Supporting Information

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SI Materials and Methods

Strain and Additional Plasmids. The *Pseudomonas aeruginosa* parent strain MPAO1 is a PAO1 isolate from the Iglewski laboratory (1). Plasmid pU1 is a derivative of pEX19Tc (2) carrying a "USER" polylinker (3); pU2 is a derivative of pU1 carrying *Escherichia coli araC*, the P_{BAD} promoter, the PAO1 *fusA1* Shine–Dalgarno sequence, and seven additional nucleotides upstream of PAO1 *fusA1*, followed by five codons of *fusA1* and seven codons derived from the plasmid multiple cloning site; pU3 is identical to pU2 with *lacI*^{q}-P_{tac} in place of *araC*-P_{BAD}. Sequence files and details of the constructions of plasmids pU1-pU3 are available by request from C.M.

Growth Media. LB agar (per liter): 10 g tryptone, 5 g yeast extract, 8 g NaCl, 15 g Bacto agar; supplemented in some cases with with tetracycline (60 μg/mL) and chloramphenicol (10 μg/mL).

LB agar, 2× (per liter): 20 g tryptone, 10 g yeast extract, 8 g NaCl, 30 g Bacto agar; supplemented with tetracycline (60 μg/mL) and chloramphenicol (10 μg/mL).

MOPS-pyruvate agar (per liter): 100 mL 10× MOPS (400 mM MOPS buffer, 40 mM tricine, 0.1 mM $FeSO_4$, 95 mM NH_4Cl, 2.76 mM K_2SO_4, 5 μM $CaCl_2$ and 5.25 mM $MgCl_2$, adjusted to pH 7.6 with ∼330 mM KOH), 10 mL 0.132 M K_2HPO_4, 10 mL 20% (wt/vol) sodium pyruvate, 15 g Bacto agar; supplemented with tetracycline (90 μg/mL) and chloramphenicol (10 μg/mL).

Cystic fibrosis sputum agar: Sputum was obtained from consenting cystic fibrosis patients under a study protocol approved by the University of Washington Institutional Review Board (#31279), and samples were stored at −20 °C. The sputum samples were thawed in cold water before addition of equal volume of sterile deionized water and then homogenized for three minutes in a Polytron PT 3100 homogenizer. The samples were further liquified using three sonication treatments (Ultrasonic Homogenizer 4710 series, Cole-Parmer Instrument Co.; power setting 5, duty cycle 50%, 50 s each). Samples were then sterilized by the addition of chloroform [2% (vol/vol) final] and vortex mixed for 30 s. Chloroform was removed by centrifuging the samples (1,000 × *g* for 10 min) and removing the aqueous sputum phase. Sputum samples were then diluted to achieve a final concentration of 10% (vol/vol) sputum from the initial sample and solidified with 1.5% (wt/vol) agar. Antibiotic supplements were tetracycline (70 μg/mL) and chloramphenicol (10 μg/mL); 2.5 μM $FeSO_4$ was added to two of the four batches of sputum agar used to increase the efficiency of *P. aeruginosa* colony formation.

LB agar, 0.1× (per liter): 1 g tryptone, 0.5 g yeast extract, 100 mL 10× MOPS, 10 mL 0.132 M K_2HPO_4, 10 mL 20% (wt/vol) sodium pyruvate, 10 mL 20% (wt/vol) glucose, 15 g Bacto agar; 10 μg/mL each adenosine, guanosine, cytidine, thymidine, and uridine; 1 μg/mL each biotin, folic acid, hemin, lipoic acid, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, thiamine, and 2,6-diaminopimelic acid; supplemented with tetracycline (50 μg/mL) and chloramphenicol (10 μg/mL).

Human serum agar (per liter): 5% (vol/vol) human serum (heat-inactivated; Sigma H3667), 2.5 μM $FeSO_4$, 15 g Bacto agar; supplemented with tetracycline (52.5 μg/mL) and chloramphenicol (10 μg/mL).

BHI agar (per liter): 52 g BHI agar (Difco 241830), 2 g yeast extract, 1 mM $MgSO_4$; 5 μg/mL each adenosine, guanosine, cytidine, thymidine and uridine; glutamine (100 μg/mL), 2,6-diaminopimelic acid (400 μg/mL), hemin (5 μg/mL); 1 μg/mL each biotin, folic acid, lipoic acid, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine; supplemented with tetracycline (60 μg/mL) and chloramphenicol (10 μg/mL).

