AOCHC, 6-amino-5-oxocyclohex-2-ene-1-carboxylic acid; HHPDC, hexahydrophenazine 1,6-dicarboxylic acid; THPCA, tetrahydrophenazine-1-carboxylic acid; DHPCA, 5,10-dihydro PCA; PCA, phenazine-1-carboxylic acid; PCN, phenazine-1-carboxamide; 5MPCA, 5-methylphenazine-1-carboxylic acid; 1-HP, 1-hydroxyphenazine; PYO, pyocyanin; L-Gln, L-glutamine; L-Glu, L-glutamic acid; NAD, nicotinamide adenine dinucleotide; AdoMet, S-adenosylmethionine.

Tab. S4: Timeline of yTREX application for generation of *P. putida* metabolite production strains

Step	Procedures	Day
DNA fragment generation	PCR or restriction hydrolysis	1
Yeast recombinational cloning	Transformation, incubation, plasmid isolation	1 - 4
Plasmid amplification in *E. coli*	Transformation, incubation, plasmid isolation, restriction analysis, sequencing	4 - 7
***P. putida* strain generation**	Conjugational transfer, selection of strains expressing biosynthetic genes	7 - 12
Production and analysis	Cultivation, extraction, analysis as suitable, *e.g.* HPLC, spectrophotometry	13 - *

* Final day depending on the substance and the associated production, extraction and analysis protocols.

Supplementary References

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