Supplementary Table 1. Derivation of *E. coli* K-12 BW25113

Name	Genotype	Pedigree	Source[a]	Source
BD792	*rpoS396*(Am) *rph-1*	W1485	CGSC6159 from B. Bachmann	(Wanner, 1983)
BW21597	DE*phoR574 rpoS396*(Am) *rph-1*	BD792	Tc^R with pAH14 then Tc^S for Pho^- (Haldimann et al., 1997)	unpublished
BW21652	DE*phoR574* DE(*creABCD*)*154 rpoS396*(Am) *rph-1*	BD792 via BW21597	Tc^R with pSK26 then Tc^S for Cre- (Metcalf et al., 1996)	unpublished
BW23186	DE*phoR574* DE(*creABCD*)*154 rpoS396*(Am) DE(*araBAD*)*567 rph-1*	BD792 via BW21652	Tc^R with pAH54 then Tc^S for Ara^- (Haldimann et al., 1998)	unpublished
BW23599	*rrnB3* DE*lacZ4787 proC*::Tn*5-132* DE(*creABCD*)*154 rpoS396*(Am) DE(*araBAD*)*567 rph-1*	BD792 via BW23186	Tc^R, Pro^-, Lac^-, Pho^+ with P1*kc* on BW22773 (Haldimann et al., 1998)	unpublished
BW23660	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* DE(*creABCD*)*154 rpoS396*(Am) DE(*araBAD*)*567 rph-1*	BD792 via BW23599	Pro^+, Pho^- with P1*kc* on BW23316	(Haldimann et al., 1997)
BW24200	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* DE(*creABCD*)*154 rpoS396*(Am) DE(*araBAD*)*567 metF159*(Am) *zii-510*::Tn*10 rph-1*	BD792 via BW23660 (Haldimann et al., 1997)	Tc^R, Met^- with P1*kc* on JW383 (Haldimann et al., 1997)	unpublished
BW24201	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* DE(*creABCD*)*154 rpoS396*(Am) DE(*araBAD*)*567* DE(*rhaBAD*)*568 rph-1*	BD792 via BW24200	Met^+, Tc^S, Rha^- with P1*kc* on BW22875 (Haldimann et al., 1998)	unpublished
BW24310	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* DE(*creABCD*)*154 rpoS396*(Am) DE(*araBAD*)*567* DE(*rhaBAD*)*568 cysC95*::Tn*10 rpoS*::*kan rph-1*	BD792 via BW24201	Tc^R, Km^R with P1*kc* on ZK1001 (Haldimann et al., 1998)	(Haldimann and Wanner, 2001)
BW24320[b]	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* DE(*creABCD*)*154* DE(*araBAD*)*567* DE(*rhaBAD*)*568 rph-1*	BD792 via BW24310 (Haldimann and Wanner, 2001)	Cys^+, Km^S with P1*kc* on MG1655 (Wanner and Boline, 1990)	(Haldimann and Wanner, 2001)
BW24353	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* DE(*zaa trpR creABCD arcA thr*)*104*::Tn*5-132* DE(*araBAD*)*567* DE(*rhaBAD*)*568 rph-1*	BD792 via BW24320 (Haldimann and Wanner, 2001)	Tc^R, Thr^- with P1*kc* on BW12070 (Fisher et al., 1995)	unpublished
BW24476	*rrnB3* DE*lacZ4787* DE(*phoBR*)*580* hsdR514 DE(*araBAD*)*567* DE(*rhaBAD*)*568 rph-1*	BD792 via BW24353	Thr^+, $HsdR^-$ with P1*kc* on BW24447 (Stern et al., 1999)	(Stern et al., 1999)
BW25083	*rrnB3* DE*lacZ4787 proC*::Tn*5-132 hsdR514* DE(*araBAD*)*567* DE(*rhaBAD*)*568 rph-1*	BD792 via BW24476 (Stern et al., 1999)	Tc^R, Pro^-, Pho^+ with P1*kc* on BW22773 (Haldimann et al., 1998)	(Haldimann and Wanner, 2001)

Supplementary Table 1. continued

Name	Genotype	Pedigree	Source[a]	Reference
BW25113[c]	*rrnB3* DE*lacZ4787 hsdR514* DE(*araBAD*)*567* DE(*rhaBAD*)*568 rph-1*	BD792 via BW25083 (Haldimann and Wanner, 2001)	Pro^+ with P1*kc* on BW24321 (Haldimann and Wanner, 2001)	(Lessard et al., 1998;Datsenko and Wanner, 2000)

[a]Tc^R, tetracycline-resistant; Tc^S, tetracycline-sensitive; Km^R, kanamycin-resistant.

