Supplementary materials

Genome-wide screens reveal *Escherichia coli* genes required for growth of T1-like phage LL5 and rV5-like phage LL12

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Determination of genes required for phage propagation

The Keio collection consists of a total of 3,985 individual gene knockout mutants in the *E. coli* K-12 strain BW25113. Each gene knockout is represented twice in the collection (the results of two independent experiments) [1], thus the total collection contains 7,970 mutants, with each independent gene knockout mutants represented with even and odd numbers. Phages LL5 and LL12 were screened against the entire odd-numbered series of 3,985 Keio single-gene knockouts as described in Materials and Methods. *E. coli* mutants that were unable to support phage growth, as indicated by their growth to an OD_{550} of at least 0.2 or 0.11 at 8 h in the presence of phage LL5 or LL12, respectively, were considered positive hits in this initial screen. Using this selection criteria, 37 knockout mutants (21 mutants for each phage) were selected for further investigation (Tables S4, S5). For each of these initial hits, the screening experiment was repeated using the same odd-numbered mutant and its even-numbered counterpart from the collection. From this second experiment, 11/21 mutants identified against LL5 and 9/21 mutants identified against LL12 were found to produce the same phenotype in at least one of the paired knockouts, and these were retained for further study.

The efficiency of plating (EOP) of phage LL5 on the retained mutant strains was determined by spot titer. The observed plating efficiency of phage LL5 was reduced by at least ~20-fold in eight mutants (Table S4). This plating defect was confirmed by titration of LL5 in full plate assays, in which only four mutants showed an EOP reduction of ~20-fold or greater. In order to confirm the plating phenotype in a clean genetic background, the kanamycin resistance cassettes from these Keio mutants were transduced by P1 into the parental *E. coli* strain BW25113. Markers could be transduced from all four Keio mutants into the parental strain, and three showed a similar plating defect as the corresponding Keio mutant, indicating the phenotype was linked to the disrupted locus (Table S4). One mutant, *rfaQ*, showed a ~25-fold reduction in EOP in the Keio mutant but its P1 transductant exhibited only a very mild EOP defect of 0.3, despite having the *rfaQ* deletion confirmed in the transductant by PCR and sequencing. This suggests an abnormality or additional defect in the original *rfaQ* Keio mutant; this mutant was not examined further.

The same approach was applied to confirm the phenotypes of the Keio mutants identified from the screens against phage LL12 (Table S5). Spot titer assays showed that the EOP of phage LL12 was reduced in only three of the nine initially identified Keio mutants. This plating defect could be replicated via full plate plaque assay in all three mutants. Only one of these mutants could be P1 transduced into the

parental strain BW25113 and same plating phenotype was observed in the P1 transductant as in the Keio mutant (Table S5). The other two Keio mutants were resistant to P1 infection and could not be transduced.

Based on the genes identified in these initial screens, additional mutants from the odd- and even-numbered Keio sets were subjected to targeted re-screening by directly determining the phage EOP by the spot method. For both phages, genes involved in LPS biosynthesis (*rfaP* for LL5, and *rfaP, rfaG* and *lpcA* for LL12, Tables S4, S5) were identified and confirmed, but these genes represented only parts of the known biosynthetic pathway. Additional Keio mutants in *lpcA*, *rfaE*, *rfaC*, *rfaF*, *rfaY*, *rfaI*, and *rfaB* were obtained and confirmed by PCR and sequencing of the mutant locus. A *tolC* mutant was obtained and tested based on the similarity between LL5 and phage TLS, which is known to use TolC as a receptor. Strong EOP defects ($< 10^{-7}$) were identified in the *lpcA*, *rfaE*, *rfaC*, *rfaF,* and *tolC* mutants against LL5, and in *rfaE*, *rfaC* and *rfaF* in LL12 (an EOP defect in *lpcA* against LL12 was already identified in the initial screen) (Table 2).

Discovery rates from gene knockout libraries

From the initial 37 "hits" identified in the untargeted screen, it was established that three *E. coli* genes were needed each for of phage LL5 and LL12 propagation (Tables S4, S5), which gives a false positive gene discovery rate of ~86% for both phages in the initial screen. It was noted that many genes predicted to play roles in phage propagation were not "hit" in the initial screen, so targeted screens were performed against additional mutants. Ultimately, eight genes affecting LL5 propagation and six genes affecting LL12 propagation were confirmed (Table 2), which translates to a false-negative gene discovery rate of at least 62% for LL5 and 50% for LL12 in the initial untargeted screen. In this process, it was discovered that in several instances the mutant genes were still intact in either the odd- or even-numbered sets, and in a few cases genes were still intact in both sets. These findings highlight the potential utility of using multiple independently-generated mutant libraries when conducting large forward genetic screens as described here. Gene discovery rates are likely to be higher for biological pathways comprised of multiple genes, as this increases the probability that at least some genes in the pathway will be detected in initial screens. This highlights that the results from high-throughput forward genetic screens should be interpreted only after rigorous confirmation of the mutant genotypes and their phenotypes.

