Supporting Information

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Supporting Materials and Methods

Reagents, Strains, and Plasmids. LB medium (1.0% tryptone, 0.5% yeast extract, 0.5% NaCl) was from Acumedia, agar was from Difco, and antibiotics, isopropyl-beta-D-thiogalactopyranoside (IPTG), L-arabinose, maltose, and $MgSO_4$ were from Sigma-Aldrich. Restriction enzymes were from New England Biolabs. Rapid ligation kit was from Roche. Bacterial strains, phages, plasmids and oligonucleotides used in this study are listed in Table S1.

Construction of Transposon-Insertion Library and Selection of Genes Essential for CRISPR Activity. Conjugation of pRL27-kan plasmid carrying the Tn5 transposon was carried out as follows. A late-exponential culture (OD_{600} 0.8) of the recipient strain *E. coli* BW25113Δ*hns araB*::T7-RNAp λ*cI857-bla* grown in LB medium with 50 μg/ml ampicillin was mixed with a late-exponential culture (OD_{600} 0.8) of the donor strain WM2672 (1) grown in LB medium containing 12 μg/ml kanamycin at a 10:1 recipient-to-donor ratio. The mixed cell suspension was incubated at 32°C for 30 min. Cells were vortexed and plated on LB agar containing 12 μg/ml kanamycin and 50 μg/ml ampicillin. Approximately 50,000 colonies were obtained from repeated conjugations. The pooled cells were supplemented with 10% vol/vol glycerol and stored in aliquots at −80°C. For the selection procedure, these cells were thawed, rendered competent, and transformed with pWUR478. Colonies grown on selection plates supplemented with 35 μg/ml chloramphenicol, 12 μg/ml kanamycin, 100 μg/ml ampicillin, 10 μg/ml tetracycline, 1% L-arabinose and 1 mM IPTG were taken for further studies as candidates for colonies with Tn5 disruption of genes essential for CRISPR function. Screening for *cas*-disrupted colonies and examining transposon insertions in *cas1* or *cas2* were performed using primers P1, P2, P3, KF, KR, Cas1-F, and Cas2-R (Table S1). Further identification of the transposon-insertion site in non-*cas* disrupted colonies was carried out by semi-random two-step PCR as described previously (2).

Transduction. Transductions were used to replace specific genes (e.g. *htpG*, *hns*) with antibiotic resistance or for insertion of *araB*::T7-RNAp. P1 lysate was prepared as follows: overnight cultures of the donor strain, either specific clones from the Keio collection (3) or BL21-AI-T7-RNAp::*tet* (Invitrogen), were diluted 1:100 in 2.5 ml LB medium containing 5 mM $CaCl_2$ and 0.2% glucose. After 1 h shaking at 37°C, 0 to 100 μl phage P1 was added. Cultures were aerated for 1 to 3 h, until lysis occurred. The obtained P1 lysate was used in transduction, where 100 μl of fresh overnight culture was mixed with 1.25 μl of 1 M $CaCl_2$ and 0 to 100 μl P1 phage lysate. After incubation for 30 min at 30°C without shaking, 100 μl of 1 M Na-citrate and 500 μl LB medium were added. Cultures were incubated at 37°C for 60 min, then 3 ml of warm LB medium supplemented with 0.7% agar was added and the suspension was poured onto a plate containing the appropriate drug. Transductants obtained on antibiotic plates were streaked several times on selection plates and verified by PCR for the presence of the transduced DNA fragment. In some cases, the kanamycin resistance marker was removed using the flippase recombination enzyme encoded by plasmid pCP20 (4).

Plasmid Construction. *htpG* was cloned under the arabinose promotor in plasmid pBAD18. The *htpG* gene was PCR-amplified from MG1655 genomic DNA using primers RE18F and RE18R (Table S1). Amplified DNA was digested by SalI/SacI and ligated into pBAD18 digested with the same restriction enzymes, to yield pBAD-HtpG. pAC-477 and pAC-478 are ampicillin-resistant (amp^r) pACYC177 derivatives with short CRISPR arrays derived from the cam^r pWUR477 and pWUR478, respectively. DNA inserts were prepared by PCR amplification of pWUR477 or pWUR478 using oligonucleotides RK8F and RK7R (Table S1). The resulting DNA inserts encode the respective arrays under the T7 promoter, but without the lacO site, and therefore do not require IPTG for induction. The amplified DNAs were digested with HindIII/XhoI and ligated into pACYC177 digested with the same restriction enzymes. All DNA inserts were confirmed by sequencing.

