Supporting Information

Lan and Liao 10.1073/pnas.1200074109

SI Text

Chemicals and Reagents. All chemicals were purchased from Sigma-Aldrich or Fisher Scientifics unless otherwise specified. iProof high-fidelity DNA polymerase was purchased from Bio-Rad. Restriction enzymes, Phusion DNA polymerase, and ligases were purchased from New England Biolabs (Ipswich, MA). T5-Exonuclease was purchased from Epicentre Biotechnologies. KOD and KOD xtreme DNA polymerases were purchased from EMD biosciences.

DNA Manipulations. All chromosomal manipulations were carried out by homologous recombination of plasmid DNA into *S. elongatus* PCC 7942 genome at neutral site I (NSI) (1) and II (NSII) (2). All plasmids were constructed using the isothermal DNA assembly method (3). Plasmids were constructed in *Escherichia coli* XL-1 strain for propagation and storage (Table S1).

Plasmid Constructions. The plasmids used and constructed in this work are listed in Table S1 and briefly described below. The sequences of primers used are listed in Table S2. Plasmid pEL29 was synthesized by Genewiz Inc. Plasmid pEL52 was synthesized by DNA 2.0.

Plasmid pEL53 was constructed by assembling a nphT7 fragment and a pEL11 without atoB fragment. nphT7 fragment was amplified by PCR with primers rEL-335 and rEL-336 with pEL52 as template. pEL11 without atoB fragment was amplified by PCR with primers rEL-333 and rEL-334 with pEL11 as template.

Plasmid pEL54 was constructed by assembling a bldh fragment, a yqhD fragment, and a pEL11 without adhE2 fragment. bldh fragment was amplified by PCR with primers rEL-329 and rEL-330 with *Clostridium saccharoperbutylacetonicum* NI-4 genome as template. yqhD fragment was amplified by PCR with primers rEL-331 and rEL-332 with *E. coli* genome as template. pEL11 without adhE2 fragment was amplified by PCR with primers rEL-327 and rEL-328 with pEL11 as template.

Plasmid pEL56 was constructed by assembling a NSII vector fragment and a pEL53 coding sequence fragment. NSII vector fragment was amplified by PCR with primers rEL-217 and rEL-253 with pEL37 as template. pEL53 coding sequence fragment was amplified by PCR with primers rEL-254 and rEL-255 with pEL53 as template.

Plasmid pEL57 was constructed by assembling a NSII vector fragment and a pEL54 coding sequence fragment. NSII vector fragment was amplified by PCR with primers rEL-217 and rEL-253 with pEL37 as template. pEL54 coding sequence fragment was amplified by PCR with primers rEL-254 and rEL-255 with pEL54 as template.

Plasmid pEL59 was constructed by assembling a NSII vector fragment, a pEL54 coding sequence without atoB fragment, and a nphT7 fragment. NSII vector fragment was amplified by PCR with primers rEL-217 and rEL-253 with pEL37 as template. pEL54 coding sequence without atoB fragment was amplified by PCR with primers rEL-352 and rEL-255 with pEL54 as template. nphT7 fragment was amplified by PCR with primers rEL-254 and rEL-351.

Plasmid pEL70 was constructed by assembling a pEL59 without crt.hbd fragment and a phaJ.phaB fragment. pEL59 without crt.hbd fragment was amplified by PCR with primers rEL-390 and rEL-391 with pEL59 as template. phaJ.phaB fragment was amplified by PCR with primers rEL-392 and rEL-393 with pEL29 as template. Plasmid pEL71 was constructed by assembling a pEL57 without crt.hbd fragment and a phaJ.phaB fragment. pEL57 without crt.hbd fragment was amplified by PCR with primers rEL-390 and rEL-391 with pEL57 as template. phaJ.phaB fragment was amplified by PCR with primers rEL-392 and rEL-393 with pEL29 as template.

Plasmid pEL73 was constructed by assembling a pEL56 without crt.hbd fragment and a phaJ.phaB fragment. pEL56 without crt.hbd fragment was amplified by PCR with primers rEL-390 and rEL-398 with pEL56 as template. phaJ.phaB fragment was amplified by PCR with primers rEL-399 and rEL-393 with pEL70 as template.