Table S1. Gene products of phage LL5 (Genbank accession# MH491968) .

Gene	start	end	strand	product
CPT_LL5_01	365	652	+	Conserved hypothetical protein
CPT_LL5_02	664	1107	+	Conserved hypothetical protein
CPT_LL5_03	1104	1211	+	Conserved hypothetical protein
CPT_LL5_04	1224	1346	-	Conserved hypothetical protein
CPT_LL5_05	1415	1678	+	Conserved hypothetical protein
CPT_LL5_06	1830	2396	+	Conserved hypothetical protein
CPT_LL5_07	2456	2770	+	Conserved hypothetical protein
CPT_LL5_08	2843	2950	+	Conserved hypothetical protein
CPT_LL5_09	2954	3496	+	Polynucleotide kinase/phosphatase
CPT_LL5_10	3493	3669	+	Conserved hypothetical protein
CPT_LL5_11	3764	3973	+	Hypothetical protein
CPT_LL5_12	4047	4301	+	Conserved hypothetical protein
CPT_LL5_13	4298	4480	+	Conserved hypothetical protein
CPT_LL5_14	4473	5048	+	Conserved hypothetical protein
CPT_LL5_15	5140	5340	+	Conserved hypothetical protein
CPT_LL5_16	5342	5506	+	Conserved hypothetical protein
CPT_LL5_17	5508	5633	+	Conserved hypothetical protein
CPT_LL5_18	5626	5871	+	Conserved hypothetical protein
CPT_LL5_19	5883	6353	+	Putative GntR-family transcriptional regulator
CPT_LL5_20	6427	6630	+	Conserved hypothetical protein
CPT_LL5_21	6634	6939	+	Conserved hypothetical protein
CPT_LL5_22	6936	7253	+	Conserved hypothetical protein
CPT_LL5_23	7320	7448	+	Conserved hypothetical protein
CPT_LL5_24	7450	7677	+	Conserved hypothetical protein
CPT_LL5_25	7860	8069	+	Conserved hypothetical protein
CPT_LL5_26	8059	8220	+	Conserved hypothetical protein
CPT_LL5_27	8204	8386	+	Conserved hypothetical protein
CPT_LL5_28	8386	8616	+	Conserved hypothetical protein
CPT_LL5_29	8704	9228	+	Terminase small subunit
CPT_LL5_30	9240	10811	+	Terminase large subunit
CPT_LL5_31	10865	12151	+	Portal protein
CPT_LL5_32	12156	12827	+	Conserved hypothetical protein
CPT_LL5_33	12824	13933	+	putative scaffold or prohead protease
CPT_LL5_34	13946	14425	+	Conserved hypothetical protein
CPT_LL5_35	14469	14909	+	Conserved hypothetical protein
CPT_LL5_36	14999	15973	+	Major capsid protein
CPT_LL5_37	16035	16307	+	Conserved hypothetical protein
CPT_LL5_38	16354	16773	+	Conserved hypothetical protein
CPT_LL5_39	16770	17141	+	Conserved hypothetical protein

