Supplementary Information for

Enhancing gold recovery from electronic waste *via* lixiviant metabolic engineering in *Chromobacterium violaceum*

Song Buck Tay[1, §], Gayathri Natarajan[2, §], Muhammad Nadjad bin Abdul Rahim[1], Hwee Tong Tan[1], Maxey Ching Ming Chung[1,3], Yen Peng Ting[2], and Wen Shan Yew[1]

[1]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore; [2]Department of Chemical and Biomolecular Engineering, Faculty of Engineering, National University of Singapore; [3]Department of Biological Sciences, Faculty of Science, National University of Singapore.

§ These authors contributed equally to the work.

Correspondence should be addressed to Y.P.T. (chetyp@nus.edu.sg) and W.S.Y. (bchyws@nus.edu.sg).

Methods

Construction of *Chromobacterium violaceum* strains. Wild-type *C. violaceum* ATCC 12472 was obtained from ATCC. The hcnABC operon was PCR amplified from the genomic DNA isolated from *C. violaceum* (ATCC 12472) using Platinum Pfx DNA polymerase (Invitrogen) and the primer sets hcnABC-XbaI-F/hcnABC-XhoI-R (Table S2). The PCR reaction (100 μL) contained 1 ng of genomic DNA, 10 μL of 10X *Pfx* Amplification Buffer, 1 mM $MgSO_4$, 0.4 mM of each dNTP, 40 pmol of each primer, and 5 U of Platinum *Pfx* DNA polymerase. The gene was amplified using a PTC-0200G Thermal Cycler (Bio-Rad Laboratories), with the following parameters: 94 ^{O}C for 1 min followed by 40 cycles of: 94 ^{O}C for 1 min, 55 ^{O}C for 1 min and 15 sec, 68 ^{O}C for 5 min, and a final extension of 68 ^{O}C for 10 min. Exogenous inducible promoters pBAD and pTac were cloned upstream of the hcnABC operon. The pBAD and pTac promoters were PCR amplified from pBAD33 and pET15b vectors, respectively. The PCR reaction (100 μL) contained 1 ng of plasmid DNA, 10 μL of 10X *Pfx* Amplification Buffer, 1 mM $MgSO_4$, 0.4 mM of each dNTP, 40 pmol of each primer, and 5 U of Platinum *Pfx* DNA polymerase. The gene was amplified using a PTC-0200G Thermal Cycler (Bio-Rad Laboratories), with the following parameters: 94 ^{O}C for 1 min followed by 40 cycles of: 94 ^{O}C for 1 min, 55 ^{O}C for 1 min and 15 sec, 68 ^{O}C for 3 min, and a final extension of 68 ^{O}C for 10 min.

Figure S1. Growth curves of wild-type and engineered *C. violaceum*. Wild-type *C. violaceum* was grown in the absence of gentamycin, while engineered *C. violaceum* strains were grown in the presence of gentamycin.