Plasmids pEL75, pEL76, pEL77, pEL78, pEL79, and pEL80 were constructed by assembling a pDK26 without adhE2 fragment and an aldehyde dehydrogenase gene from Clostridium saccharoperbutylacetonicum NI-4, Clostridium Kluyveri, Geobacillus thermoglucosidasius, Escherichia coli, Clostridium beijerinckii NCIMB 8052, and Clostridium saccharobutylicum ATCC BAA-117, respectively. pDK26 without adhE2 fragment was amplified by PCR using primers rEL-403 and rEL-404 with pDK26 as template. C. saccharoperbutylacetonicum NI-4 bldh fragment was amplified by primers rEL-332 and rEL-394 with C. saccharoperbutylacetonicum NI-4 genome as template. C. Kluyveri bldh fragment was amplified by primers rEL-405 and rEL-406 with C. kluyveri genome as template. G. thermoglucosidasius bldh fragment was amplified by primers rEL-407 and rEL-408 with G. thermoglucosidasius genome as template. E. coli EutE fragment was amplified by primers rEL-409 and rEL-410 with E. coli genome as template. C. beijerinckii NCIMB 8052 bldh fragment was amplified by primers rEL-411 and rEL-412 with C. beijerinckii NCIMB 8052 genome as template. C. saccharobutylicum ATCC BAA-117 bldh fragment was amplified by primers rEL-413 and rEL-414 with C. saccharobutylicum ATCC BAA-117 genome as template.

Plasmids pEL90 to pEL96 were constructed by assembling the KASIII-like genes with a vector fragment. Vector fragment was amplified with primers rEL-455 and rEL-456 with pCS27 as the template. *bamb6224* was amplified with primers rEL-457 and rEL-458 with *Burkholderia ambifaria* gDNA as template. *gox0115* was amplified with primers rEL-459 and rEL-460 with *Gluconobacter oxydans* gDNA as template. *hp0202* was amplified with primers rEL-461 and rEL-462 with *Helicobacter pylori* gDNA as template. *lm02202* was amplified with primers rEL-463 and rEL-464 with *Listeria monocytogenes* gDNA as template. *pae-fabH2* was amplified with primers rEL-467 and rEL-468 with *Pseudomonas aeruginosa* gDNA as template. *sav-fabH4* was amplified with primers rEL-469 and rEL-470 with *Streptomyces avermitilis* gDNA as template. *sco5888* was amplified with primers rEL-471 and rEL-472 with *Streptomyces coelicolor* gDNA as template.

Strain Construction and Transformation. The strains used and constructed are listed in Table S1. Briefly, strain EL18 was constructed by recombination of plasmids pEL57 NSII of Strain EL9 (Table S1 for relevant genotypes). Strain EL20 was constructed by recombination of plasmids pEL56 into NSII of strain EL9. Strain EL21 was constructed by recombination of plasmids pEL56 into NSII of strain EL23 was constructed by recombination of plasmids pEL70 into NSII of strain EL23 was constructed by recombination of plasmids pEL70 into NSII of strain EL23 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL24 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL24 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL24 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL24 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL24 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL24 was constructed by recombination of plasmids pEL71 into NSII of strain EL9. Strain EL94 was constructed by recombination of plasmids pEL73 into NSII of strain EL9.

S. elongatus PCC 7942 *strains* were transformed by incubating cells at mid-log phase (OD₇₃₀ of 0.4 to 0.6) with 2 μ g of plasmid DNA overnight in dark. The culture was then spread on BG-11 plates supplemented with appropriate antibiotics for selection of successful recombination. For selection and culture maintenance, 20 μ g/mL spectinomycin and 10 μ g/mL kanamycin were added into BG-11 agar plates and BG-11 medium where appropriate. Colonies grown on BG-11 agar plates were grown in liquid culture. Genomic DNA was then prepared from the liquid culture and analyzed by PCR using gene-specific primers (Table S2) to verify integration of inserted genes into the recombinant strain. In all cases, four individual colonies were analyzed and propagated for downstream tests.