CPT_LL5_40	17134	17574	+	Conserved hypothetical protein
CPT_LL5_41	17564	17857	+	Minor tail protein
CPT_LL5_42	17884	18087	+	Minor tail protein
CPT_LL5_43	18102	18764	+	tail tube protein
CPT_LL5_44	18842	19156	+	tapemeasure chaperone protein
CPT_LL5_45	18842	19473	+	tapemeasure chaperone protein frasmeshift product
CPT_LL5_46	19511	22420	+	tail tape measure protein
CPT_LL5_47	22420	22767	+	Minor tail protein
CPT_LL5_48	22835	23593	+	Minor tail protein
CPT_LL5_49	23590	24312	+	Tail tip assembly protein
CPT_LL5_50	24305	24904	+	Tail assembly protein
CPT_LL5_51	24986	28762	+	Tail fiber protein
CPT_LL5_52	29018	29161	+	Hypothetical protein
CPT_LL5_53	29224	30279	+	Exodeoxyribonuclease VIII
CPT_LL5_54	30357	30647	+	Conserved hypothetical protein
CPT_LL5_55	30694	31356	+	Recombinase
CPT_LL5_56	31395	31820	+	Single-stranded DNA binding protein
CPT_LL5_57	31853	34363	-	Tail fiber protein
CPT_LL5_58	34495	35421	-	DNA primase/helicase
CPT_LL5_59	35479	36087	-	Putative transcriptional regulator
CPT_LL5_60	36176	38149	+	ATP-dependent helicase
CPT_LL5_61	38152	38559	+	VRR-NUC domain protein
CPT_LL5_62	38549	38683	-	Conserved hypothetical protein
CPT_LL5_63	38631	38909	+	Conserved hypothetical protein
CPT_LL5_64	38911	39651	+	Dam methylase
CPT_LL5_65	39653	39883	+	Conserved hypothetical protein
CPT_LL5_66	39922	40119	+	Conserved hypothetical protein
CPT_LL5_67	40201	40341	+	Conserved hypothetical protein
CPT_LL5_68	40344	40823	+	HNH endonuclease
CPT_LL5_69	40907	42022	+	Conserved hypothetical protein
CPT_LL5_70	42152	42358	+	Putative holin
CPT_LL5_71	42358	42798	+	glycoside hydrolase endolysin
CPT_LL5_72	42845	43249	+	unimolecular spanin protein
CPT_LL5_73	43266	43460	-	Conserved hypothetical protein
CPT_LL5_74	43384	43776	-	Conserved hypothetical protein
CPT_LL5_75	43779	45359	-	Helicase
CPT_LL5_76	45427	45675	-	Conserved hypothetical protein
CPT_LL5_77	45672	46061	-	Conserved hypothetical protein
CPT_LL5_78	46063	46257	-	Hypothetical protein
CPT_LL5_79	46318	47013	-	Site-specific DNA methylase
CPT_LL5_80	47209	47448	-	Conserved hypothetical protein

CPT_LL5_81	47454	47675	-	Conserved hypothetical protein
CPT_LL5_82	47734	47901	-	Conserved hypothetical protein
CPT_LL5_83	48003	48134	+	Hypothetical protein
CPT_LL5_84	48131	48400	-	Conserved hypothetical protein
CPT_LL5_85	48428	48700	+	Hypothetical protein
CPT_LL5_86	48684	48878	-	Conserved hypothetical protein
CPT_LL5_87	48875	49078	-	Conserved hypothetical protein
CPT_LL5_88	49150	49428	-	Conserved hypothetical protein

Table S2. Gene products of phage LL12 (Genbank accession# MH491969)

Gene	start	end	strand	product
CPT_LL12_001	1	1983	-	rIIA protector from prophage-induced early lysis
CPT_LL12_002	1980	2480	-	Hypothetical conserved protein
CPT_LL12_003	2530	2910	-	Hypothetical conserved protein
CPT_LL12_004	2920	4104	-	MoxR ATPase
CPT_LL12_005	4104	4373	-	Hypothetical conserved protein
CPT_LL12_006	4418	4798	-	Hypothetical conserved protein
CPT_LL12_007	4801	5058	-	Hypothetical conserved protein
CPT_LL12_008	5058	5414	-	Hypothetical conserved protein
CPT_LL12_009	5428	5700	-	Hypothetical conserved protein
CPT_LL12_010	5703	6602	-	Putative alpha 1-3 fucosyltransferase
CPT_LL12_011	6745	7722	+	Anti-sigma factor
CPT_LL12_012	7756	9216	+	Putative metallopeptidase
CPT_LL12_013	9232	9495	+	Hypothetical conserved protein
CPT_LL12_014	9495	9758	+	Hypothetical conserved protein
CPT_LL12_015	9758	10021	+	Hypothetical conserved protein
CPT_LL12_016	10023	10256	+	Hypothetical conserved protein
CPT_LL12_017	10272	10721	+	Hypothetical conserved protein
CPT_LL12_018	10718	10963	+	Hypothetical conserved protein
CPT_LL12_019	10966	11532	+	Hypothetical conserved protein
CPT_LL12_020	11588	11863	+	Hypothetical conserved protein
CPT_LL12_021	11866	12339	+	Hypothetical conserved protein
CPT_LL12_022	12336	12506	+	Hypothetical conserved protein
CPT_LL12_023	12537	12917	+	Hypothetical conserved protein
CPT_LL12_024	13081	13488	+	Hypothetical conserved protein
CPT_LL12_025	13492	13692	+	Hypothetical conserved protein
CPT_LL12_026	13740	19376	-	Hypothetical conserved protein
CPT_LL12_027	19425	21170	-	Tail fiber protein