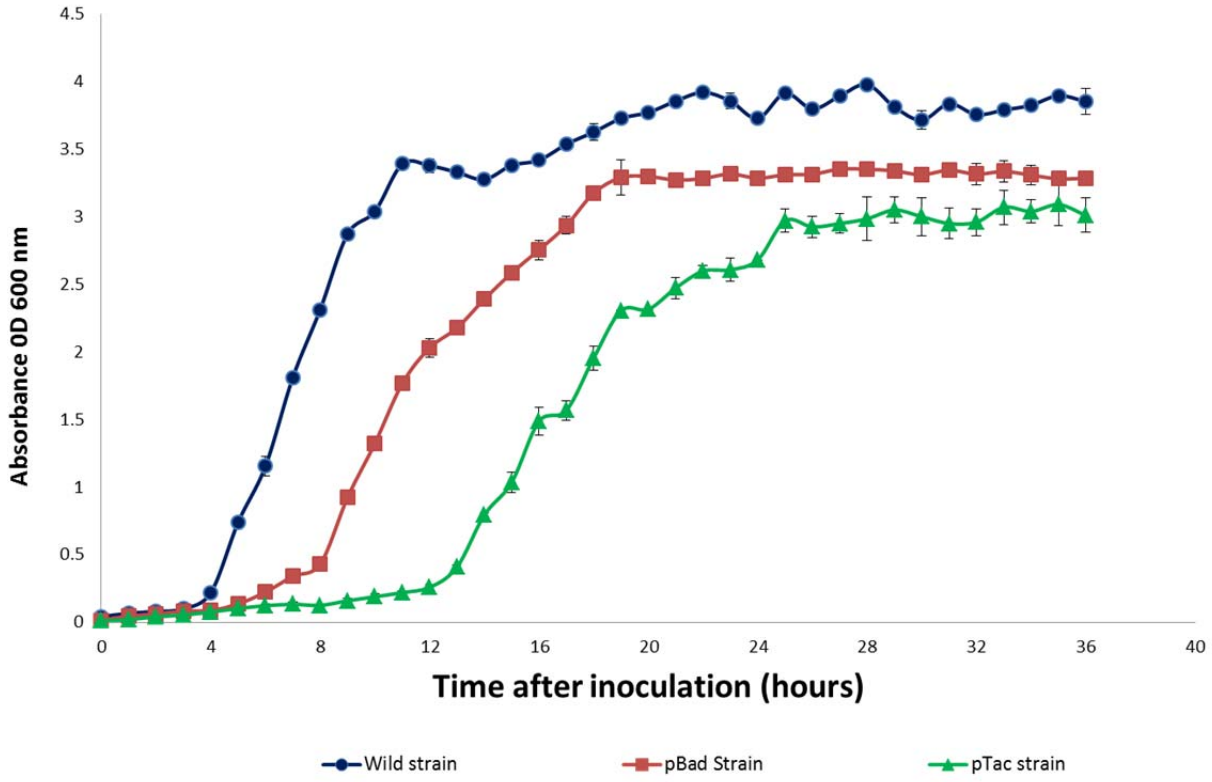

Figure S2. Growth curves of wild-type and engineered *C. violaceum* in the absence of gentamycin. Due to the production of violaceum, growth progress was monitored as a function of colony forming units per mL of bacterial culture (CFU/mL).