Mutant Selection. Mating mixtures were thawed for 1 h before plating. For selection on LB and BHI agar, thawed cell mixtures (originally frozen in LB-DMSO) were plated directly on selection agar. For selection on media other than LB agar, thawed cell mixtures (originally frozen in MOPS-pyruvate-DMSO or LB-DMSO) were plated directly or resuspended in 40 mM MOPS + 10 mM $MgSO_4$ pH 7.6 buffer and plated. Mating mixes were plated on Bioassay QTrays (Molecular Devices, part #X6023; 240 × 240 × 20 mm) aiming for 30,000 mutants per QTray. Incubation times varied depending on the selective medium: LB and 0.1× LB agars, 24 h; MOPS-pyruvate, sputum, and serum agars, 36 h; and BHI agar, 19.5 h. Cells were harvested after flooding plates with 24-mL buffer (or media) and suspending bacteria.

Sequence Data Analysis. Processing of Illumina sequence reads was carried out as described previously (4), with reads per insertion location calculated using a custom script (www.nwrce.org/software-utilities, "Tn-seq"). In a few cases for which reads could not be mapped to an exact location because of repetitive genome sequences, they were randomly assigned one of the possible locations. (Presumably, most repetitive sequences are not essential because of redundancy.) A second script was used to annotate the insertion locations and tabulate the number of hits per gene (www.nwrce.org/software-utilities, "Tn-seq") using the current genome annotation for PAO1-UW (NC_002516) (5). Tn-seq results were normalized to 10,000,000 reads per pool.

For each Tn-seq assay, two values were calculated for each locus: transposon insertions per kilobase ("hits/kb") and transposon sequence reads per kilobase ("reads/kb"). To discount non-disruptive insertions in the 5′ and 3′ ends of loci, only insertions within the 5–90% of each locus were considered. Hit and read density was calculated by dividing hits (5–90%) and reads (5–90%) by locus length (5–90%). Hit and read densities were calculated for a total of 5,573 annotated coding sequences.

Next, the distributions of hits per gene and reads per gene for each Tn-seq assay were calculated. Genes with zero values were considered essential. The remaining assignments (reads per gene and hits per gene) were $\log_2$-transformed and data were fit to a normal distribution (Fig. S3). Loci with values falling below a $P = 0.001$ cut-off for each distribution were added to the 0-value set for the final set of candidate essential loci for the corresponding Tn-seq run. In practice, the essential gene assignments derived from the reads per gene and hits per gene analyses were nearly the same for each assay.

The final step of the analysis was to determine the consensus sets of essential genes for the three primary growth conditions analyzed using multiple pools (LB, MOPS-pyruvate, and sputum media). First, for two of the mutant pools for which multiple technical replicates were available (LB-1, and LB-2), genes that were scored as essential in at least two of three assays (LB-1) or at least one of two assays (LB-2) were included. Second, the consensus sets of essential genes for each of the three growth conditions were derived from those genes found for at least two of three LB pools, two of three MOPS-pyruvate pools, or three of four sputum agar pools.

Identifying Essential Regions by Gap Size Analysis. As an independent procedure to detect genes with essential domains and unidentified essential genes, we analyzed the distribution of gaps

between transposon insertions in the genome (6). We examined the combined insertions from all 13 mutant pools (corresponding to 1,043,276 unique insertion sites). (For the LB-1 and LB-2 mutant pools, the hits and reads values for the technical replicates were averaged to derive a representative dataset for each.) A histogram of gaps of different lengths for the experimental values is shown in Fig. S5 (blue bars) compared with the distribution seen for a simulation based on random positioning of the same number of unique insertion sites (red bars). The simulation allowed a unique hit in either orientation at each nucleotide and assumed every position in the genome and was equally likely to be hit.

As gap sizes increased, the disparity between the experimental and simulated distributions increased, reflecting the essential regions that did not tolerate insertions. For gaps larger than 100 bp, virtually all (>99.9%) of those seen experimentally could not be accounted for by chance. Recognizing that mutagenesis may not be completely random, even for nonessential regions, we used a conservative cut-off of 150 bp to assign essential regions. There were 833 gaps of at least this length in the combined mutant pool. The gaps overlapped with 303 of 329 general essential coding genes identified using the method described in the previous section. It also identified 22 new genes with both essential and nonessential regions, and 1 gene that had not been recognized in the genome sequence annotation. The total number of general essential genes including those with essential domains was thus 352 (329 + 22 + 1).