Protein Purification and SDS/PAGE. Protein purification was done by using His-Spin Protein miniprep purification kit from Zymo following manufacturer's manual. Overnight culture of XL-1 blue strains harboring individual plasmid of pEL90 to pEL96, each encodes for a particular KAS III-like enzyme, was used to inoculate fresh 20 mL LB. The newly inoculated culture was incubated at 37 °C until OD_{600nm} reaches 0.6, which was then induced with 1 mM IPTG. The induced culture were then incubated in 30 °C shaker for 2 hr to allow protein expression. The culture was then harvested by centrifugation at $5,250 \times g$ for 20 min. The pellet was then resuspended with 1 mL of 100 mM Tris-HCl pH 7.6 and mixed with 1 mL of 0.1 mm glass beads (Biospec). The sample was then homogenated using mini bead beater (biospec). Total protein was then collected. Soluble protein was collected after centrifugation. Purified protein was collected after His-spin column purification. The protein samples were then ran on SDS/ PAGE using commercially available precast gels (Biorad) following standard protocol.

Enzyme Assays. Enzyme assays were conducted by using Bio-Tek PowerWave XS microplate spectrophotometer. Thiolase activity was measured via both condensation and thiolysis direction. The enzymatic reaction was monitored by the increase or decrease of absorbance at 303 nm, which corresponded to the result of Mg²⁺ coordination with the diketo moiety of acetoacetyl-CoA (4). The enzymatic reaction was initiated by the addition of the enzyme. For purified enzyme reaction, the reaction mixture contained 100 mM Tris-HCl (pH 8.0), 20 mM MgCl₂, equimolar acetoacetyl-CoA and CoA. For the crude cyanobacteria extract assay, the same buffer was used with 200 µM acetoacetyl-CoA and 300 µM CoA. Crude extract of strains EL22 (2.7 µg), EL14 (5.0 µg), and Wild-type (2.4 µg) were used for assay. Concentration of acetoacetyl-CoA was calculated based on a constructed standard curve. Cyanobacteria crude extract was obtained using the same bead beater method described above in protein purification. Protein concentration was measured by Coomassie Plus Assay (Thermo Scientific).

- Bustos SA, Golden SS (1992) Light-regulated expression of the psbd gene family in synechococcus-sp strain Pcc-7942—evidence for the role of duplicated psbd genes in cyanobacteria. *Mol Gen Genet* 232:221–230.
- Andersson CR, et al. (2000) Application of bioluminescence to the study of circadian rhythms in cyanobacteria. J Biolumin Chemilumin 305:527–542.
- Gibson DG, et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 6:343–345.

Acetoacetyl-CoA synthase activity was measured by monitoring the increase of absorbance at 303 nm, which corresponds to appearance of acetoacetyl-CoA. The reaction buffer is the same as that used for thiolase assay. Equimolar malonyl-CoA and acetyl-CoA were used for purified enzyme assay, while 400 μ M of both malonyl-CoA and acetyl-CoA were used for crude extract assay. Crude extract of strains EL22 (27 μ g), EL14 (50 μ g), and wild type (24 μ g) were used for assay.

Enzyme assays for the other enzymes were conducted as previously described (5).

Alcohol Production by E. coli Expressing Butyraldehyde Dehydrogenase. E. coli wild type is based on strain BW25113 (6). Transformed E. coli strain JCL299 ($\Delta adhE$, $\Delta ldhA$, Δfrd , Δpta) was selected on LB plate supplemented with ampicillin (100 µg/mL) and kanamycin (50 µg/mL). Three colonies were picked from the plate to make overnight preculture. The precultures were then used to inoculate 5 mL of Terrific broth (TB; 12 g tryptone, 24 g yeast extract, 2.31 g KH₂PO₄, 12.54 g K₂HPO₄, 4 mL glycerol per liter of water) supplemented with 20 g/L glucose. Growing culture was then induced with IPTG at OD_{600 nm} of 0.6 with 0.1 mM final IPTG concentration. After allowing 1 hr of protein expression, the cultures were then switched to anaerobic condition by purging with N_2 gas using anaerobic chamber. After two days of fermentation, culture sample (2 mL) was centrifuged for 5 min at $21,130 \times g$. The supernatant was analyzed by GC following the same method as that described above in 1-butanol quantification.

