Supplementary material to Bächler *et al.*

Materials and methods

Strain construction by gene interruption, and plasmid construction

Genes were inactivated with PCR-products according to (1) as follows: (i) MC4100 or BW25113 were transformed with the temperature-sensitive plasmid pKD46 expressing the λ Red recombination system; (ii) cells were electroporated with a PCR products of the kanamycin or chloramphenicol resistence flanked by ends homologues to the target gene, and plated at permissive temperature (30°C) to select for chromosome integration of the resistance gene; (iii) resistant colonies were plated at non-permissive temperature (37°C) to allow for loss of pKD46 plasmid; (iv) correct integration of the antibiotic resistance tag was verified by PCR. The tagged gene disruption was crossed into *E. coli* CBZ and CBZΔI strains by P1*vir* transduction. To remove the antibiotic resistance transductants were transformed with the temperature-sensitive plasmid pCP20 encoding the yeast recombinase and grown at non-permissive temperature of 42°C. Colonies were tested for pCP20 plasmid loss and loss of chromosomal antibiotic resistance, and antibiotic-tag free gene disruptions verified by PCR. Details on construction and primers can be obtained from supplementary material. The P_{dhaK}-*lacZ* and and P_{dhaR}-*lacZ* reporter genes were integrated in the bacteriophage λ attachment site *attB* of *E. coli* MC4100 and its isogenic deletion derivatives using the integration system provided by Boyed et al. (2000). The intergenic region between *dhaR* and *dhaK* containing the *dhaK and the dhaR* promoter and the *lacZ* coding region were joined by overlapping primer extension PCR and then ligated with the vector fragment of pBR322 to afford the reporter plasmid pBRP_{dhaK}-lacZ and pBR P_{dhaR}-lacZ. These plasmids were used to transform *E. coli* DHB6521 which carries the lambda InCh vector with a *c*I857 repressor and an amber mutation in gene *S* (2). An exponentially growing culture of this temperature sensitive lysogen was induced at 42°C resulting in plasmid-phage recombination. During lytic phage production the reporter gene and *bla* from the pBR322 vector recombine with the lambda InCh phage and the kanamycin resistance of the phage is lost. The low-frequency transducing (LFT) lysate was used to lysogenize *E. coli* TH074 (3) and thus insert by site-specific recombination reporter gene and *bla* into the chromsosme at the *attB* site. Lysogens were selected for ampicillin resistance and screened for kanamycin sensitivity. A HFT lysates from such a lysogen was then used to lysogenize *E. coli* MC4100 and TH074. Lysogens were grown on ampicillin at 42°C to select for loss of phage DNA. Phage DNA is lost by recombination between flanking phage DNA sequences resulting in temperature tolerance.

References

1. Datsenko, K. A. and Wanner, B. L. (2000) *Proc.Natl.Acad.Sci.U.S.A* **97,** 6640-6645
2. Boyd, D., Weiss, D. S., Chen, J. C., and Beckwith, J. (2000) *J.Bacteriol.* **182,** 842-847
3. Hesterkamp, T. and Erni, B. (1999) *J.Mol.Microbiol.Biotechnol.* **1,** 309-317

Strain construction

Table S1 Oligonucleotide primers for deletions

Name	Description	Sequence[a]
P101	*dhaR* upstream	5'-tccgccacagcgtagtgcggccgatcccaagtaaaggtgtaggctggagctgcttc
P102	*dhaR* downstream	5'-tatggcaccgcgagagtgtgcgctgtttatcctcgattaacatatgaatatcctccttag
P103	*dhaM* upstream	5'-tgtttatgatgcaaatgttggcgttagccgcaaaagagtaaggaattgggtgtaggctggagctgcttc
P104	*dhaM* downstream	5'-aaccattagtgctgagtaaattgccggatgacatcagaacgatgccatccttccggggatccgtcgacc
P105	*dhaK* upstream	5'-atggattcatcggcagtcagtggtcgccgtgtcgttgaacatcatccgtgtaggctggagctgcttc
P106	*dhaL* downstream	5'-aatcgtaatcgtactttgtgcagcggattcggcgatgctactggcagcttcgagatgaatatcctccttag
P107	*rpoN* upstream	5'-tagggtagaagtttgcgacgttttagcaggagagtacgattctgaacgtgtaggctggagctgcttc
P108	*rpoN* downstream	5'-cgttatttccggtaatgttgagctgcatagtgtcttccttatcggttgggttccggggatccgtcgacc

[a] Underlined bases are complementary to the template, the rest of the sequence is complementary to targeted genes.