Construction of Strains with Conditionally Expressed Essential Genes. To create strains with essential genes expressed from inducer-dependent promoters, we used a plasmid single-integration procedure in which an inducible promoter replaced the native promoter. Plasmids carried an inducible promoter (P_{BAD} or P_{tac}) adjacent to sequences corresponding to the 5′ regions of essential operons, oriented such that integration at homologous sequences replaced the native promoter in the genome with one of the inducible promoters. The plasmids used for the integrations were constructed from pU2 and pU3 by insertion of PCR fragments using uracil ("USER") cloning (3). The final constructs were designed to carry an inducible promoter (P_{BAD} or P_{tac}), the PAO1 *fusA1* Shine–Dalgarno sequence and seven additional nucleotides upstream of PAO1 *fusA1*, followed by five codons of *fusA1*, seven codons derived from the plasmid multiple cloning site, and finally sequences corresponding to the target essential gene starting at its second codon. (The N-terminal sequence of each final protein product is expected to be MARTTGTAEETF followed by the second amino acid of the target gene product.) For most genes (>600-bp long), the 5′ sequences inserted into the plasmid were 0.4–0.6 kbp; for small genes, shorter sequences were inserted corresponding to the essential 5′ regions that had been identified by Tn-seq. Fragments were amplified from MPAO1 genomic DNA using Taq polymerase and cloned into the linearized pU2 and pU3 vectors by the "USER" method (3). Products were electroporated into DH5α cells, then plated on LB supplemented with tetracycline (15 μg/mL) and 5-bromo-4-chloro-3-indolyl galactoside (40 μg/mL). White colonies were screened by PCR for plasmid inserts of an appropriate size; the corresponding plasmids were isolated and transformed into the *E. coli* strain SM10 λ pir for use in conjugation. For conjugation with MPAO1, donor *E. coli* strains were grown overnight at 30 °C, diluted to OD_{600} 0.3, then grown at 37 °C with aeration to OD_{600} 1.0 (~3 h). MPAO1 colonies grown on LB agar were suspended to an OD_{600} of 0.2 in LB and grown for 22–24 h at 42 °C without agitation. MPAO1 cells were mixed with *E. coli* donor cells in a 1:1 ratio in 1.5-mL microcentrifuge tubes, pelleted, washed with 10 mM $MgSO_4$, and then resuspended in 50 μL of LB + 10 mM $MgSO_4$. Mixtures were spotted on 0.45-μm Millipore filters on LB agar, and incubated 3 h at 37 °C. Bacteria were removed from filters by vortex mixing in LB and small volumes were plated on selective media containing appropriate inducers [LB agar supplemented with tetracycline (60 μg/mL), chloramphenicol (10 μg/mL) and either isfopropyl-β-D-thiogalactopyranoside (2 mM) or arabinose (0.2%)]. Plates were incubated 24–36 h, and single colonies that grew were colony-purified and tested for inducer-dependence by streaking onto LB agar + tetracycline with and without appropriate inducer.

Construction of LPS O-Antigen and Pyocin R2 Deletion Mutants. Three MPAO1 deletion mutants were constructed using integration-excision of appropriate sequences generated by PCR and cloned into pU1 (2). Mutants were constructed on MOPS-pyruvate agar, a permissive condition, because of the expected essentiality status of the deleted genes on LB agar. The Δ*waaL* (PA4999) mutant was deleted of coding sequence from the sixth codon of the 5′ end to the seventh codon from the 3′ end. The Δ*wbpM* (PA3141) mutant was deleted of coding sequence from the 3rd codon to the 13th codon from the end. The Δ*wbp-operon* (PA3141-PA3157) mutant was deleted of coding sequence from the 6th codon of the 5′ end of PA3157 to the 13th codon from the 3′ end of PA3141. All three deletions were in-frame and carried SacI sites at their deletion sites.