1-Butanol Quantification. Culture samples (5 mL) were centrifuged for 20 min at 5,250 × g. The supernatant (900 μ L) was then mixed with 0.1% v/v 2-methyl-pentanol (100 μ L) as internal standard. The mixture was then vortexed and directly analyzed on Agilent GC 6850 system with flame ionization detector and DB-FFAP capillary column (30 m, 0.32 mm i.d., 0.25 film thickness) from Agilent Technologies. 1-Butanol in the sample was identified and quantified by comparing to 0.001% v/v 1-butanol standard. 1-Butanol standard of 0.001% v/v was prepared by 100fold dilution of a 0.1% v/v solution. The GC result was analyzed by Agilent software Chem Station (Rev.B.04.01 SP1). Amount of 1-butanol in the sample was then calculated based on the ratio of its integrated area and that of the 0.001% 1-butanol standard.

Helium gas was used as the carrier gas with 9.52 psi inlet pressure. The injector and detector temperatures were maintained at 225 °C. Injection volume was 1 μ L. The GC oven temperature was initially held at 85 °C for 3 minutes and then raised to 235 °C with a temperature ramp of 45 °C/min. The GC oven was then maintained at 235 °C for 1 min before completion of analysis. Column flow rate was 1.7 ml/min.

- Nishimura T, Saito T, Tomita K (1978) Purification and properties of beta-ketothiolase from Zoogloea ramigera. Arch Microbiol 116:21–27.
- Lan El, Liao JC (2011) Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. *Metab Eng* 13:353–363.
- Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci USA 97:6640–6645.



Fig. S1. Combining ATP hydrolysis to thiolase reaction yields a favorable net reaction.



Fig. S2. Protein SDS/PAGE of His-tag purified KASIII-like enzymes.



Fig. S3. In vitro assay of purified acetoacetyl-CoA synthase (NphT7). Equimolar acetyl-CoA and 4 malonyl-CoA of different concentration were used with 100 mM Tris-HCl and 20 mM MgCl₂. Reaction was conducted at room temperature using spectrophotometer.

