Supplementary material

Genetic methods

Plasmids were maintained in the host *E. coli* DH5α (F^-, φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk^-, mk^+), *phoA*, *supE*44, λ^-, *thi*-1, *gyrA*96, *relA*1).

Plasmids. pKD46 (Red helper plasmid, Ampicillin resistance), pKD3 (contains an FRT-flanked chloramphenicol resistance (*cat*) gene), pKD4 (contains an FRT-flanked kanamycin resistance (*kan*) gene), and pCP20 (expresses FLP recombinase activity) plasmids were obtained from Prof. Dr. J-P Hernalsteens (Vrije Universiteit Brussel, Belgium). The plasmid pBluescript (Fermentas, St. Leon-Rot, Germany) was used to construct the derivates of pKD3 and pKD4 with a promoter library, or with alleles carrying a point mutation.

Mutations. The mutations consisted in gene disruption (knock-out, KO), replacement of an endogenous promoter by an artificial promoter (knock-in, KI) (De Mey *et al.*, 2007). They were introduced using the concept of Datsenko and Wanner (2000). The primers for the mutation strategies are described in Table 1.

Transformants carrying a Red helper plasmid were grown in 10 ml LB media with ampicillin (100 mg/l) and L-arabinose (10 mM) at 30 °C to an OD_{600nm} of 0.6. The cells were made electrocompetent by washing them with 50 ml of ice-cold water, a first time, and with 1 ml ice-cold water, a second time. Then, the cells were resuspended in 50 µl of ice-cold water. Electroporation was done with 50 µl of cells and 10–100 ng of linear double-stranded-DNA product by using a Gene Pulser™ (BioRad) (600 Ω, 25 µFD, and 250 volts).

After electroporation, cells were added to 1 ml LB media incubated 1 h at 37 °C, and finally spread onto LB-agar containing 25 mg/l of chloramphenicol or 50 mg/l of kanamycin to select antibiotic resistant transformants. The selected mutants were verified by PCR with primers upstream and downstream of the modified region and were grown in LB-agar at 42 °C for the loss of the helper plasmid (Table 2). The mutants were tested for ampicillin sensitivity

Linear double-stranded-DNA. The linear ds-DNA amplicons were obtained by PCR using pKD3, pKD4 and their derivates as template. The primers used had a part of the sequence complementary to the template and another part complementary to the side on the chromosomal DNA where the recombination has to take place. For the KO, the region of homology was designed 50-nt upstream and 50-nt downstream of the start and stop codon of the gene of interest. For the KI, the transcriptional starting point (+1) had to be respected. PCR products were PCR-purified, digested with *Dpn*I, repurified from an agarose gel, and suspended in elution buffer (5 mM Tris, pH 8.0).

Elimination of the antibiotic resistance gene. The selected mutants (chloramphenicol or kanamycin resistant) were transformed with pCP20 plasmid, which is an ampicillin and chloramphenicol resistant plasmid that shows temperature-sensitive replication and thermal induction of FLP synthesis. The ampicillin-resistant transformants were selected at 30 °C, after which a few were colony purified in LB at 42 °C and then tested for loss of all antibiotic resistance and of the FLP helper plasmid. The gene knock outs and knock ins are checked with control primers. These primers are given in Table 2.