To create double mutants, pyocin R2 deletion mutations [Δ(PA0615-PA0628)] were introduced into the O-antigen deletion mutants using a previously described plasmid (7) by integration-excision. The deletion extends from the 111th codon of PA0615 to the 88th codon from the 3′ end of PA0628 (8, 9).

1. Jacobs MA, et al. (2003) Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 100(24):14339–14344.
2. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP (1998) A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: Application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* 212(1):77–86.
3. Bitinaite J, et al. (2007) USER friendly DNA engineering and cloning method by uracil excision. *Nucleic Acids Res* 35(6):1992–2002.
4. Gallagher LA, Shendure J, Manoil C (2011) Genome-scale identification of resistance functions in *Pseudomonas aeruginosa* using Tn-seq. *MBio* 2(1):e00315–e10.
5. Winsor GL, et al. (2011) Pseudomonas Genome Database: Improved comparative analysis and population genomics capability for *Pseudomonas* genomes. *Nucleic Acids Res* 39(Database issue):D596–D600.
6. Christen B, et al. (2011) The essential genome of a bacterium. *Mol Syst Biol* 7:528.
7. Penterman J, et al. (2014) Rapid evolution of culture-impaired bacteria during adaptation to biofilm growth. *Cell Reports* 6(2):293–300.
8. Baba T, et al. (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol Syst Biol* 2:2006.0008.
9. Barquist L, et al. (2013) A comparison of dense transposon insertion libraries in the *Salmonella* serovars Typhi and Typhimurium. *Nucleic Acids Res* 41(8):4549–4564.

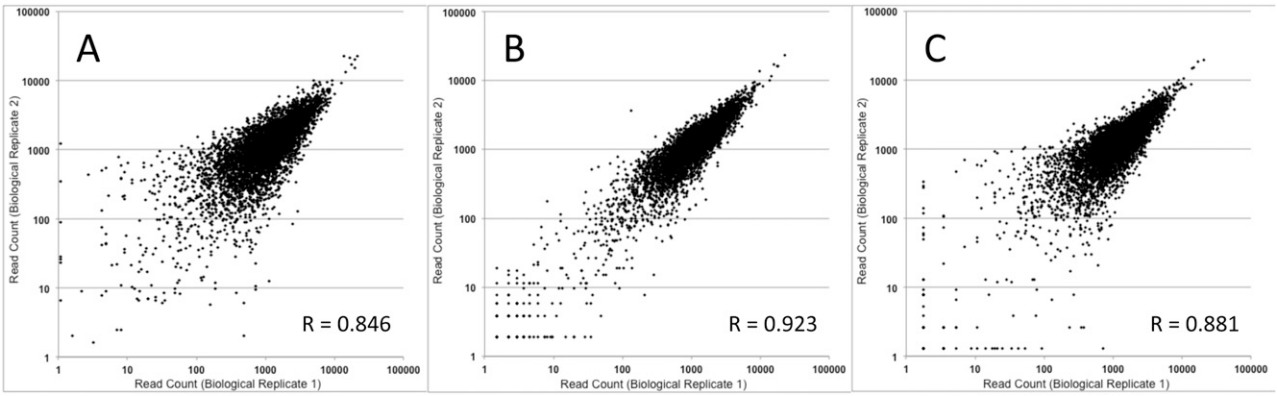

Fig. S1. Tn-seq biological replicates. The sequence read counts per gene for two independent mutant pools for each of the three primary growth conditions analyzed are compared. (*A*) LB agar; (*B*) MOPS-pyruvate agar; (*C*) Sputum agar. R, correlation coefficient.

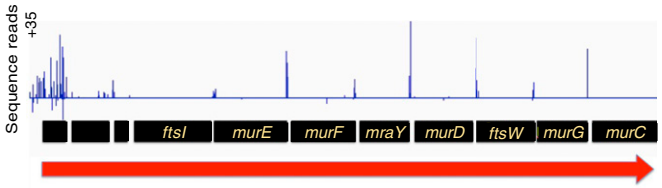

Fig. S2. Transposon insertions in a long operon with multiple essential genes. Except for the first gene of the operon, which is nonessential, nearly all of the insertions fall in regions between genes. The insertions show an extreme orientation bias, such that the outward-facing promoter in the transposon allows expression of downstream genes, evidently overcoming polar effects associated with transposon insertions.

