PccD regulates branched-chain amino acid degradation, and exerts a negative effect on erythromycin production in *Saccharopolyspora erythraea*

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Supplemental Materials

Table S1. Predicted PccD-binding genes in *S. erythraea*

Name	Function	*p*-value	PccD box	pos
SACE_3400	acetyl/propionyl-CoA carboxylase alpha subunit	1.39E-08	ATGACGGTGTTGT	80
SACE_1905	alcohol dehydrogenase, zinc-binding domain protein	1.64E-07	ATGTCGGTGGTGT	179
SACE_7326	twin-arginine translocation pathway signal	2.38E-07	ATGACGGTGCAGT	-105
SACE_4036	putative para-aminobenzoic acid synthase	4.14E-07	ATGACGATGTTGT	-39
SACE_1233	Putative 2-hydroxyhepta-2,4-diene-1,7-dioate isomerase	4.14E-07	ATGTCGGTGGTGA	59
SACE_2882	possible transcriptional regulator, ArsR family	6.22E-07	ATGGCGGTGTTGT	59
SACE_2881	flavodoxin/nitric oxide synthase	6.22E-07	ATGGCGGTGTTGT	1
SACE_0073	probable copper resistance protein	6.22E-07	ATGACGCTGATGT	-57
SACE_0057	monooxygenase, FAD-binding	6.22E-07	ATGACGCTGATGT	-10
SACE_6922	hypothetical protein	7.34E-07	ATGACGGTGCTGG	-52
SACE_3897	putative S-adenosyl-L-homocysteine hydrolase	7.34E-07	ATGACGGTGCAGA	179
SACE_3880	aldehyde dehydrogenase	7.34E-07	ATGTCGGTGGAGT	83
SACE_2890	LuxR-family transcriptional regulator	7.34E-07	ATGACGGTGCAGA	0
SACE_5879	hypothetical protein	8.28E-07	ATGACCGTGTTGT	53
SACE_3143	aromatic acid symporter, MFS superfamily	8.28E-07	ATGACGGTGCCGT	77
SACE_0174	transcriptional regulator, PadR-like family	9.75E-07	ATGACGGTTTTGT	116
SACE_0173	putative dioxygenase	9.75E-07	ATGACGGTTTTGT	134
SACE_3223	transcriptional regulator, TetR family	1.20E-06	ATGTCGCTGCTGA	43
SACE_7025	hypothetical protein	1.34E-06	ATCACGGTGGTGT	-129
SACE_1196	probable ATP-dependent DNA helicase-related protein	1.34E-06	ATGACGGTGGTCT	-73
SACE_0568	MoaD2	1.54E-06	ATGACGGCGGTGT	184
SACE_6541	cation antiporter	2.04E-06	CTGACGCTGGTGT	181
SACE_3915	NAD-dependent epimerase/dehydratase	2.04E-06	ATGACGCTGCCGT	119
SACE_3497	probable transcriptional regulator, MarR family	2.04E-06	ATGACGGTGGTGC	40
SACE_1094	hypothetical protein	2.04E-06	ATGACCCTGTTGT	155
SACE_0312	GatB/YqeY, uncharacterized protein	2.29E-06	GTGACGGTGTTGT	196
SACE_3562	formate dehydrogenase alpha subunit	2.48E-06	ATGACGATGCAGT	-115
SACE_7013	hypothetical protein	2.91E-06	AAGACGGTGCTGT	64
SACE_5095	site-specific recombinase, phage integrase family	2.91E-06	TTGACGCTGGTGA	25
SACE_6914	naphthoate synthase	3.20E-06	ATGTCGGTGAAGT	106
SACE_6895	NADH dehydrogenase I chain H	3.20E-06	ATGACGCTGTTCT	-5
SACE_3722	terpene synthase, metal-binding	3.20E-06	ACGACGGTGCTGT	184
SACE_3397	hypothetical protein	3.20E-06	TTGACGCTGCTGA	152
SACE_1503	hypothetical protein	3.20E-06	TTGTCGGTGTTGA	140
SACE_3042	dicarboxylate carrier protein	3.75E-06	ATCACGCTGCTGT	-112
SACE_5642	hypothetical protein	4.44E-06	ATGTCGTTGGTGT	-303
SACE_0222	hypothetical protein	4.91E-06	ATGAGGGTGTAGT	-8
SACE_1082	hypothetical protein	5.93E-06	ATATCGGTGTTGT	1
SACE_1277	peptide/nickel transport system ATP-binding protein	8.93E-06	ATGACCGTGCTGG	31
SACE_1638	dihydrolipoamide succinyltransferase	1.09E-05	TTGTCGGTGGAGA	88
SACE_2228	probable multidrug resistance transporter	1.54E-05	CTGACGCTGGTGG	14
SACE_1550	LamB/YcsF, UPF0271 protein	2.25E-05	TTGGCGCTGGTGA	1
SACE_7008	hypothetical protein	2.40E-05	ATGACGACGGTGA	-170
SACE_4063	metabolite transporter, MFS superfamily	2.58E-05	ATGACGGTGTGTT	25
SACE_0951	protein of unknown function DUF195	2.78E-05	CTGTCGGTGGGGT	-10
SACE_3318	possible esterase	5.53E-05	ATGTGGGTGTCGA	-11