Table 1: Overview of the primers used for the mutation strategies

Primer name	sequence
Fw-*dctA*-P1	caggggtcaattatgcgcaaacacccgcactcggggaagggagtgcgggcataagtgatgaga gtgtaggctggagctgcttc
Rv-*dctA*-P2	caggtaccccataaccttacaagacctgtggttttactaaaggacaccct catatgaatatcctccttag
Fw-*yfbS*-P1	Gagtctgcgtcgcatcaggcaataagcgccggatgcgacatcaggctcttg gtgtaggctggagctgcttc
Rv-*yfbS*-P2	gggctttttttatggcagaatcaagtcatccccctcaattaacaaggataagtt catatgaatatcctccttag
Fw-*sstT*-P1	atgcgtcgacagaacgcaccagggatgtgcgacaacacaatgaaaggatcgaaaa gtgtaggctggagctgcttc
Rv-*sstT*-P2	gaattgatccgtttaaagttgagaaaaccccttccgccgtagacgaaaggggttaaacaa catatgaatatcctccttag
Fw-*ydjN*-P1	gcgccaacactatgactgctacgcagtgatagaaataataagatcaggagaacgggg gtgtaggctggagctgcttc
Rv-*ydjN*-P2	ggaacatttaaaaagtaaggcaacggcccctatacaaaacggaccgttgccagcataagaa catatgaatatcctccttag
Fw-*ybhI*-P1	aaggacaatcaataaaggacttctgtatgagtcatacagaaagaacaggattttaa gtgtaggctggagctgcttc
Rv-*ybhI*-P2	gactggggtgagcgaacgcagacgcagcacatgcaacttgaagtatgacgagaatat catatgaatatcctccttag
Fw-*yhjE*-P1	cacttttgctgtgcgtaatatggctattcgttagccaaaaaataagaaaagatt gtgtaggctggagctgcttc
Rv-*yhjE*-P2	tatattgtatggaaattcaggcctgataagcgtagcgcatcaggcttttcactctta catatgaatatcctccttag
Fw-*ydfJ*-P1	atgttttgttatatgaataaaaatcccctctccggtaagagaagggattaagggt gtgtaggctggagctgcttc
Rv-*ydfJ*-P2	aaaacctagaaaacgccaaggaaaccacaggatgggaaaaaacacctgtgaatt catatgaatatcctccttag
Fw-*ttdT*-P1	tcaaataaccctcccggagaggctcacccctctccttttttcgcaggcataacacg gtgtaggctggagctgcttc
Rv-*ttdT*-P2	gctgcgtaaaactattgggtgccggagagcaatttccggcaccgtcctcac catatgaatatcctccttag
Fw-*citT*-P1	aggtgggcttacctggcgggcgtgatgatttattcagcgtttggcgaacgta gtgtaggctggagctgcttc
Rv-*citT*-P2	taattatttaagcacttgataaatttggaaatattaattttcggagaacccgt catatgaatatcctccttag
Fw-*sdhAB*-P1	cactggtggtttacgtgatttatggattcgttgtggtgtggggtgtgtgagtgtaggctggagctgcttc
Rv-*sdhAB*-P2	tccggcactggttgcctgatgcgacgcttgcgcgtcttatcaggcctacggtcatatgaatatcctccttag
Rv-d*cuC*-P2	gctacaattaacataaccttaatagacccaacataaagaataatctgaatagccatatgaatatcctccttag
Fw-d*cuC*-pro37	tgcccaataaattggcgatgaatgctgattaaaatcaagaaaaactgccataaaaatgacatataccacatgga

Table 2: Overview of the control primers used to check knock outs and knock ins

Primer name	sequence
Fw-*dctA*-out	tgcccgttgtcggtcagtc
Rv-*dctA*-out	atcttcgaagagagttacctgg
Fw-*yfbS*-out	aggagatggactacttcatgga
Rv-*yfbS*-out	tttggtcctccacagtctgg
Fw-*sstT*-out	agcatttttcgcttcccgaag
Rv-*sstT*-out	gggtgattcacaacttctggg
Fw-*ydjN*-out	ataccttgcagatccagtttcatcg
Rv-*ydjN*-out	gtatgaggttttaagcttctcg
Fw-*ybhI*-out	aggtcaggatgccacgacg
Rv-*ybhI*-out	agatatctgcgcaccgtg
Fw-*yhjE*-out	ttcatagaagaaaaatcactggc
Rv-*yhjE*-out	attgccggatgtctgacatcc
Fw-*ydjN*-out	ataccttgcagatccagtttcatcg
Rv-*ydjN*-out	gtatgaggttttaagcttctcg
Fw-*ybhI*-out	aggtcaggatgccacgacg
Rv-*ybhI*-out	agatatctgcgcaccgtg
Fw-*ydfJ*-out	Aatgaacaattcttggagccagg
Rv-*ydfJ*-out	tatctgttggggagttttttgg
Fw-*ttdT*-out	tgattgtctctattgatacccac
Rv-*ttdT*-out	cggaatggtgcggtttaaagc
Fw-*citT*-out	gcagaagagaagggaagcagag
Rv-*citT*-out	ttatctccggcagttcgacag
Fw-*sdhAB*-out	catgtggcaggtgttgaccg
Rv-*sdhAB*-out2	tcctgcatttctttgttacgcct
Fw-*DcuC*-out	cgagctacacccacaataacc
Rv-*DcuC*-out	tcaggctgttgccaggtttgt