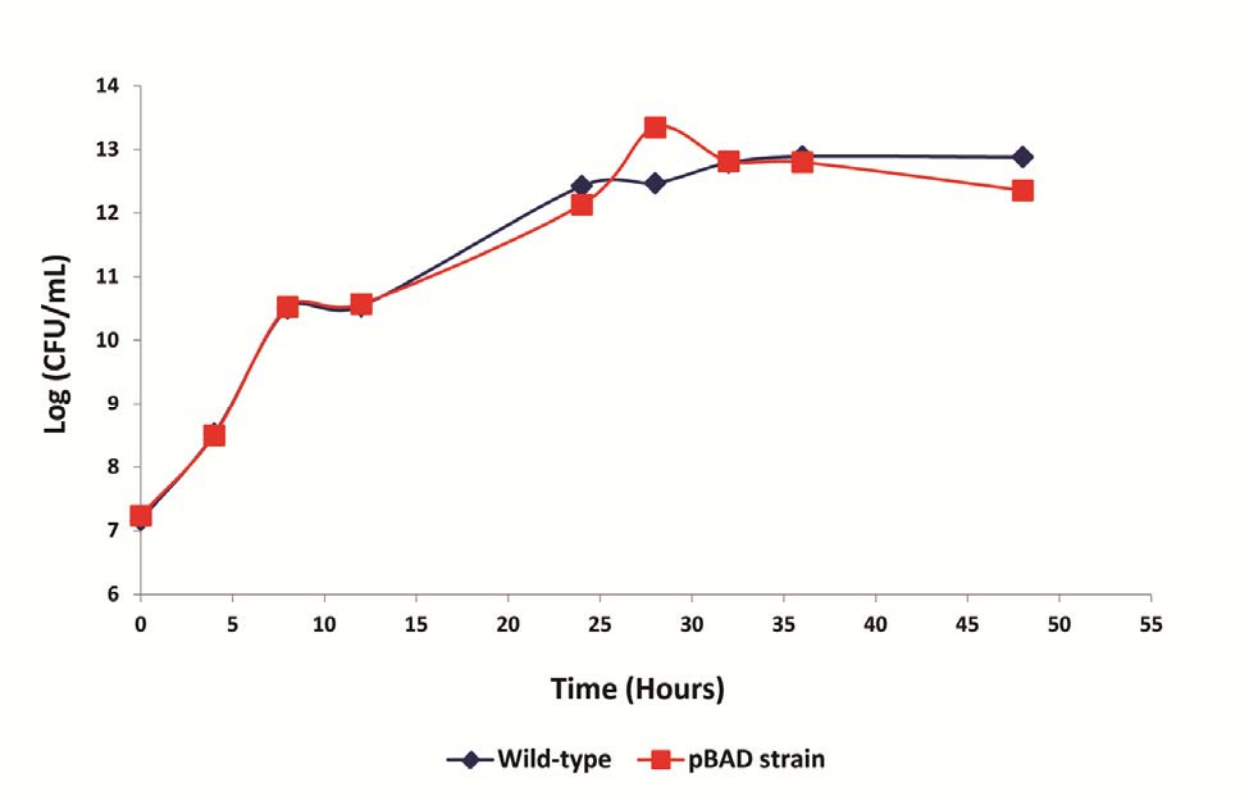

Figure S3. Cyanide production by wild-type and engineered *C. violaceum.*

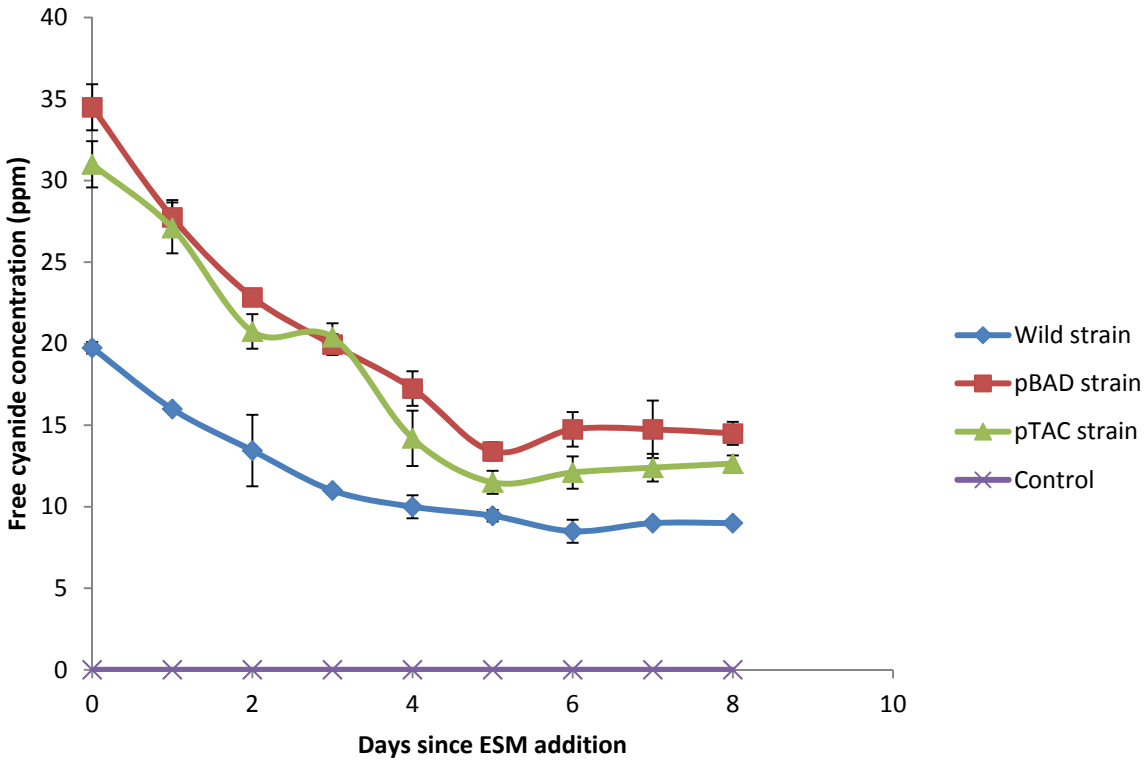

Table S1. Comparative Proteomics Results

Uniprot Accession #	Name	Relative expression to wild-type	S.D.	Function
Proteins Up-regulated				
Q7M7F1	Elongation factor Tu (*tuf1*)	1.477	0.186	Promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis
Q7NQ65	Glutathione synthetase (*gshB*)	1.366	0.245	Catalyzes the reaction: ATP + gamma-L-glutamyl-L-cysteine + glycine = ADP + phosphate + glutathione.
Q7NQG7	50S ribosomal protein L6 (*rplF*)	1.759	0.508	Binds to the 23S rRNA, and is important in its secondary structure. It is located near the subunit interface in the base of the L7/L12 stalk, and near the tRNA binding site of t
Q7NQG9	30S ribosomal protein S5 (*rpsE*)	1.456	0.270	With S4 and S12 plays an important role in translational accuracy. Located at the back of the 30S subunit body where it stabilizes the conformation of the head with respec
Q7NXP1	Transcription termination factor Rho (*rho*)	1.537	0.254	Facilitates transcription termination by a mechanism that involves Rho binding to the nascent RNA, activation of Rho's RNA-dependent ATPase activity, and release of the r
Q7NXX7	N-succinylglutamate 5-semialdehyde dehydrogenase (*astD*)	1.562	0.401	Catalyzes the NAD-dependent reduction of succinylglutamate semialdehyde into succinylglutamate
Q7NY10	Purine nucleoside phosphorylase (*pnp*)	2.094	0.548	Involved in RNA degradation
Q7NYY5	Acetaldehyde dehydrogenase (*adhE*)	1.838	1.141	Catalyzes the conversion of acetaldehyde into acetic acid; also facilitates the interconversion between alcohols and aldehydes or ketones with the reduction of NAD+
Q7P1M2	Transketolase 1 (*tktA*)	2.457	1.334	Involved in the pentose phosphate pathway
Q7P255	Probable site-specific DNA-methyltransferase, cytosine-specific (*CV_0006*)	3.064	1.889	A large group of enzymes involved in transferring methyl group to DNA.
Q7NRN7	Enoyl-[acyl-carrier-protein] reductase [NADH] (*CV_3743*)	1.333	0.118	Essential enzyme in the biosynthesis of saturated straight-chain fatty acids
Proteins with no detected change				
Q7NRJ9	Arginine deiminase (*arcA*)	1.115	0.385	Catalyses the reaction: L-arginine + H2O <=> L-citrulline + NH3
Q7NUY9	ATP-dependent Clp protease proteolytic subunit (*clpP*)	1.043	0.190	Cleaves peptides in various proteins in a process that requires ATP hydrolysis. Has a chymotrypsin-like activity. Plays a major role in the degradation of misfolded proteins
Q7NWN7	Chaperone protein ClpB (*clpB*)	0.899	0.142	Part of a stress-induced multi-chaperone system.
Q7NXU5	Acetate kinase (*ackA*)	1.153	0.272	Facilitates the production of acetyl-CoA by phosphorylating acetate in the presence of ATP and a divalent cation
Q7NYC6	Threonyl-tRNA synthetase (*thrS*)	0.926	0.073	This enzyme participates in glycine, serine and threonine metabolism and aminoacyl-tRNA biosynthesis
Q7NYI8	Serine hydroxymethyltransferase (*glyA*)	1.012	0.189	Catalyzes the pyridoxal phosphate-dependent, interconversion of serine and glycine
Q7NYR7	Methionyl-tRNA synthetase (*metG*)	0.888	0.189	Required for elongation of protein synthesis and the initiation of all mRNA translation through initiator tRNA(fMet) aminoacylation
Q7NZF7	Adenosylhomocysteinase (*ahcY*)	1.118	0.186	May play a key role in the regulation of the intracellular concentration of adenosylhomocysteine