[b]BW24320 and its descendents carry the same pseudorevertant *rpoS*(Q33) allele as MG1655 (Hayashi et al., 2005)

[c]We recently discovered that BW25113, like its immediate ancestors, is $lacI^+$ and not $lacI^q$, as had been previously reported (Lessard et al., 1998;Datsenko and Wanner, 2000;Haldimann and Wanner, 2001;Khlebnikov et al., 2001;Zhou et al., 2003;Ismail et al., 2003;Zhou et al., 2004).

References

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

Fisher SL, Jiang W, Wanner BL, Walsh CT (1995) Cross-talk between the histidine protein kinase VanS and the response regulator PhoB: characterization and identification of a VanS domain that inhibits activation of PhoB. J Biol Chem 270:23143-23149

Haldimann A, Daniels LL, Wanner BL (1998) Use of new methods for construction of tightly regulated arabinose and rhamnose promoter fusions in studies of the *Escherichia coli* phosphate regulon. J Bacteriol 180:1277-1286

Haldimann A, Fisher SL, Daniels LL, Walsh CT, Wanner BL (1997) Transcriptional regulation of the *Enterococcus faecium* BM4147 vancomycin resistance gene cluster by the VanS-VanR two-component regulatory system in *Escherichia coli*. J Bacteriol 179:5903-5913

Haldimann A, Wanner BL (2001) Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies in bacteria. J Bacteriol 183:6384-6393

Hayashi K, Morooka N, Yamamoto Y, Fujita K, Isono K, Choi S, Ohtsubo E, Baba T, Wanner BL, Mori H, Horiuchi T (2005) Highly accurate genome sequences of the *Escherichia coli* K-12 strains MG1655 and W3110. Molecular Systems Biology MSB-05-095:

Ismail W, Mohamed MES, Wanner BL, Datsenko KA, Eisenreich W, Rohdich F, Bacher A, Fuchs G (2003) Functional genomis by NMR spectorscopy. Phenylacetate catabolism in *Escherichia coli*. J Biol Chem

Khlebnikov A, Datsenko KA, Skaug T, Wanner BL, Keasling JD (2001) Homogeneous expression of the P_{BAD} promoter in *Escherichia coli* by constitutive expression of the low-affinity high-capacity AraE transporter. Microbiol 147:3241-3247

Lessard IAD, Pratt SD, McCafferty DG, Bussiere DE, Hutchins C, Wanner BL, Katz L, Walsh CT (1998) Homologs of the vancomycin resistance D-ala-D-ala dipeptidase VanX in *Streptomyces toyocaensis*, *Escherichia coli*, and *Synechocystis*: Attributes of catalytic efficiency, stereoselectivity, and regulation with implications for function. Chemistry & Biology 5:489-504

Metcalf WW, Jiang W, Daniels LL, Kim S-K, Haldimann A, Wanner BL (1996) Conditionally replicative and conjugative plasmids carrying *lacZ*α for cloning, mutagenesis, and allele replacement in bacteria. Plasmid 35:1-13

Stern RJ, Lee T-Y, Lee T-J, Yan W, Scherman MS, Vissa VD, Kim S-K, Wanner BL, McNeil MR (1999) Conversion of dTDP-4-keto-6-deoxyglucose to free dTDP-4-keto-rhamnose by the *rmlC* products of *Escherichia coli* and *Mycobacterium tuberculosis*. Microbiol 145:663-671

Wanner BL (1983) Overlapping and separate controls on the phosphate regulon in *Escherichia coli* K-12. J Mol Biol 166:283-308

Wanner BL, Boline JA (1990) Mapping and molecular cloning of the *phn* (*psiD*) locus for phosphonate utilization in *Escherichia coli*. J Bacteriol 172:1186-1196

Zhou L, Kim S-K, Avramova L, Datsenko KA, Wanner BL (2003) Use of Conditional-replication, integration, and modular CRIM plasmids to make single-copy *lacZ* fusions. In: Blot, M. (ed) Methods and Tools in Biosciences and Medicine. Prokaryotic Genomics. Birkhäuser Verlag, Basel, Switzerland, pp 65-89

Zhou L, Zhang K, Wanner BL (2004) Chromosomal expression of foreign and native genes from regulatable promoters in *E. coli*. Methods in Molecular Biology 267:123-134