CPT_LL12_028	21180	21446	-	Hypothetical conserved protein
CPT_LL12_029	21458	22501	-	Tail fiber protein
CPT_LL12_030	22591	22905	-	Hypothetical conserved protein
CPT_LL12_031	22917	23501	-	Tail fiber assembly protein
CPT_LL12_032	23516	24556	-	Tail fiber protein
CPT_LL12_033	24569	26641	-	Tail fiber protein
CPT_LL12_034	26641	27327	-	Hypothetical conserved protein
CPT_LL12_035	27339	28829	-	Baseplate protein
CPT_LL12_036	28935	32534	-	Tail fiber protein
CPT_LL12_037	32534	32959	-	Hypothetical conserved protein
CPT_LL12_038	32959	33510	-	Hypothetical conserved protein
CPT_LL12_039	33512	34102	-	Hypothetical conserved protein
CPT_LL12_040	34200	34532	-	Hypothetical conserved protein
CPT_LL12_041	34532	38971	-	Tail fiber protein
CPT_LL12_042	39007	41604	-	Tail fiber protein
CPT_LL12_043	41606	42274	-	Hypothetical conserved protein
CPT_LL12_044	42284	43000	-	Hypothetical conserved protein
CPT_LL12_045	43013	43690	-	Hypothetical conserved protein
CPT_LL12_046	43690	44694	-	Hypothetical conserved protein
CPT_LL12_047	44694	45074	-	Hypothetical conserved protein
CPT_LL12_048	45086	45970	-	Hypothetical conserved protein
CPT_LL12_049	46075	48414	-	Hypothetical conserved protein
CPT_LL12_050	48469	48717	-	Hypothetical conserved protein
CPT_LL12_051	48729	49202	-	Hypothetical conserved protein
CPT_LL12_052	49366	49839	-	Hypothetical conserved protein
CPT_LL12_053	49850	51226	-	Tail sheath protein
CPT_LL12_054	51299	51850	-	Hypothetical conserved protein
CPT_LL12_055	51850	52275	-	Hypothetical conserved protein
CPT_LL12_056	52291	52749	-	Hypothetical conserved protein
CPT_LL12_057	52803	53423	-	Hypothetical conserved protein
CPT_LL12_058	53483	54307	-	Hypothetical conserved protein
CPT_LL12_059	54392	55399	-	Major capsid protein
CPT_LL12_060	55448	55837	-	Head stabilization/decoration protein
CPT_LL12_061	55858	56835	-	Hypothetical conserved protein
CPT_LL12_062	56835	57254	-	Prohead protease
CPT_LL12_063	57322	58878	-	Portal protein
CPT_LL12_064	58981	60847	-	Terminase large subunit
CPT_LL12_065	60847	61134	-	O-spanin
CPT_LL12_066	61131	61529	-	I-spanin
CPT_LL12_067	61635	61904	-	Hypothetical conserved protein
CPT_LL12_075	62861	63199	-	Conserved hypothetical protein

CPT_LL12_076	63451	63561	-	Conserved hypothetical protein
CPT_LL12_077	63546	63788	-	Conserved hypothetical protein
CPT_LL12_078	63865	64053	-	Conserved hypothetical protein
CPT_LL12_079	64064	64642	-	Conserved hypothetical protein
CPT_LL12_080	65303	65653	-	Putative transcriptional regulator
CPT_LL12_081	65701	66255	-	Phosphoesterase
CPT_LL12_082	66263	66673	-	ATP-binding protein
CPT_LL12_083	66792	67709	+	RNA ligase and tail attachment protein
CPT_LL12_084	67706	68056	+	Hypothetical conserved protein
CPT_LL12_085	68065	68826	+	Putative Sir2-like protein
CPT_LL12_086	68840	69553	+	Putative Sir2-like protein
CPT_LL12_087	69616	69924	+	Conserved hypothetical protein
CPT_LL12_088	69911	70180	+	Conserved hypothetical protein
CPT_LL12_089	70180	71463	+	DNA ligase
CPT_LL12_090	71587	72057	+	Endolysin
CPT_LL12_091	72121	72327	+	Conserved hypothetical protein
CPT_LL12_092	72337	72801	+	HNH endonuclease
CPT_LL12_093	72794	73084	+	Conserved hypothetical protein
CPT_LL12_094	73144	73452	+	Conserved hypothetical protein
CPT_LL12_095	73445	74584	+	Exodeoxyribonuclease
CPT_LL12_096	74584	74916	+	Conserved hypothetical protein
CPT_LL12_097	74913	75542	+	Hypothetical conserved protein
CPT_LL12_098	75494	76099	+	EndoVII packaging and recombination endonuclease
CPT_LL12_099	76038	76259	+	Conserved hypothetical protein
CPT_LL12_100	76272	77255	+	Putative DNA polymerase/exonuclease
CPT_LL12_101	77301	78119	+	Putative DNA N6-adenine methyltransferase
CPT_LL12_102	78082	78540	+	Hypothetical conserved protein
CPT_LL12_103	78568	78759	+	Hypothetical conserved protein
CPT_LL12_104	78759	79373	+	Hypothetical conserved protein
CPT_LL12_105	79367	79960	+	Hypothetical conserved protein
CPT_LL12_106	79962	80162	+	Hypothetical conserved protein
CPT_LL12_107	80162	81145	+	Thymidylate synthase
CPT_LL12_108	81244	81705	+	Hypothetical conserved protein
CPT_LL12_109	81716	81946	+	Hypothetical conserved protein
CPT_LL12_110	81943	84258	+	Ribonucleoside triphosphate reductase alpha chain
CPT_LL12_111	84298	85386	+	Ribonucleoside diphosphate reductase beta chain
CPT_LL12_112	85390	85668	+	Glutaredoxin 1
CPT_LL12_113	85665	87788	+	Anaerobic ribonucleoside triphosphate reductase