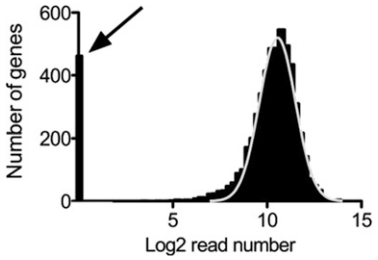

Fig. S3. Distribution of sequence reads per gene in a representative Tn-seq assay. The distribution of reads per gene for one Tn-seq assay (LB-1) is shown. Reads corresponding to 5–90% of the predicted ORF for each gene (log_2-transformed) are plotted, normalized for gene length, and to 10 million reads total. Reads per gene (minus outliers at the origin corresponding to genes with no reads) follows an approximately normal distribution, as indicated. A gene was classified as a candidate essential in this Tn-seq run example if the *x*-axis value was less than 6.3 ($P < 0.001$), although most candidate essentials lacked reads altogether (indicated by the arrow).

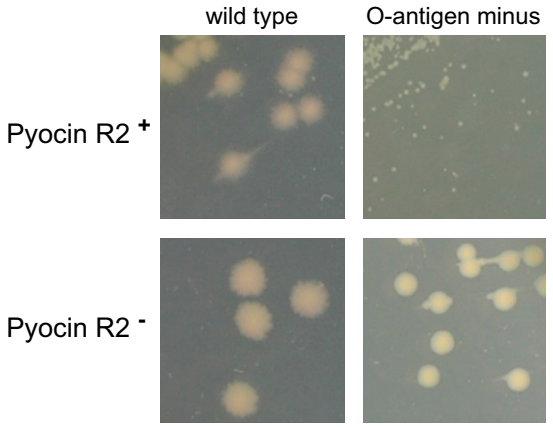

Fig. S4. Pyocin R2 toxicity toward LPS O-antigen mutants. Mutants defective in LPS O-antigen synthesis because of a large deletion mutation (Δ(PA3141-3157)) grew poorly in a Pyocin $R2^+$ genetic background but not in a $Pyocin^-$ background [Δ(PA0615-PA0628)]. Bacteria were grown on LB agar overnight at 37 °C. Surprisingly, the $Pyocin^+$ O-antigen deletion mutants grew almost as well as the $Pyocin^-$ mutants at a more elevated temperature (42 °C). Culture supernatants from wild-type bacteria grown at 42 °C were depleted of pyocin R2 activity relative to cells grown at 37 °C, suggesting a pyocin production defect at the higher temperature. (Magnification: 1.5×.)

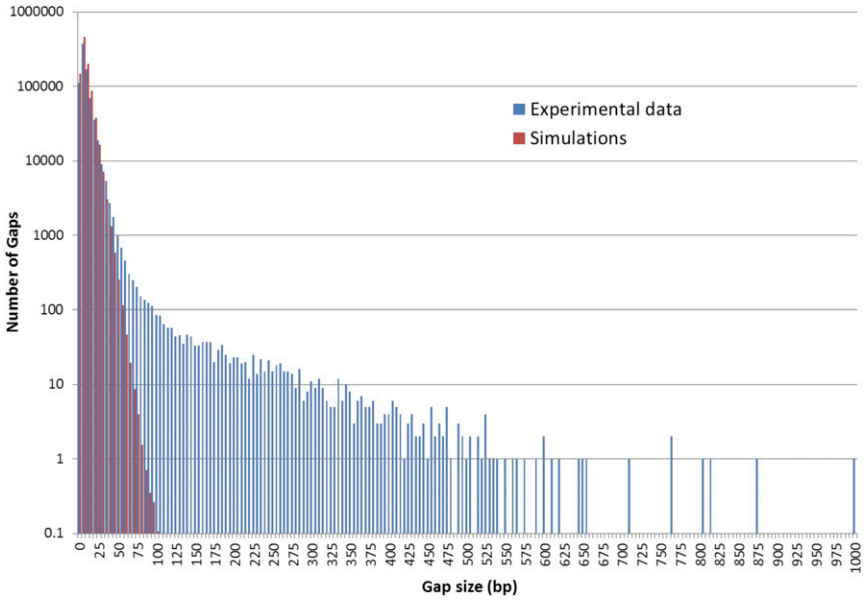

Fig. S5. Distributions of genome gap lengths. The distribution of gaps lacking transposon insertions for the pooled transposon insertion data for 13 mutant pools (blue bars) and a simulation with an equivalent number of unique insertion sites (red bars) shows the extreme overrepresentation of long gaps in the experimental distribution.