Q7P0W5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase (*dapD*)	1.207	0.123	Catalyzes the reaction: Succinyl-CoA + (S)-2,3,4,5-tetrahydropyridine-2,6-dicarboxylate + H2O <=> CoA + N-succinyl-L-2-amino-6-oxoheptanedioate
Q7P1M4	Phosphoglycerate kinase (*pgk*)	0.977	0.203	Catalyses the reaction: ATP + 3-phospho-D-glycerate --> ADP + 3-phospho-D-glyceroyl phosphate
Q7NQP9	Probable transcriptional regulator (*CV_4088*)	0.944	0.165	Transcriptional regulator
Q7NR07	Putative uncharacterized protein (*CV_3978*)	0.941	0.320	Unknown. Top three blastp hits are 'type VI secretion protein'
Q7NR66	Probable glutaryl-CoA dehydrogenase (*CV_3918*)	1.037	0.172	An acyl-CoA dehydrogenase that acts on glutaryl-CoA, creating crotonyl-CoA. It plays a role in the metabolism of lysine, hydroxylysine and tryptophan.
Q7NRJ7	NAD(P)H-flavin reductase (*ubiB*)	1.108	0.172	Catalyzes the reaction: reduced riboflavin + NADP <=> riboflavin + NADPH + H+
Q7NRS1	Phosphoenolpyruvate synthase (*ppsA*)	0.938	0.221	Catalyzes the phosphorylation of pyruvate to phosphoenolpyruvate
Q7NS39	Putative uncharacterized protein (*CV_3588*)	0.950	0.215	Unknown. Top three blastp hits are 'Rhodanese domain protein'
Q7NS77	Catalase (*katE*)	0.921	0.265	Catalyzes the decomposition of hydrogen peroxide to water and oxygen.
Q7NSI0	Chemotaxis protein CheV (*cheV2*)	1.092	0.226	Involved in chemotaxis
Q7NTH1	Glutamate dehydrogenase (*gdhA*)	0.935	0.160	Catalyzes the conversion glutamate to α-ketoglutarate
Q7NU29	L-serine dehydratase (*sdaA1*)	1.065	0.170	Catalyzes the pyridoxal phosphate-dependent, deamination of L-serine to yield pyruvate, with the release of ammonia
Q7NUM7	Putative uncharacterized protein (*CV_2670*)	0.963	0.129	Unknown. Top four hits 'Amidohydrolase 3'
Q7NV74	Aconitate hydratase 2 (*acnB*)	1.042	0.212	Catalyses the stereo-specific isomerization of citrate to isocitrate via cis-aconitate in the TCA cycle
Q7NWD9	Probable zinc-containing alcohol dehydrogenase (*CV_2051*)	1.058	0.274	Probable alcohol dehydrogenase
Q7NWT8	Probable outer membrane protein (*CV_1891*)	0.933	0.097	Unknown. Outer membrane protein belonging to OmpA family
Q7NXH6	Glycine C-acetyltransferase (*kbl*)	0.881	0.102	Associated with the biosynthesis of threonine
Q7NZ48	Dihydrolipoyl dehydrogenase (*lpdA2*)	0.955	0.207	Catalzyes the reaction: Protein N(6)-(dihydrolipoyl)lysine + NAD+ <=> protein N(6)-(lipoyl)lysine + NADH.
Q7NZ51	2-oxoglutarate dehydrogenase E1 component (*sucA*)	1.084	0.281	Part of α-ketoglutarate dehydrogenase complex
Q7NZ52	Citrate synthase (*gltA*)	0.984	0.127	Catalyzes the reaction: Acetyl-CoA + H2O + oxaloacetate --> citrate + CoA for TCA cycle
Q7P022	Probable transformylase (*CV_0747*)	0.978	0.219	Possible transformylase in nucleotide biosynthesis
Q7P0M6	Putative uncharacterized protein (*CV_0540*)	1.153	0.131	Unknown. High similarity with uncharacterised proteins in *P.aeruginosa*
Q7P182	Aromatic-amino-acid transaminase (*tyrB2*)	0.882	0.215	Aminotransferase
Q7P1T7	Ribonuclease G (*cafA*)	0.920	0.168	Catalyzes degradation of RNA
Q7P210	Uroporphyrin-III C-methyltransferase (*hemX*)	1.275	0.362	Catalyzes two methylation reactions forming precorrin-1 and precorrin-2
Q7NQE4	50S ribosomal protein L10 (*rplJ*)	0.979	0.220	Part of 70S ribosome
Q7NQF7	50S ribosomal protein L22 (*rplV*)	0.841	0.145	Part of 70S ribosome