CPT_LL12_114	87852	87968	+	Hypothetical conserved protein
CPT_LL12_115	87984	88088	+	Hypothetical protein
CPT_LL12_116	88120	88356	+	Hypothetical conserved protein
CPT_LL12_117	88353	88826	+	Anaerobic ribonucleoside triphosphate reductase activating protein
CPT_LL12_118	88883	88996	+	Hypothetical conserved protein
CPT_LL12_119	88993	89343	+	Hypothetical conserved protein
CPT_LL12_120	89382	90170	+	PhoH-like protein
CPT_LL12_121	90269	90580	+	Hypothetical conserved protein
CPT_LL12_122	90640	91689	+	Clp ATP-dependent protease subunit
CPT_LL12_123	91741	92274	+	DNA methyltransferase
CPT_LL12_124	92271	92555	+	Hypothetical conserved protein
CPT_LL12_125	92590	92829	+	Hypothetical conserved protein
CPT_LL12_126	92826	93224	+	Hypothetical conserved protein
CPT_LL12_127	93264	93497	+	Hypothetical conserved protein
CPT_LL12_128	93503	94027	+	Hypothetical conserved protein
CPT_LL12_129	94058	94294	+	Hypothetical conserved protein
CPT_LL12_130	94278	94430	+	Hypothetical conserved protein
CPT_LL12_131	94514	94699	+	Hypothetical conserved protein
CPT_LL12_132	94702	95157	+	Hypothetical conserved protein
CPT_LL12_133	95218	95565	+	Hypothetical conserved protein
CPT_LL12_134	95562	95849	+	Hypothetical conserved protein
CPT_LL12_135	95846	96040	+	Hypothetical conserved protein
CPT_LL12_136	96060	96245	+	Hypothetical conserved protein
CPT_LL12_137	96341	96637	+	Hypothetical conserved protein
CPT_LL12_138	96640	96921	+	Hypothetical conserved protein
CPT_LL12_139	96921	97145	+	Hypothetical conserved protein
CPT_LL12_140	97135	97389	+	Hypothetical conserved protein
CPT_LL12_141	97402	97800	+	Hypothetical conserved protein
CPT_LL12_142	97818	98108	+	Hypothetical conserved protein
CPT_LL12_143	98191	98508	+	Sigma 54 modulation factor
CPT_LL12_144	98646	98915	+	Hypothetical conserved protein
CPT_LL12_145	99007	99270	+	Hypothetical conserved protein
CPT_LL12_146	99275	99607	+	Hypothetical conserved protein
CPT_LL12_147	99607	100002	+	Hypothetical conserved protein
CPT_LL12_148	100082	100339	+	Hypothetical conserved protein
CPT_LL12_149	100340	100690	+	Hypothetical conserved protein
CPT_LL12_150	100690	101019	+	Hypothetical conserved protein
CPT_LL12_151	101019	101306	+	Hypothetical conserved protein
CPT_LL12_152	101396	101659	+	Hypothetical conserved protein
CPT_LL12_153	101688	101828	+	Hypothetical conserved protein