Table S1. Genomic categories of essential genes

Genomic category	General essential (352 genes)		LB essential (101 genes)		Minimal essential (103 genes)		Sputum essential (43 genes)	
	Genes	Enrichment	Genes	Enrichment	Genes	Enrichment	Genes	Enrichment
Cell division	12	**6.8**	3	**5.9**	0	0	2	**9.3**
Chaperones and heat-shock proteins	8	2.3	1	1.0	0	0	1	2.3
Antibiotic resistance and susceptibility	0	0	0	0	0	0	0	0.0
Chemotaxis	0	0	1	0.8	0	0	0	0.0
Motility and attachment	0	0	2	0.8	1	0.4	0	0.0
Related to phage, transposon, or plasmid	0	0	0	0	0	0	0	0.0
DNA replication, recombination repair	20	3.6	4	2.5	0	0	3	4.4
Secreted factors (toxins, enzymes, alginate)	2	0.3	0	0	0	0	1	1.2
Two-component regulatory systems	0	0	1	0.5	0	0	0	0.0
Protein secretion/export apparatus	9	1.1	3	1.3	1	0.4	2	2.0
Adaptation, protection	7	0.6	1	0.3	0	0	2	1.4
Cell wall/LPS/capsule	39	3.3	19	**5.5**	2	0.6	2	1.4
Carbon compound catabolism	5	0.4	2	0.6	3	0.8	3	2.0
Translation, posttranslational modification, degradation	92	**7.5**	4	1.1	4	1.1	5	3.3
Amino acid biosynthesis and metabolism	33	2.2	6	1.4	49	**11.1**	1	0.5
Putative enzymes	6	0.2	13	1.5	2	0.2	4	1.1
Transcriptional regulators	6	0.2	4	0.5	1	0.1	1	0.3
Transport of small molecules	10	0.3	3	0.3	0	0	2	0.4
Membrane proteins	13	0.3	11	0.9	0	0	4	0.8
Hypothetical, unclassified, unknown	41	0.3	24	0.7	7	0.2	8	0.5
Energy metabolism	31	2.4	8	2.1	5	1.3	5	3.1
Biosynthesis of cofactors, prosthetic groups and carriers	46	4.5	8	2.7	21	**7.0**	7	**5.6**
Fatty acid and phospholipid metabolism	14	3.6	3	2.7	4	3.5	1	2.1
Central intermediary metabolism	10	1.6	3	1.7	3	1.6	1	1.3
Transcription, RNA processing and degradation	13	3.7	4	4.0	2	1.9	2	4.7

The enrichments of genes in each category relative to all coding genes in the *P. aeruginosa* PAO1 genome are given. Greatest enrichments are in boldface. Genomic categories are from PseudoCAP (5).

Table S2. Inducer growth-dependence of strains carrying essential operons controlled by arabinose (P_{BAD}) and *trp-lac* (P_{tac}) promoters