PNAS PNAS

Table S1 Strain and plasmid list

Strain	Relevant genotypes	Reference		
Cvanobacteria strains				
PCC 7942	Wild-type Synechococcus elongatus PCC 7942	S.S.Golden		
EL9	P _{Trc} ::His-tagged <i>T. denticola ter</i> integrated at NSI in PCC7942 genome	(1)		
EL14	P _{Trc} ::His-tagged <i>T. denticola ter</i> integrated at NSI and	(1)		
	Pulaco1::atoB. adhE2. crt. hbd integrated at NSII in PCC7942 genome			
EL18	P _{Trc} ::His-tagged <i>T. denticola ter</i> integrated at NSI and	This work		
	Pulaco1::atoB, bldh, vahD, crt, hbd integrated at NSII in PCC7942 genome			
EL20	P _{Trc} ::His-tagged <i>T. denticola ter</i> integrated at NSI and	This work		
	Pulaco1::nphT7, adhE2, crt. hbd integrated at NSII in PCC7942 genome			
EL21	P _{Trc} ::His-tagged <i>T. denticola ter</i> integrated at NSI and	This work		
	Pulaco1::nphT7, bldh, vahD, crt, hbd integrated at NSII in PCC7942 genome			
EL22	$P_{Tre:}$ His-tagged T. denticola ter integrated at NSI and	This work		
	Pulaco1::nphT7, bldh, vahD, phaJ, phaB integrated at NSII in PCC7942 genome			
EL23	P_{Trc} ::His-tagged <i>T. denticola ter</i> integrated at NSI and	This work		
	Pulaco1::atoB, bldh, vghD, phaJ, phaB integrated at NSII in PCC7942 genome			
EL24	$P_{Tre:}$ His-tagged T. denticola ter integrated at NSI and	This work		
	Pulaco1::nphT7, adhE2, phaJ, phaB integrated at NSII in PCC7942 genome			
E. coli strains				
BW25113	$rrnB_{T14} \Delta lacZ_{WJ16} hsdR514 \Delta araBAD_{AH33} \Delta rhaBAD_{LD78}$	(2)		
XL-1 blue	recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F' proAB lacl $^{9}Z\Delta M15$ Tn10 (Tet [*])]	Stratagene		
JCL299	BW25113 Δ IdhA Δ adhE Δ frdBC Δ pta/F' [traD36, proAB+, lacl ^q Z Δ M15 (Tet ^k)]	(3)		
Plasmid	Genotypes	Reference		
pCDFDuet	Spec ^R ; CDF ori; P _{T7} ::MCS	Novagen		
pCDF-nphT7	Spec ^R : CDF ori: P _{T7} :: <i>nphT7</i> (his-tagged)	This work		
pCDF-atoB	Spec ^R : CDF ori: P _{TT} ::atoB (his-tagged)	This work		
pCS27	Kan ^R : P15A ori: Pulaco1::MCS	This work		
pCS138	Cm ^R : SC101 ori: Pupeo1::fdh	(3)		
pDK26	Amp ^R : ColE1 ori: Pusco1::bktB.adhE2.crt.paaH1	This work		
pEL11	Amp ^R : ColE1 ori: Pulseo::atoB.adhE2.crt.hbd	(3)		
pEL29	Kan ^R : pUC ori: ccr-phaJ-phaB	This work		
pEL37	Kan ^R : NSII targeting: Pulacol::atoB.adhE2.crt.hbd	(1)		
pEL52	Amp ^R : pUC ori: P_{TS} :: <i>nphT7</i>	This work		
pEL53	Amp ^R : ColE1 ori: Pupeo1::nphT7.adhE2.crt.hbd	This work		
pEL54	Amp ^R : ColE1 ori: Pulaco1::atoB.bldh.vghD.crt.hbd	This work		
pEL56	Kan ^R : NSII targeting: Pueco1::nphT7.adhE2.crt.hbd	This work		
pEL57	Kan ^R : NSII targeting: Placot::atoB.bldh.vghD.crt.hbd	This work		
pEL59	Kan ^R : NSII targeting: Pulacot::nphT7.bldh.vghD.crt.hbd	This work		
pEL70	Kan ^R ; NSII targeting; Pulaco1::nphT7.bldh.vghD.phaJ.phaB	This work		
pEL71	Kan ^R : NSII targeting: Pulacot::atoB.bldh.vghD.phaJ.phaB	This work		
pEL73	Kan ^R : NSII targeting: Pulaco1::nphT7.adhE2.phaJ.phaB	This work		
pEL75	Amp ^R : ColE1 ori: Pueco1::bktB.bldh.vghD.crt.paaH1	This work		
pEL76	Amp ^R : ColE1 ori: Pulaco1::bktB.aldh(CK).vghD.crt.paaH1	This work		
pEL77	Amp ^R : ColE1 ori: Pulaco1::bktB.aldh(GT).vghD.crt.paaH1	This work		
pEL78	Amp ^R ; ColE1 ori; Pulaco1::bktB.eutE.vghD.crt.paaH1	This work		
pEL79	Amp ^R : ColE1 ori: Pulaco1::bktB.aldh(CB).crt.paaH1	This work		
pEL80	Amp ^R : ColE1 ori: Pulaco:::bktB.aldh(BAA117).vghD.crt.paaH1	This work		
pEL90	Kan ^R ; P15A ori; P _{Llaco1} ::bamb6224 (his-tagged)	This work		
pEL91	Kan ^R ; P15A ori; P _{Llaco1} ::gox0115 (his-tagged)	This work		
pEL92	Kan ^R ; P15A ori; P _{Llaco1} ::hp0202 (his-tagged)	This work		
pEL93	Kan ^R : P15A ori: Pulaco::/mo2202 (his-tagged)	This work		
pEL94	Kan ^R : P15A ori: Pulacoi::pae-fabH2 (his-tagged)	This work		
pEL95	Kan ^R ; P15A ori; P _{Llaco1} ::sav-fabH4 (his-tagged)	This work		
pEL96	Kan ^R : P15A ori: Pulacol::sco5888 (his-tagged)	This work		
	· · · · · · · · · · · · · · · · · · ·			

Kan^R, kanamycin resistance; Amp^R, ampicillin resistance.