CPT_LL12_154	101815	102066	+	Hypothetical conserved protein
CPT_LL12_155	102095	102400	+	Hypothetical conserved protein
CPT_LL12_156	102453	102779	+	Hypothetical conserved protein
CPT_LL12_157	102788	103087	+	Hypothetical conserved protein
CPT_LL12_158	103355	103660	+	Hypothetical conserved protein
CPT_LL12_159	103782	103997	+	Hypothetical conserved protein
CPT_LL12_160	106517	106936	-	Hypothetical conserved protein
CPT_LL12_161	107082	107345	-	Hypothetical conserved protein
CPT_LL12_162	107406	107678	-	Hypothetical conserved protein
CPT_LL12_163	107897	108208	-	Hypothetical conserved protein
CPT_LL12_164	108205	108396	-	Hypothetical conserved protein
CPT_LL12_165	108413	108703	-	Hypothetical conserved protein
CPT_LL12_166	108795	109010	-	Hypothetical conserved protein
CPT_LL12_167	109020	109124	-	Hypothetical conserved protein
CPT_LL12_168	109202	109447	-	Hypothetical conserved protein
CPT_LL12_169	109540	109719	-	Hypothetical conserved protein
CPT_LL12_170	109795	110028	-	Hypothetical conserved protein
CPT_LL12_171	110083	110367	-	Hypothetical conserved protein
CPT_LL12_172	110570	110920	-	Hypothetical conserved protein
CPT_LL12_173	110938	111237	-	Hypothetical conserved protein
CPT_LL12_174	111331	111531	-	Hypothetical conserved protein
CPT_LL12_175	111679	111798	-	Hypothetical conserved protein
CPT_LL12_176	111819	112058	-	Hypothetical conserved protein
CPT_LL12_177	112018	112389	-	Hypothetical conserved protein
CPT_LL12_178	112454	112834	-	Hypothetical conserved protein
CPT_LL12_179	112923	113546	-	Hypothetical conserved protein
CPT_LL12_180	113622	113882	-	Hypothetical conserved protein
CPT_LL12_181	114102	114299	-	Hypothetical conserved protein
CPT_LL12_182	114289	114642	-	Hypothetical conserved protein
CPT_LL12_183	114717	114962	-	Hypothetical conserved protein
CPT_LL12_184	115052	115393	-	Hypothetical conserved protein
CPT_LL12_185	115473	115592	-	Hypothetical conserved protein
CPT_LL12_186	115673	116068	-	Hypothetical conserved protein
CPT_LL12_187	116151	116549	-	Hypothetical conserved protein
CPT_LL12_188	116614	116811	-	Hypothetical conserved protein
CPT_LL12_189	117664	117810	+	Hypothetical conserved protein
CPT_LL12_190	117913	118242	-	Hypothetical conserved protein
CPT_LL12_191	118239	118433	-	Hypothetical conserved protein
CPT_LL12_192	118426	118671	-	Hypothetical conserved protein
CPT_LL12_193	118681	119013	-	Hypothetical conserved protein
CPT_LL12_194	119023	119214	-	Hypothetical conserved protein

CPT_LL12_195	119278	119472	-	Hypothetical conserved protein
CPT_LL12_196	119469	119669	-	Hypothetical conserved protein
CPT_LL12_197	119723	120181	-	Hypothetical conserved protein
CPT_LL12_198	120171	120596	-	Hypothetical conserved protein
CPT_LL12_199	120665	120862	-	Hypothetical conserved protein
CPT_LL12_200	120958	121374	-	Hypothetical conserved protein
CPT_LL12_201	121437	122663	-	Hypothetical conserved protein
CPT_LL12_202	122663	123028	-	Hypothetical conserved protein
CPT_LL12_203	123112	123357	-	Hypothetical conserved protein
CPT_LL12_204	123375	123677	-	Hypothetical conserved protein
CPT_LL12_205	123667	124056	-	Hypothetical conserved protein
CPT_LL12_206	124056	124328	-	Hypothetical conserved protein
CPT_LL12_207	124392	124646	-	Hypothetical conserved protein
CPT_LL12_208	124723	125094	-	Hypothetical conserved protein
CPT_LL12_209	125105	125299	-	Hypothetical conserved protein
CPT_LL12_210	125296	125523	-	Hypothetical conserved protein
CPT_LL12_211	125520	125825	-	Hypothetical conserved protein
CPT_LL12_212	125854	128907	-	DNA polymerase A
CPT_LL12_213	128966	131044	-	DNA replicative helicase/primase
CPT_LL12_214	131034	131798	-	DNA cytosine methyltransferase
CPT_LL12_215	131850	132332	-	Hypothetical conserved protein
CPT_LL12_216	132345	132740	-	Hypothetical conserved protein
CPT_LL12_217	132740	133042	-	Hypothetical conserved protein
CPT_LL12_218	133103	133444	-	Hypothetical conserved protein
CPT_LL12_219	133441	134811	-	Helicase
CPT_LL12_220	134811	136016	-	rIIB-like protein

Table S3: Homologs of putative LL12 tail fibers identified in related phage genomes.