Table S2 Strain constructions

Strain	Construction[a]
CBZ	Integrated P_{dhaK}-lacZ into *attBλ* site of MC4100, details in "material and methods"
CBZΔI	Integrated P_{dhaK}-lacZ into *attBλ* site of TH074, details in "material and methods"
CBZΔR	Deletion with *dhaR::cat* PCR fragment generated by using P101 and P102 with pKD3 as template. P1 transduced into CBZ
CBZΔIΔR	Deletion with *dhaR::cat* PCR fragment generated by using P101 and P102 with pKD3 as template. P1 transduced into CBZΔI
CBZΔKL	Deletion with *dhaKL::cat* PCR fragment generated by using P105 and P106 with pKD3 as template. P1 transduced into CBZ
CBZΔIΔKL	Deletion with *dhaKL::cat* PCR fragment generated by using P105 and P106 with pKD3 as template. P1 transduced into CBZΔI
CBZΔKLM	Deletion with *dhaKLM::kan* PCR fragment generated by using P105 and P104 with pKD13 as template. P1 transduced into CBZ
CBΔKLM	Deletion with *dhaKLM::kan* PCR fragment generated by using P105 and P104 with pKD13 as template. P1 transduced into MC4100
CBZΔIΔKLM	Deletion with *dhaKLM::kan* PCR fragment generated by using P105 and P104 with pKD13 as template. P1 transduced into CBZΔI
CBZΔM	Deletion with *dhaM::kan* PCR fragment generated by using P103 and P104 with pKD13 as template. P1 transduced into CBZ
CBZΔIΔM	Deletion with *dhaM::kan* PCR fragment generated by using P103 and P104 with pKD13 as template. P1 transduced into CBZΔI
CBZΔrpoN	Deletion with *rpoN::kan* PCR fragment generated by using P107 and P108 with pKD13 as template. P1 transduced into CBZ
CBZR	Integrated $P_{dhaR\ E.\ coli}$-lacZ into *attBλ* site of MC4100, details in "material and methods"
CBZRΔR	Integrated $P_{dhaR\ E.\ coli}$-lacZ into *attBλ* site of MC4100ΔR, details in "material and methods"
CBZRΔKLM	Integrated $P_{dhaR\ E.\ coli}$-lacZ into *attBλ* site of MC4100ΔKLM, details in "material and methods"

[a] All deletions were made in pKD46 transformants of BW25113. Oligonucleotide primers are shown in Table S1. The plasmids pKD46, pKD3 and pKD13 are of (1)

Plasmid construction

Table S3 Oligonucleotide primer

Name	Desription	Sequence [a]
P3	*tetR* upstream	5'-gaaagcgggcagtgagcgcaacgcaa
P4	*tetR* downstream	5'-accct**gacgtc**gtcatcaaacctgtc
P5	*araC* P_{BAD} upstream	5'-tgcttac**gagctc**ccccttc
P6	*araC* downstream	5'-caattgc**catatg**cttactccatc
P7	p15A kan upstream	5'-tgg**gaattc**ttgctaatctcatg
P8	p15A kan downstream	5'-aaggggga**gagctc**ttattcaacaaag
P9	H230A forward	5'-gcgtcggcattgccggcgagccgggtattgacc
P10	H230A backward	5'-caatacccggctcgccggcaatgccgacgccaaac
P11	*dhaR Ec* downstream	5'-aaccg**gaattc**tttctgtctt
P12	*dhaR Cf* upstream	5'-tgacgacgcacactcaggacat
P13	*dhaR Cf* downstream	5'-aaccg**gaattc**gaggccaggcgtt
P14	*dhaK* upstream	5'-tgccctaccgtaattgct
P15	*dhaK* downstream	5'-caggc**gaattc**tcagtgacattgctt
P20	*dhaR* upstream	5'-tgcgggatatgagtggcgctt
P21	*dhaR* (318)downstream	5'-aacgc**ggatcc**tcaactggtcatcaac
P25	*lacZ* upstream	5'-gaacatcatcc**atgcat**atgattacgga
P26	*lacZ* downstream	5'-cgacccggttattattattttg
P27	*dhaR* upstream	5'-aacgc**ggatcc**tttctgtctttcata
P28	P_{dha} downsteam	5'-atccgtaatct**atgcat**ggatgatgttcaa
P31	P_{dhaR} upstream	5'-aacgc**ggatcc**tgatgcagt
P32	P_{dhaR} downstream	5'-gtcca**atgcat**ccgaccatcgttgtt
P33	P_{dhaK} upstream	5'-aacgc**ggatcc**cgtgccgtcattgaatcc
P34	P_{dhaK} downsteam	5'-attttgctccagcaattacggtagggcatggatgatgttcaac
P40	*dhaK* upstream	5'-**gtacc**atgccctaccgtaattgct
P41	*dhaM* downstream	5'-gatca**ctgcag**tgacatcagaacgat
P42	*dhaL* upstream	5'-**gtacc**atgtcactgagcagaactc
P43	*dhaL* downstream	5'-gatca**ctgcag**gaccaggtttaccat
P121	*dhaR Ec* downstream	5'-gacgc**agatct**tacccggcgcttaaactg

[a] Underlined bases are complementary to the template (s). Those in bold indicate restriction enzyme sites.

