Table S1. Bacterial strains and plasmids.

E. coli and plasmids

DH5α	*supE44 ΔlacU169 (80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1λpir*	Laboratory collection
DH5α λpir	*supE44 ΔlacU169 (80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1 λpir*	Laboratory collection
DY330	W3110 Δ*lacU169 gal490 λcI857 Δ(cro-bioA)*	(7)
	pGEM-T easy	Promega
	pKD3 (*FRT-cat-FRT* allele)	(2)
	pKD4 (*FRT-kan-FRT* allele)	(2)
MB838	pR6Kγ*FRT-gent-FRT*	This work
	pCP20 (source of *flp* gene)	(2)
	pMM237 (source of *sacB* gene)	(4)
	pBluescript KS- (source of ColE1 *ori*)	Stratagene
	pMMB206 Δmob	(6)
	pMMBGent-empty	(5)
	pMMBGent-FliA	(5)
MB741	pMMBGent (pMMB206 Δmob with gentamycin resistance cassette replacing *cat*)	This work
MB789	pMMBbve (pMMBGent with *sacB* cloned downstream of the MCS)	This work
MB790	pMMBFlp (pMMBbve with *flp* cloned under ptac control)	This work
MB791	pBSFlp (pMMBFlp with ColE1 *ori* replacing RSF1010 *ori*)	This work
MB792	pGEM*letA*	This work
MB793	pGEMΔ*letA::FRT-cat-FRT*	This work
MB750	pGEM*fliA*	This work
MB751	pGEMΔ*fliA::FRT-cat-FRT*	This work
MB748	pGEM*xseA*	Bryan A and Swanson MS, submitted for publication
MB749	pGEMΔ*xseA::FRT-kan-FRT*	Bryan A and Swanson MS, submitted for publication
MB747	pGEMΔ*recJ:FRT-cat-FRT*	Bryan A and Swanson MS, submitted for publication
MB794	pGEM*lpg0558*	This work
MB795	pGEMΔ*lpg0558::FRT-cat-FRT*	This work
MB796	pGEM*lpg1116*	This work
MB797	pGEMΔ*lpg1116::FRT-cat-FRT*	This work
MB798	pGEM*lpg1889*	This work
MB799	pGEMΔ*lpg1889::FRT-cat-FRT*	This work
MB800	pGEM*lpg1918*	This work
MB801	pGEMΔ*lpg1918::FRT-cat-FRT*	This work
MB802	pGEM*lpg2217*	This work
MB803	pGEMΔ*lpg2217::FRT-cat-FRT*	This work
MB804	pGEM*lpg2343*	This work

MB805	pGEMΔ*lpg2343::FRT-cat-FRT*	This work
MB806	pGEM*lpg2918*	This work
MB807	pGEMΔ*lpg2918::FRT-cat-FRT*	This work

*L. pneumophila**

MB110	Lp02 wild type, *thyA, hsdR, rpsL* (Str^R)	(1)
MB413	*letA::kan* transposon insertion	(3)
MB808	Lp02 *fliA*::*kan* transposon insertion, pMMBGent-empty	(5)
MB510	Lp02 *fliA*::*kan* transposon insertion, pMMBGent-FliA	(5)
MB560	Lp02 *motAB*::*gent*	(5)
MB809	Lp02 Δ*letA::FRT-cat-FRT*, pMMBbve (vector control)	This work
MB810	Lp02 Δ*letA::FRT-cat-FRT*, pMMBFlp	This work
MB811	Lp02 Δ*fliA*::*FRT-cat-FRT*	This work
MB812	Lp02 Δ*fliA*::*FRT-cat-FRT*, pMMBFlp	This work
MB813	Lp02 Δ*fliA*::*FRT-cat-FRT*, pBSFlp	This work
MB814	Lp02 Δ*fliA*::*FRT-cat-FRT*, pMMBGent-FliA	This work
MB815	Lp02 Δ*fliA*::*FRT*, Supplemental Method	This work
MB816	Lp02 Δ*fliA*::*FRT*, Supplemental Method, pMMBGent-empty	This work
MB817	Lp02 Δ*fliA*::*FRT*, Supplemental Method, pMMBGent-FliA	This work
MB818	Lp02 Δ*fliA*::*FRT*	This work
MB758	Lp02 Δ*recJ::FRT-cat-FRT*	Bryan A and Swanson MS, submitted for publication
MB819	Lp02 Δ*recJ::FRT*	This work
MB759	Lp02 Δ*xseA::FRT-kan-FRT*	Bryan A and Swanson MS, submitted for publication
MB820	Lp02 Δ*xseA::FRT*	This work
MB760	Lp02 Δ*recJ::FRT-cat-FRT*, Δ*xseA::FRT-kan-FRT*	Bryan A and Swanson MS, submitted for publication
MB821	Lp02 Δ*recJ::FRT*, Δ*xseA::FRT*	This work
MB822	Lp02 Δ*lpg0558::FRT-cat-FRT*	This work
MB823	Lp02 Δ*lpg0558::FRT*, Supplemental Method	This work
MB824	Lp02 Δ*lpg1116::FRT-cat-FRT*	This work
MB825	Lp02 Δ*lpg1116::FRT*, Supplemental Method	This work
MB826	Lp02 Δ*lpg1889::FRT-cat-FRT*	This work
MB827	Lp02 Δ*lpg1889::FRT*, Supplemental Method	This work
MB828	Lp02 Δ*lpg1918::FRT-cat-FRT*	This work
MB829	Lp02 Δ*lpg1918::FRT*, Supplemental Method	This work
MB830	Lp02 Δ*lpg2217::FRT-cat-FRT*	This work
MB831	Lp02 Δ*lpg2217::FRT*, Supplemental Method	This work
MB832	Lp02 Δ*lpg2217::FRT*	This work
MB833	Lp02 Δ*lpg2343::FRT-cat-FRT*	This work
MB834	Lp02 Δ*lpg2343::FRT*, Supplemental Method	This work
MB835	Lp02 Δ*lpg2918::FRT-cat-FRT*	This work
MB836	Lp02 Δ*lpg2918::FRT*, Supplemental Method	This work