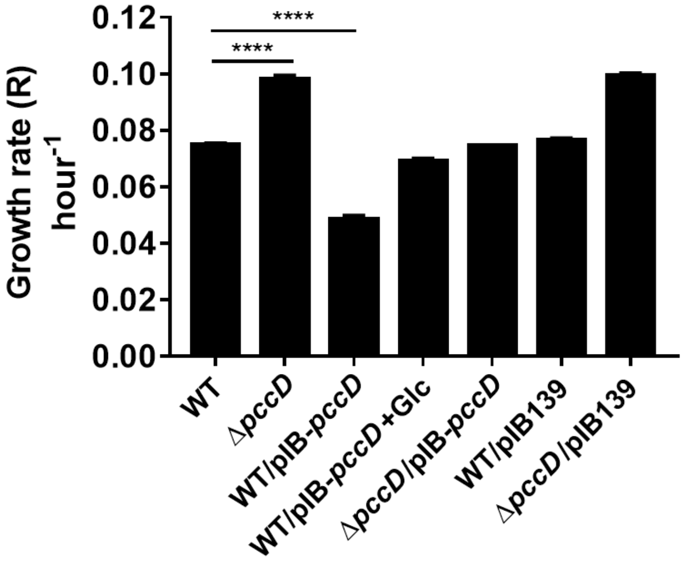

Figure S1. Growth rates of *S. erythraea* wide type strains (WT), *pccD* deletion strains (△*pccD*), *pccD* complementation strain (△*pccD*/pIB-*pccD*), and *pccD* overexpression strains (WT/pIB-*pccD*) grown with basic Evans medium with 30 mM valine and the growth rate of WT/pIB-*pccD* grown with basic Evans medium with 30 mM valine plus 140 mM glucose. ****P<0.0001.