Table S4 Plasmid construction

Name	Construction
pET28 *R(N)*	Generated a PCR product of *dhaR* (AA1-318) by using P20 and P21 with pAC *R Ec* as template. Digested with BamHI and ligated into pET28a (cut with NdeI, blunt with Klenow and cut with BamHI).
pJF *K(H230A)L*	Generated a *dhaK(H230)* mutation by using PCR Quick change method with P9 and P10
pJF *M*(H9A,H169A, H430A)	Replaced *NsiI* to *NruI* fragment of pJF *M*(H169A) with similar fragment of pJF *M*(H9A). Replaced the *ScaI to PvuI* fragement of pJF *M*(H9A, H169A) with the similar fragment of pJF *M*(H430A).
pJF *R Ec* H6	Generated a PCR product of *dhaR* by using P20 and P121 with genomic DNA as template. Digested with *BglII* and ligated into pJF fruAH6 (cut with NdeI, blunt with Klenow and cut with *BglII*).
pAC *K*	Replaced *NdeI* to *EcoRI* fragment of pAC *M* plasmid with PCR fragment generated by P14 and P15 with pJF*dhaKL* as template.
pAC *R Ec*	Replaced *NdeI* to *EcoRI* fragment of pAC *M* plasmid with PCR fragment generated by P20 and P11 with W3110 genomic DNA as template.
pAC *R Cf*	Replaced *NdeI* to *EcoRI* fragment of pAC *M* plasmid with PCR fragment generated by P12 and P13 with *C.freundii* genomic DNA as template.
pAC *M*	Generated a PCR product of P_{BAD} *araC* by using P5 and P6 with genomic DNA as template and a PCR product of p15Aori kan by using P7 and P8 with pACYC177 as template. Triple ligation of this two fragments with *dhaM* fragment of pJF*dhaM* (cut *HindIII*, made blunt with T4 polymerase, cut with *NdeI*)
pAC *M(H9A)*	Replaced *NdeI* to *BglII* fragment of pAC *M* plasmid with the *NdeI* to *BglII* fragment of pJF*dhaM*(H9A)(4)
pAC *M(H169A)*	Replaced *NdeI* to *BglII* fragment of pAC *M* plasmid with the *NdeI* to *BglII* fragment of pJF*dhaM*(H169A)(4)
pAC *M(H430A)*	Replaced *NdeI* to *HindIII* fragment of pAC *M* plasmid with the *NdeI* to *HindIII* fragment of pJFdhaM (H430A)(4)
pAC*ptsI*	Digested pMSEH2 EI_ecoli with *HindIII*, made blunt with Klenow and digested with *NdeI*. Ligated with pAC *M* (digested with *EcoRI*, made blunt with Klenow and cut with *NdeI*)
pBRdhaR$_{Pdha}$-lacZ	Generated a PCR product of lacZ by using P25 and P26 and a PCR product of dhaR-P_{dha} using P27 and P28 with genomic DNA as template. Joined the two fragments by overlapping PCR by using P26 and P28. Digested with *BamHI* and ligated into pBR322 (cut with *BamHI* and *NruI*).
pBR P_{dhaK}-lacZ	Generated a PCR product by using P33 and P34 with genomic DNA as template. Digested with BamHI and ligated into pBRdhaR-P_{dha}-lacZ (cut with *NsiI*, made blunt with T4 polymerase and cut with *BamHI*). Digested plasmid with *BamHI* and *HindIII* followed by filling-in with T4 polymerase and religation.
pBR P_{dhaR}-lacZ	Replaced *NsiI* to *BamHI* fragment of pBR dhaR P_{dha}-lacZ plasmid with PCR fragment generated by using P31 and P32 with genomic DNA of *E. coli* as template.
pZS*tetRluc	Generated a PCR product of *tetR* by using P3 and P4 with genomic DNA of DH5αZ. pZE21MCS (5) was used as template. Digested with *AatII* and ligated into pZA31-luc (cut with XhoI, made blunt with klenow enzyme and cut with AatII). Replaced a *SacI* to *AvrII* fragment of pZA31tetR-luc with the origin of pZS*24-MCS-1.
pZStetR *MCS*	Replaced *BlnI* to *KpnI* fragment of pZS*tetRluc plasmid with similar fragment from pZE21MCS (5)
pZS *KLM*	Generated a PCR product of *dhaKLM* by using P40 and P41 with genomic DNA as template. Digested with *PstI* and ligated into pZStetR *MCS* (cut with *KpnI*, made blunt with T4 polymerase and cut with *PstI*)
pZS *KL*	Generated a PCR product of *dhaKL* by using P40 and P43 with pJF*dhaKL* (4) as template. Digested with *PstI* and ligated into pZStetR *MCS* (cut with *KpnI*, made blunt with T4 polymerase and cut with *PstI*).
pZS *L*	Generated a PCR product of *dhaL* by using P42 and P43 with pJF*dhaKL* as template. Digested with *PstI* and ligated into pZStetR *MCS* (cut with *KpnI*, made blunt with T4 polymerase and cut with *PstI*)
pZS *K(H230K)L*	Generated a PCR product of *dhaK(H230K)L* by using P40 and P43 with pJF *dhaK(H230K)L* as template (6) Digested with *PstI* and ligated into pZStetR *MCS* (cut with *KpnI*, made blunt with T4 polymerase and cut with *PstI*)

[a]Oligonucleotide primers are shown in Table S3.

1. Datsenko, K. A. and Wanner, B. L. (2000) *Proc.Natl.Acad.Sci.U.S.A* **97,** 6640-6645
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6. Baechler unpublished