*Strains were constructed as described in text, expect for those listed as constructed with the Supplemental Method (also see Fig 1 and Fig S1).

Supplemental Method:

To induce Flp expression and excision of resistance cassettes, pMMBFlp was transferred by electroporation to strains containing *FRT*-flanked deletion constructs, which were then cultured overnight in AYET containing IPTG (200 μM) and gentamycin (10 μg/mL). To remove IPTG and antibiotic, cells were washed twice and then cultured overnight in AYET to mid-exponential phase. To enrich for bacteria that had lost pMMBFlp, cells were diluted into AYET + 5% sucrose, cultured overnight or until growth was visible, and then isolated on CYET + 5% sucrose. Finally, colonies which were likely to have lost or inactivated *sacB,* identified by their non-mucoid morphology were screened for gentamycin and chloramphenicol sensitivity, and the size of the putative unmarked deletion allele was verified by PCR. The predicted scar sequence following Flp-mediated excision was confirmed by sequencing two independent deletions. To test the fidelity of the method, deletions were constructed in numerous loci that were relevant to other projects in our laboratory: *letA, fliA, lpg0558, lpg1116, lpg1889, lpg1918, lpg2217, lpg2343* and *lpg2918.* Although we occasionally observed unusual growth characteristics after the antibiotic cassette was excised by this method (Fig S1), we nevertheless describe the unmarked strains here in the event they are of interest to others in the community. On the other hand, we have not observed unusual growth patterns for the corresponding antibiotic-resistant progenitors or the unmarked mutants generated using the less stable plasmid pBSFlp as described in the main text.

Strain References

1. **Berger, K. H., and R. R. Isberg.** 1993. Two distinct defects in intracellular growth complemented by a single genetic locus in *Legionella pneumophila*. Mol. Microbiol. **7:**7-19.
2. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl Acad. Sci. USA **97:**6640-6645.
3. **Hammer, B. K., E. S. Tateda, and M. S. Swanson.** 2002. A two-component regulator induces the transmission phenotype of stationary-phase *Legionella pneumophila*. Mol. Microbiol. **44:**107-118.
4. **McClain, M. S., M. C. Hurley, J. K. Brieland, and N. C. Engleberg.** 1996. The *Legionella pneumophila hel* locus encodes intracellularly induced homologs of heavy-metal ion transporters of *Alcaligenes* spp. Infect. Immun. **64:**1532-1540.
5. **Molofsky, A. B., L. M. Shetron-Rama, and M. S. Swanson.** 2005. Components of the *Legionella pneumophila* flagellar regulon contribute to multiple virulence traits, including lysosome avoidance and macrophage death. Infect. Immun. **73:**5720-5734.
6. **Molofsky, A. B., and M. S. Swanson.** 2003. *Legionella pneumophila* CsrA is a pivotal repressor of transmission traits and activator of replication. Mol. Microbiol. **50:**445-461.
7. **Yu, D., H. M. Ellis, E. C. Lee, N. A. Jenkins, N. G. Copeland, and D. L. Court.** 2000. An efficient recombination system for chromosome engineering in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:**5978-5983.