1. Larsen RA, Wilson MM, Guss AM, Metcalf WW (2002) Genetic analysis of pigment biosynthesis in Xanthobacter autotrophicus Py2 using a new, highly efficient transposon mutagenesis system that is functional in a wide variety of bacteria. *Arch Microbiol* 178(3):193–201.
2. Walker CB, et al. (2009) The electron transfer system of syntrophically grown Desulfovibrio vulgaris. *J Bacteriol* 191(18):5793–5801.
3. Baba T, et al. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* 2:1–11.
4. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci U S A* 97(12):6640–6645.

Table S1. Bacterial strains, phages, plasmids, and oligonucleotides used in this study

	Description/sequence	Source
Bacterial strains		
NEB5α	F⁻ ϕ80*lacZ*ΔM15Δ*(lacZYA-argF)* U169 *deoR recA1 endA1 hsdR17* (r_k^-, m_k^+) *gal*⁻ *phoA supE44* λ⁻*thi*⁻*1 gyrA96 relA1*	New England Biolabs
BW25113	F⁻ Δ*(araD-araB)567* Δ*lacZ4787*(::rrnB-3) λ⁻ *rph-1* Δ(*rhaD-rhaB*)*568 hsdR514*	(1)
IYB5163	BW25113 Δ*hns araB*::T7-RNAp-tetA λ*cI857*-kan	This study
RE1093	BW25113 Δ*hns araB*::T7-RNAp-tetA λ*cI857-bla*	This study
IYB5165	BW25113 Δ*hns* Δ*htpG araB*::T7-RNAp-tetA λ*cI857*-kan	This study
IYB5237	BW25113 *araB*::T7-RNAp-tetA λ *cI857*-kan	This study
JW0462	BW25113 Δ*htpG*::kan	(2)
Phages		
λvir	Obligatory lytic	(3)
λcI857-bla	*cI857* s100 nin5, amp^r	Stratagene
λcI857-kan	*cI857*, kan^r	(4)
Plasmids		
pWUR477	pACYCDuet-1 (Novagen) cloned with control spacers under T7 promoter, cam^r	(5)
pWUR478	pACYCDuet-1 (Novagen) cloned with anti λ spacers under T7 promoter, cam^r	(5)
pAC-477	pACYC184-derived, cloned with control spacers under T7 promoter, amp^r	This study
pAC-478	pACYC184-derived, cloned with anti λ spacers under T7 promoter, amp^r	This study
pBAD18	Arabinose-inducible expression vector, amp^r	(6)
pBAD-HtpG	pBAD18 cloned with *htpG*, amp^r	This study
pCA24N-CasA	pCA24N cloned with *casA*, cam^r	(7)
pCA24N-CasB	pCA24N cloned with *casB*, cam^r	(7)
pCA24N-CasC	pCA24N cloned with *casC*, cam^r	(7)
pCA24N-CasD	pCA24N cloned with *casD*, cam^r	(7)
pCA24N-CasE	pCA24N cloned with *casE*, cam^r	(7)
pCA24N-Cas3	pCA24N cloned with *cas3*, cam^r	(7)
pCA24N-HtpG	pCA24N cloned with *htpG*, cam^r	(7)
pCA24N-CasA-GFP	pCA24N cloned with *casA-gfp*, cam^r	(7)
pCA24N-CasB-GFP	pCA24N cloned with *casB-gfp*, cam^r	(7)
pCA24N-CasC-GFP	pCA24N cloned with *casC-gfp*, cam^r	(7)
pCA24N-CasD-GFP	pCA24N cloned with *casD-gfp*, cam^r	(7)
pCA24N-CasE-GFP	pCA24N cloned with *casE-gfp*, cam^r	(7)
pCA24N-Cas3-GFP	pCA24N cloned with *cas3-gfp*, cam^r	(7)
Oligonucleotides	5′→3′	
RE17F	TTAAGAGCTCTTGAGGAAGACCTACATGAAAGGAC	
RE17R	ATTGTCGACTCAGGAAACCAGCAGCTGGTTC	
RK7R	CAGACTCGAGGGTACCGCGTC	
RK8F	GATAAAGCTTGAAATTAATACGACTCACTATAGGGAGAGGAAACAAAGAATTAGCTGATC	
P1	TTTGGGATTTGCAGGGATGAC	
P2	CTTCAGCTGGCGGCCGCGGCGCCTCGCCATTATTACGAA	
P3	CTTCAGCTGGCGGCCGCGGGCTACTCCGATGGCCTGCAT	
KF	ACCTACAACAAAGCTCTCATCAACC	
KR	GCAATGTAACATCAGAGATTTTGAG	
Cas1-F	GGGCTAGCGAATTCGAGCTC GCTCCACTGTGATTGAGGTG	
Cas2-R	CATCACCTTTGGCTTCGGCTG	

1. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci U S A* 97(12):6640–6645.
2. Baba T, et al. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* 2:1–11.
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