NG SANG

atoB (E. coli), thiolase; nphT7 (Streptomyces sp. strain CL190), acetoacetyl-CoA synthase; phaB (R. eutropha), acetoacetyl-CoA reductase; phaJ (A. caviae), (R)-specific enoyl-CoA hydratase; hbd (C. acetobutylicum), 3-hydroxybutyryl-CoA dehydrogenase; crt (C. acetobutylicum), crotonase; ter (T. denticola), Trans-2-enoyl-CoA reductase; bldh (C. saccharoperbutylacetonicum), butyraldehyde dehydrogenase; paaH1 (R. eutropha), 3-hydroxybutyryl-CoA dehydrogenase; yqhD (E. coli), NADP-dependent alcohol dehydrogenase; adhE2 (C. acetobutylicum), bifunctional alcohol/aldehyde dehydrogenase. bktb (R. eutropha), thiolase; aldh (C. kluyveri, C. beijerinckii, C. saccharobutylicum, or G. thermoglucosidasius), aldehyde dehydrogenase; eutE (E. coli), aldehyde dehydrogenase; KASIII-like enzymes: bamb6224 (Burkholderia ambifaria), gox0115 (Gluconobacter oxydans), hp0202 (Helicobacter pylori), Imo2202 (Listeria monocytogenes), pae-fabH2 (Pseudomonas aeruginosa), sav-fabH4 (Streptomyces avermitilis), sco5888 (Streptomyces coelicolor).

1. Lan El, Liao JC (2011) Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. Metab Eng 13:353-363.

Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97:6640–6645.
Shen CR, et al. (2011) Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. *Appl Environ Microbiol* 77:2905–2915.