Gene or operon*		Promoter	
Locus tag	Gene name	P_{BAD}	P_{tac}
PA3126 (control)	*ibpA*	—	—
PA0001-PA0002-(PA0003)-PA0004	*dnaA-dnaN-(recF)-gyrB*	NA	—
PA0004†	*gyrB*	++	+
PA0006-(PA0005)	*yaeD-(lptA)*	—	—
PA0019	*def*	+	+
PA0363	*coaD*	++	—
PA0376	*rpoH*	++	+
PA0548-(PA0549)	*tktA*-(PA0549)	++‡	++
PA0555-(PA0556)	*fda*-(PA0556)	NA	+
PA0576	*rpoD*	NA	+
PA0577	*dnaG*	++	—
PA0580	*gcp*	+	—
PA0595-PA0594-PA0593	*lptD-surA-pdxA*	NA	+
PA0655†	PA0655	+‡	—
PA0759	PA0759	+	—
PA0903	*alaS*	+	+
PA0947	PA0947	++	—
PA0969-PA0970-PA0971-PA0972-PA0973-(PA0974)	*tolQ-tolR-tolA-tolB-oprL*-(PA0974)	NA	++
PA0973-(PA0974)†	*oprL*-(PA0974)	++	++‡
PA1151	*imm2*	NA	++
PA1156	*nrdA*	NA	++‡
PA1165	*pcpS*	—	—
PA1528	*zipA*	NA	++
PA1585-PA1586-PA1587	*sucA-sucB-lpdG*	NA	+
PA2952-PA2951	*etfB-etfA*	+	+
PA2953	PA2953	++	—
PA2968-PA2967	*fabD-fabG*	NA	+
PA3171-(PA3172)-(PA3173)	*ubiG*-(PA3172)-(*yciK*)	+‡	—
PA3397	*fpr*	++	NA
PA3639-PA3638	*accA-mesJ*	NA	+
PA3655	*tsf*	NA	++
PA3686	*adk*	NA	++
PA3746	*ffh*	++	—
PA3828-PA3827	*yjgP-yjgQ*	++	NA
PA3984	*lnt*	+	—
PA4031	*ppa*	NA	++
PA4044	*dxs*	++‡	—
PA4366	*sodB*	NA	++
PA4403	*secA*	++	+
PA4406	*lpxC*	NA	++
PA4481-PA4480-PA4479	*mreB-mreC-mreD*	NA	++
PA4569	*ispB*	+	+‡
PA4762-PA4761	*grpE-dnaK*	NA	+
PA4764	*fur*	NA	++
PA4765	*omlA*	NA	+
PA4991-PA4992	PA4991-PA4992	+	++
PA5239	*rho*	+	+

Constructs with the following genes could not be created with either regulated promoter: PA0442; PA2619 (*infA*); PA2740-PA2739-(PA2738) [*pheS-pheT-(himA)*]; PA4264-PA4250 (*rpsJ-rpsN*); PA4270-PA4269 (*rpoB-rpoC*); PA4386-PA4385 (*groES-groEL*). Primer sequences used for constructing regulated essential genes are available from the authors.

*Locus tags and genes shown in parentheses are nonessential. Operons predictions are from the *Pseudomonas* Genome Database (5).

†Genes are predicted to be internal to an operon.

‡Two growth phenotypes observed (stronger phenotype reported).

Table S3. Central carbon metabolism essential functions

Function	Gene	Essentiality		
		LB		All media*
		PA	EC†	PA
EMP				
Fructose-1,6-bisphosphatase	*fbp*	+	–	1–6
Fructose bisphosphate aldolase	*fda*	+	+	1–6
Triosephosphate isomerase	*tpiA*	+	–	1–3, 5, 6
Glyceraldehyde-3-phosphate dehydrogenase (putative)	PA3001	–	+	2–6
Phosphoglycerate kinase	*pgk*	–	+	2–6
Phosphoglycerate mutase	*pgm*	–	–	2–6
Enolase	*eno*	–	+	2–6
PPP				
Transketolase	*tktA*	+	–	1–6
Ribose 5-phosphate isomerase	*rpi*	+	–	1–6
D-ribulose-5-phosphate 3-epimerase	*rpe*	+	–	1–6
TCA				
Citrate synthase	*gltA*	–	–	2, 5, 6
Aconitase	*acnB*	+	–	1–6
2-oxoglutarate dehydrogenase	*sucAB lpdG*	+	–	1–6
Succinyl-CoA synthetase	*sucCD*	+	–	1, 3, 5, 6
Succinate dehydrogenase	*sdhABCD*	+	–	1–6
Fumarase	PA4333	+	–	1, 3, 5, 6

The functions essential for *P. aeruginosa* (PA) growth on LB nutrient medium are compared with those essential for *E. coli* (EC). *P. aeruginosa* essentiality on other media is also shown. Remarkably, three functions of lower glycolysis (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and enolase) are essential on LB for *E. coli* but not *P. aeruginosa*, even though they are essential for *P. aeruginosa* on other media.
*1 = LB; 2 = MOPS-pyruvate; 3 = sputum agar; 4 = 0.1X LB; 5 = serum agar; 6 = BHI.
†*E. coli* essential for growth on LB agar (8). An analysis of *S. typhimurium* by Tn-seq gave an identical pattern with the exception that ribose 5-phosphate isomerase was also essential for growth on LB (9).

Other Supporting Information Files

Dataset S1 (XLSX)