LL12 Protein	Related phage containing tail fiber homolog	NCBI Genome Accession #	NCBI Protein Accession #	BLASTp E-value (vs. nr)	Dice similarity coefficient
CPT_LL12_027	rV5	NC_011041.1	YP_002003530.1	0	0.99
	APCEc02	KR698074.1	AKO61946.1	0	0.98
	phi92	NC_023693.1	YP_009012483.1	7.00E-23	0.08
	phi92	NC_023693.1	YP_009012482.1	2.00E-22	0.07
CPT_LL12_029	APCEc02	KR698074.1	AKO61944.1	0	0.99
	rV5	NC_011041.1	YP_002003532.1	0	0.99
	rV5	NC_011041.1	YP_002003535.1	2.00E-90	0.44
	APCEc02	KR698074.1	AKO61941.1	2.00E-90	0.44
	phi92	NC_023693.1	YP_009012479.1	6.00E-18	0.25
CPT_LL12_032	APCEc02	KR698074.1	AKO61941.1	0	0.99
	rV5	NC_011041.1	YP_002003535.1	0	0.99
	APCEc02	KR698074.1	AKO61944.1	6.00E-91	0.44
	rV5	NC_011041.1	YP_002003532.1	1.00E-88	0.44
	phi92	NC_023693.1	YP_009012479.1	9.00E-29	0.30
CPT_LL12_033	rV5	NC_011041.1	YP_002003536.1	0	1.00
	APCEc02	KR698074.1	AKO61940.1	0	1.00
	phi92	NC_023693.1	YP_009012474.1	0	0.41
CPT_LL12_036	APCEc02	KR698074.1	AKO61937.1	0	0.99
	rV5	NC_011041.1	YP_002003539.1	0	0.86
CPT_LL12_041	APCEc02	KR698074.1	AKO61932.1	0	0.96
	rV5	NC_011041.1	YP_002003543.1	0	0.70
	rV5	NC_011041.1	YP_002003545.1	3.00E-15	0.04
	APCEc02	KR698074.1	AKO61930.1	3.00E-15	0.04
	phi92	NC_023693.1	YP_009012483.1	3.00E-07	0.02
CPT_LL12_042	rV5	NC_011041.1	YP_002003544.1	0	0.99
	APCEc02	KR698074.1	AKO61931.1	0	0.99
	phi92	NC_023693.1	YP_009012473.1	2.00E-173	0.36

Table S4. Results of initial (untargeted) screening and targeted re-screening of phage LL5 against the Keio *E. coli* knockout collection. In the initial screen, all mutants yielding a positive result were screened a second time against both independently-generated gene knockouts present in the Keio collection, denoted as the representatives from the even- and odd-numbered plate sets. Mutants with a positive result from either set were then tested for their efficiency of plating (EOP) by both spot titer and full-plate titration methods. Mutants exhibiting a significant EOP defect (less than ~0.05, highlighted green) in full-plate titers were used for further study. The presence of the appropriate gene deletion was confirmed by PCR and sequencing. One knockout from each even/odd pair was selected for P1 transduction of the kan-marked deletion into the parental E. coli strain BW25113 background, re-tested for EOP defects and complemented in trans. Selected gene knockouts from the collection that were not identified in the initial screen were targeted for re-screening (bottom panel). Mutants were cultured from the Keio collection, the presence of the appropriate gene deletion confirmed by PCR, and the EOP determined in the knockout and its complemented counterpart. Blank cells denote that data was not collected, usually because the desired EOP defects were not observed.

Initial screen of Keio collection

Gene deletion	Results of first screen	Results of second screen		EOP (spot titer)		EOP (plate titer)		Keio mutant used for further work	Deletion confirmed by PCR	Transducible by P1*vir*	EOP (plate titer) in P1 transductant	EOP (plate titer) in complemented strain
		Odd	Even	Odd	Even	Odd	Even					
nuoM	+	+	+	0.06	0.04	0.3	0.3					
yehQ	+	-	-									
ycdB	+	+	-	0.4	0.2							
ydcR	+	+	-	1	1							
ygcN	+	-	-									
rof	+	-	-									
crcB	+	-	-									
ydjO	+	-	-									
holD	+	-	-									
aroC	+	-	-									
ydgL	+	-	-									
idi	+	-	-									
rfaP	+	+	+	< 8E-8	1.0	< 7.5E-8	1.1	Odd	Confirmed	Yes	< 7.5E-8	2.2
rfaG	+	+	-	1.1	0.3				Confirmed			
rfaF	+	+	+	0.3	0.03	1.3	0.3					
rfaQ	+	+	+	0.01	0.4	0.04	0.04	Odd	Confirmed	Yes	0.3	
rfaH	+	-	+	0.003	0.01	0.6	0.2					
rfaY	+	-	-									
secB	+	+	+	0.01	0.03	0.05	0.02	Odd	Confirmed	Yes	0.06	0.2
ppiB	+	+	-	0.003	0.3	0.04	0.4	Odd	Confirmed	Yes	0.09	1.5
ylaC	+	+	-	0.07	0.03	0.4	0.8					
Total passed	**21**	**10**	**6**	**7**	**5**	**4**	**2**				**3**	

Table S4. Continued. Targeted re-screening. Based on the results of the initial screening, individual mutants were confirmed for gene deletions and obtained from other sources as necessary. The genes *rfaC, rfaF, and tolC* were found to be intact in the copy of the Keio collection used for initial screening.