Lan and Liao www.pnas.org/cgi/doi/10.1073/pnas.1200074109

Table S2. Primer sequences

Primers	Sequence (5 '- > 3 ')	Used for plasmid
rFI -333	TTGCGCTGATCGAGTGGTAAGCATGCAGGAGAAAGGTACCATGAAAG	pEL 53
rEL-334		nFL53
rEL-335		nEL53: nnhT7 gene-specific
rEL 333	ΔGGΔGATATACCATGGΔΔCTΔΔΔCΔΔTGTCATCC	nFI 54
rEL-328	ΤΤΑΔΤΤΓΑΔΟΓΩΤΤΓΑΔΤΓΑΓΓΑΤΤΟΟΓ	nFI 54
rEL 320	GGTTGAATTAAGCATGCAGGAGAAAAGGTACCATGATTAAAGACACGCTAGTTTCTATAAC	nFI 54
rEL-330	GTIGTICATGGTATATCTCCTTTAACCGGCGAGTACACATCTTCTTTGTC	nFI 54
rEL-331	GTACTCGCCGGTTAAAGGAGATATACCATGAACAACTTTAATCTGCACACCCC	nEl 54: vahD specific
rEL-332	TIGTTTAGTTCCATGGTATATCTCCTTCTAGATTAGCGGGCGG	pELS (,) grib specific
rEL 332		nELS6 nELS7 nELS9
rEL-253	ΔΓΩΓΩΤΩΓΩΔΩΩ	nEL56 nEL57 nEL59
rEL-255		pELS6, pELS7, pELS5
rEL-255	TTTATTTGATGCCTCTAGCACGCGTTTATTTTGAATAATCGTAGAAACCTTTTCCTG	pELS6, pELS7, pELS5
rEL-351	CATGGTACCTTTCTCCTGCATGCTTACCACTCGATCAGCGCAAAGCTCGC	p=100, p=101, p=100
rEL-352	TAAGCATGCAGGAGAAAGGTACCATGATTAAAGACACGCTAGTTTC	pE233
rEL-390	ΤΔΔΔCGCGTGCTΔGΔGGCΔTCΔΔΔΤΔ	pEL235
rEL-391	GCAGACATGGTATATCTCCTTTAGCGGGCGGCTTCGTATATACGGC	pEL70, pEL71
rFL-392	ACGAAGCCGCCGCTAAAGGAGATATACCATGTCTGCGCAATC	pEL70, pEL71
rFL-393	TTGATGCCTCTAGCACGCGTTTAACCCATGTGCAGACCACCGTTC	pEL70, pEL71, pEL73
rFL-398	CATGGTATATCTCCTTTAAAATGATTTTATATAGATATCCTTAAGTTCAC	pEL73
rFI -399	ATATCTATATAAAATCATTTTAAAGGAGATATACCATGTCTGCGC	pEL73
rEL-403	TAAAGGAGATATACCATGAACAACTTTAATCTGC	pEL75, pEL76, pEL77, pEL78, pEL79, pEL80
rFI -404	CTTTCTCCTGCATGCTTAGATACGC	pEL75, pEL76, pEL77, pEL78, pEL79, pEL80
rEL-332	TTGTTTAGTTCCATGGTATATCTCCCTTCTAGATTAGCGGGCGG	pEL75
rEL-394	ATGCAGGAGAAAGGTACCATGATTAAAGACACGCTAGTTTCTATAAC	pEL75
rEL-405	AGCGTATCTAAGCATGCAGGAGAAAGGTACCATGGAGATAATGGATAAGGACTTACAGTC	pEL76
rEL-406	TAAAGTTGTTCATGGTATATCTCCTTTAAAGATTTAATTTAGCCATTATAGCTTTTAC	pEL76
rEL-407	GTATCTAAGCATGCAGGAGAAAGGTACCATGGATGCACAAAAAATTGAGAAACTTG	pEL77
rEL-408	AGTTGTTCATGGTATATCTCCTTTATCTATCGACAAAGCATCCACTAGG	pEL77
rEL-409	CGTATCTAAGCATGCAGGAGAAAGGTACCATGAATCAACAGGATATTGAACAGGTG	pEL78
rEL-410	TTGTTCATGGTATATCTCCTTTAAACAATGCGAAACGCATCGACTA	pEL78
rEL-411	TCTAAGCATGCAGGAGAAAGGTACCATGAATAAAGACACACTAATACCTACAACTAAAG	pEL79
rEL-412	TAAAGTTGTTCATGGTATATCTCCTTTAGCCGGCAAGTACACATCTTCTTTG	pEL79
rEL-413	GTATCTAAGCATGCAGGAGAAAGGTACCATGAATAATAATTATTCGTGTCACCAGAAAC	pEL80
rEL-414	TAAAGTTGTTCATGGTATATCTCCTTTAGCCTACGAACACACAC	pEL80
rEL-455	GCTGTGGTGATGATGGTGATGGCTGCTGCCCATGGTACCTTTCTCCTCTTTAATGAATTC	pEL90–96
rEL-456	CGCGTGCTAGAGGCATCAAATAAAAC	pEL90–96
rEL-457	ATCACCATCATCACCACAGCATGGCGGAAATCACCGGCGCGGGGA	pEL90
rEL-458	TTTGATGCCTCTAGCACGCGCTACCAGCGAATCAACGCCGCCCCCA	pEL90
rEL-459	ATCACCATCATCACCACAGCATGTCCGATCCCATTCGTGTCCGCCT	pEL91
rEL-460	TTTGATGCCTCTAGCACGCGTTACATCCGGATAAGGGCGGATCCCCA	pEL91
rEL-461	ATCACCATCATCACCACAGCATGGAATTTTACGCCTCTCTTAAATCCATT	pEL92
rEL-462	TTTGATGCCTCTAGCACGCGCTAACTTCCTCCAAAATACACCAACGCT	pEL92
rEL-463	ATCACCATCATCACCACAGCATGAACGCAGGAATTTTAGGAGTAGGTAAA	pEL93
rEL-464	TTTGATGCCTCTAGCACGCGTTACTTACCCCAACGAATGATTAGGGC	pEL93
rEL-467		pEL94
rEL-468	TTTGATGCCTCTAGCACGCGTCAGTCCATTGTCGGAACGATCTTC	pEL94
rEL-469		pEL95
rEL-470		pEL95
rEL-4/1		pEL96
rEL-472		pEL96
rEL-148		N/A; atoB gene-specific
rEL-149		N/A; atob gene-specific
TEL-15/		N/A; crt.nbd tragment specific
rEL-158		N/A; crt.nbd tragment specific
rel-160		IN/A; ter gene-specific
FEL-101		IV/A; ter gene-specific
rel 162		N/A; adnE2 gene-specific
FEL-163		IN/A; adnez gene-specific
IEL-323		N/A, phas.phab tragment specific
1EL-320		N/A, phas.phab fragment specific
rEl_349	GGGAAAAAACGCACCIAUTTCTATAC	N/A: hldh gene-specific
		Mr., Man gene-specific

PNAS PNAS