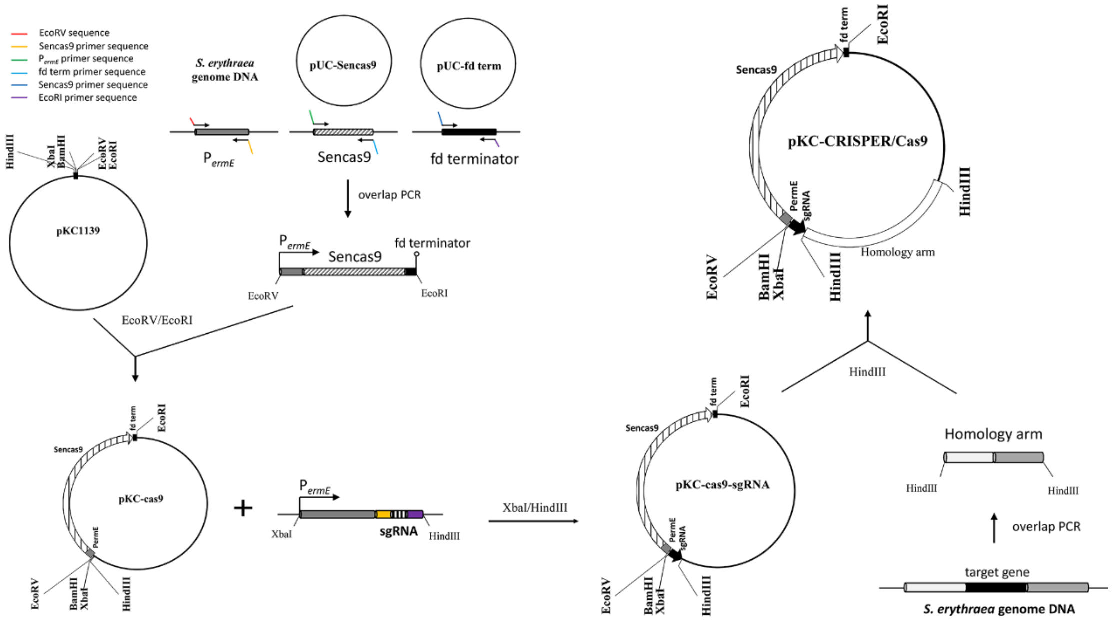

Figure S2. **Flowchart for pKC-CRISPR/Cas9 construction.**

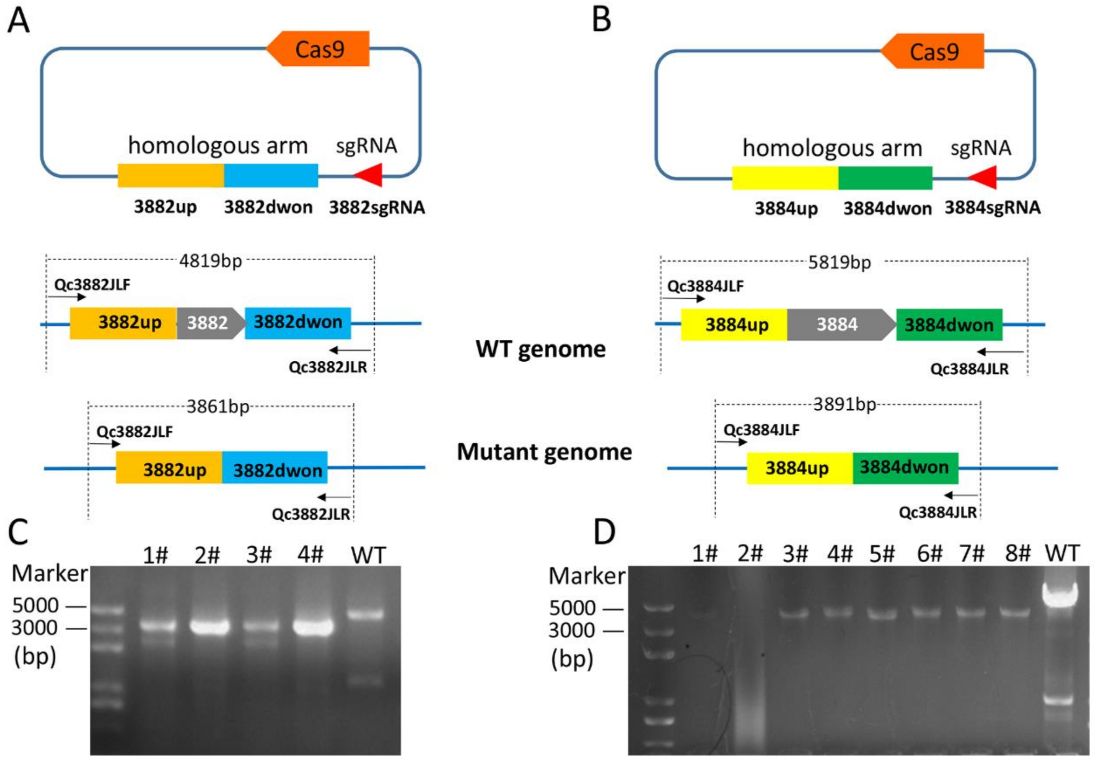

Figure S3. Identification of *gdhA1*-deleted and *bcdhE1*-deleted mutant. **A** and **B.** Strategy of CRISPR/Cas9-mediated deletion of *gdhA1* (SACE_3882) and *bcdhE1* (SACE_3884). **C** and **D.** PCR screening of *gdhA1*-deleted and *bcdhE1*-deleted mutants using primer Qc3882JLF/R and Qc3884JLF/R.

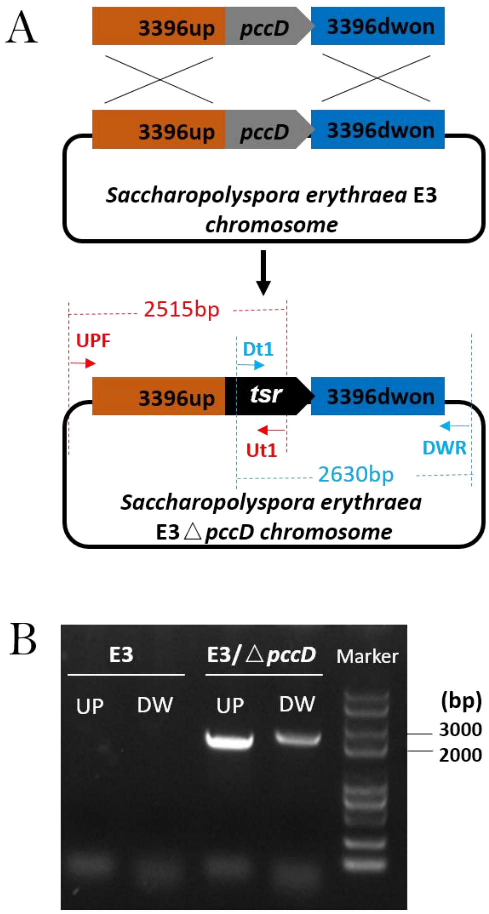

Figure S4. Identification of *S. erythraea* E3△*pccD*.

A. Strategy of double crossover gene knock-out method for *pccD* (SACE_3396).

B. PCR screening of double-exchanged clones using primer UPF/Ut1 and primer Dt1/DWR.