Gene deletion	Deletion confirmed by PCR	Keio mutant used for further work	EOP (spot titer)	EOP (plate titer)	EOP (plate titer) in complemented strain (average ± S.D.)
lpcA	Confirmed	Even	< 8E-8	< 7.5E-8	0.8 ± 0.2
rfaE	Confirmed	Even	< 8E-8	< 7.5E-8	1.0 ± 0.5
rfaC	Confirmed	CGSC	< 2E-8	< 5.3E-7	0.7 ± 0.3
rfaF	Confirmed	Dharmacon, Inc.	< 3E-7	< 7.9E-7	0.7 ± 0.3
rfaY	Confirmed	Odd	1.4		
tolC	Confirmed	CGSC	< 3E-7	< 5.3E-7	0.4 ± 0.3

Table S5. Results of initial (untargeted) screening and targeted re-screening of phage LL12 against the Keio *E. coli* knockout collection. In the initial screen, all mutants yielding a positive result were screened a second time against both independently-generated gene knockouts present in the Keio collection, denoted as the representatives from the even- and odd-numbered plate sets. Mutants with a positive result from either set were then tested for their efficiency of plating (EOP) by both spot titer and full-plate titration methods. Mutants exhibiting a significant EOP defect (less than ~0.05, highlighted green) in full-plate titers were used for further study. The presence of the appropriate gene deletion was confirmed by PCR and sequencing. One knockout from each even/odd pair was selected for P1 transduction of the *kan*-marked deletion into the parental *E. coli* strain BW25113 background, re-tested for EOP defects and complemented *in trans*. Selected gene knockouts from the collection that were not identified in the initial screen were targeted for re-screening (bottom panel). Mutants were cultured from the Keio collection, the presence of the appropriate gene deletion confirmed by PCR, and the EOP determined in the knockout and its complemented counterpart. Blank cells denote that data was not collected, usually because the desired EOP defects were not observed.

Initial screen of Keio collection

Gene deletion	Results of first screen	Results of second screen		EOP (spot titer)		EOP (plate titer)		Keio mutant used for further work	Deletion confirmed by PCR	Transducible by P1*vir*	EOP (plate titer) in P1 transductant	EOP (plate titer) in complemented strain
		Odd	Even	Odd	Even	Odd	Even					
pflC	+	-	-									
cusB	+	-	-									
ompR	+	+	-	0.5	0.5							
envZ	+	-	-									
ompC	+	+	+	0.3	0.3							
yncJ	+	-	-									
ycbL	+	-	-									
yqjB	+	nt	nt	1.9	1.1							
yaaW	+	-	-									
yggT	+	+	+	0.1	0.1							
rfaP	+	+	+	0.06	1.1	0.04	1.2	Odd	Yes	Yes	0.02	1.0
rfaD	+	-	-									
rfaG	+	+	-	7E-06	1.0	5.1E-06	1.3	Odd	Yes	No		1.1
rfaF	+	-	-									
rfaH	+	+	-	0.4	0.8							
rfaY	+	+	+	0.3	0.2							
rffE	+	+	-	0.2	0.1							
lpcA	+	-	+	0.8	< 4E-9	1.5	< 4.4E-9	Even	Yes	No		1.1
trmC	+	-	-									
ygbF	+	-	-									
etp	+	-	-									
Total passed	**21**	**8**	**5**	**2**	**1**	**2**	**1**				**1**	

Table S5. Continued. Targeted re-screening. Based on the results of the initial screening, individual mutants were confirmed for gene deletions and obtained from other sources as necessary. The genes *rfaC* and *rfaF* were found to be intact in the copy of the Keio collection used for initial screening.

Gene deletion	Deletion confirmed by PCR	Keio mutant used for further work	EOP (spot titer)	EOP (plate titer)	EOP (plate titer) in complemented strain (Average ± S.D.)
rfaE	Confirmed	Even	< 4E-9	< 4.4E-9	1.1 ± 0.5
rfaI	Confirmed	Odd	0.6		
rfaB	Confirmed	Even	0.4		
rfaC	Confirmed	CGSC	< 7E-8	< 5.1E-9	0.9 ± 0.4
rfaF	Confirmed	Dharmacon, Inc.	< 8E-8	< 6.5E-9	1.5 ± 0.5
rfaY	Confirmed	Odd	0.1		

References

1. Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K.A.; Tomita, M.; Wanner, B.L.; Mori, H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* **2006**, *2*, 2006 0008, doi:10.1038/msb4100050.