Q7NRX0	Cyanide insensitive terminal oxidase (cioA)	1.002	0.066	Cyanide-insensitive terminal cytochrome oxidase in the respiratory electron transport chain
Q7NQ31	L-allo-Threonine aldolase (*CV_4309*)	1.135	0.214	Catalyzes the pyridoxal phosphate-dependent, reversible reaction between threonine and acetaldehyde plus glycine
Q7NSJ5	Glycine dehydrogenase [decarboxylating] (*gcvP*)	1.223	0.625	Catalyzes the degradation of glycine as part of the glycine cleavage system
Proteins Down-regulated				
Q7P190	Urocanate hydratase (*hutU*)	0.651	0.114	Involved in the degradation of Histidine
Q9ZHI1	Acetyl-CoA acetyltransferase (*phaA*)	0.445	0.099	Competes with fatty acid synthesis for acetyl-CoA; produces acetoacetyl-CoA from acetyl-CoA
Q7NPX8	Putative uncharacterized protein (*CV_4364*)	0.426	0.208	Unknown. Top few blastp hits are also uncharacterized proteins from a range of bacterial species
Q7NR08	Putative uncharacterized protein (*CV_3977*)	0.387	0.042	Unknown. Top three blastp hits are 'Type VI secretion system effector'
Q7NS55	Outer membrane protein A (*rmpM*)	0.250	0.160	Outer membrane protein found in Neisseria species
Q7NSF9	Transmembrane protein (*elaB*)	0.434	0.059	Unknown function. Ribosome binding, probably membrane-anchored.
Q7NTK8	30S ribosomal protein S1 (*rpsA*)	0.723	0.123	Binds mRNA; thus facilitating recognition of the initiation point. It is needed to translate mRNA with a short Shine-Dalgarno (SD) purine-rich sequence.
Q7NUH8	3-hydroxyacyl-CoA dehydrogenase (fadB)	0.637	0.096	Associated with β-oxidation of fatty acids
Q7NV31	Amidophosphoribosyltransferase (*purF*)	0.704	0.136	Catalyzes the conversion of α-phosphoribosylpyrophosphate (α-PRPP) into 5-β-phosphoribosylamine
Q7NV42	Superoxide dismutase (*sodB1*)	0.551	0.068	Destroys radicals normally produced within cells
Q7NVG0	Putative uncharacterized protein (*CV_2383*)	0.637	0.403	Unknown. Low identity blastp hits.
Q7NWA2	Acetyl-CoA C-acetyltransferase (*atoB*)	0.523	0.163	Competes with fatty acid synthesis for acetyl-CoA; produces acetoacetyl-CoA from acetyl-CoA
Q7NWA4	3-hydroxybutyryl-CoA dehydrogenase (*paaH*)	0.637	0.156	Involved in breakdown of metabolites in butanoate metabolism into acetoacetyl-CoA
Q7NWH0	Probable aldehyde dehydrogenase (*CV_2019*)	0.676	0.085	Catalyzes the oxidation of aldehydes
Q7NX40	Protein kinase (*prkA*)	0.745	0.113	Phosphoryltaes protein
Q7NXP2	Thioredoxin (*trxA*)	0.435	0.194	Redox protein playing critical roles in reducing oxidative stress and as an electron donor to ribonucleotide reducatase
Q7NYA8	Probable phasin (*CV_1366*)	0.523	0.150	Poly(hydroxyalcanoate) granule associated protein; storage polyesters
Q7NYB1	Probable trans-acting regulatory HvrA protein (*CV_1363*)	0.412	0.076	Protein that modulates protein expression
Q7NYU5	Probable small heat shock protein (*CV_1177*)	0.338	0.099	Involved in the proper folding of proteins and can protect proteins from heat-induced denaturation
Q7NZK6	NADP-dependent malate dehydrogenase (*maeB*)	0.535	0.106	Catalyzes the reversible oxidative decarboxylation of L-malate using NAD(P)(+) as a cofactor
Q7NZQ3	Putative uncharacterized protein (*CV_0868*)	0.288	0.086	Unknown. Top few blastp hits are also uncharacterized proteins from a range of bacterial species
Q7P1C4	Glutathione S-transferase family protein (*gst2*)	0.386	0.263	Probably has a role in cell signalling.
Q7NQX1	60 kDa chaperonin 2 (*groL2*)	0.561	0.185	Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions

Table S2. Primers used in strain construction.

Name of Primer	Sequence	Remarks
hcnABC-XbaI-F hcnABC-XhoI-R	5’ GACACCTGTATCGCTCTAGAGAACCGGGGCAATGGCGGAACCC 3’ 5’ GGCTCAGTGAGAGGACTCGAGTCAGGCGTGCAGCGCGAAATCC 3’	Forward and reverse primers for cloning *hcnABC* into pUC18-mini-Tn7T-Gm
pBAD BamHI pBAD XbaI	5’ ATTGTCTGATTCGTGGATCCTTATGACAACTTGACGGCTAC 3’ 5’ CAAAATTATTTGAGCTCTCTAGAGCTAGCCCAAAAAAACGGG 3’	Forward and reverse primers for cloning PBAD promoter into pUC18-mini-Tn7T-Gm
pTAC BamHI pTAC XbaI	5’ CGTTGGGATCCCTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGC 3’ 5’ CTAGTCTAGAACATTATACGAGCCGATGATTAATTGTCAGTGAAACCAGTAACGTTATACG 3’	Forward and reverse primers for cloning Ptac promoter into pUC18-mini-Tn7T-Gm
P-Tn7L P-0678	5’ ATTAGCTTACGACGCTACACCC 3’ 5’ TCGAGCCAAGGCCGA CGAAC 3’	Forward and reverse sequencing primers specific for site of Tn7L integration