

Supplemental information

The intestinal gut microbiota facilitates drifts in the genetic diversity and infectivity of bacterial viruses

Luisa De Sordi, Varun Khanna and Laurent Debarbieux

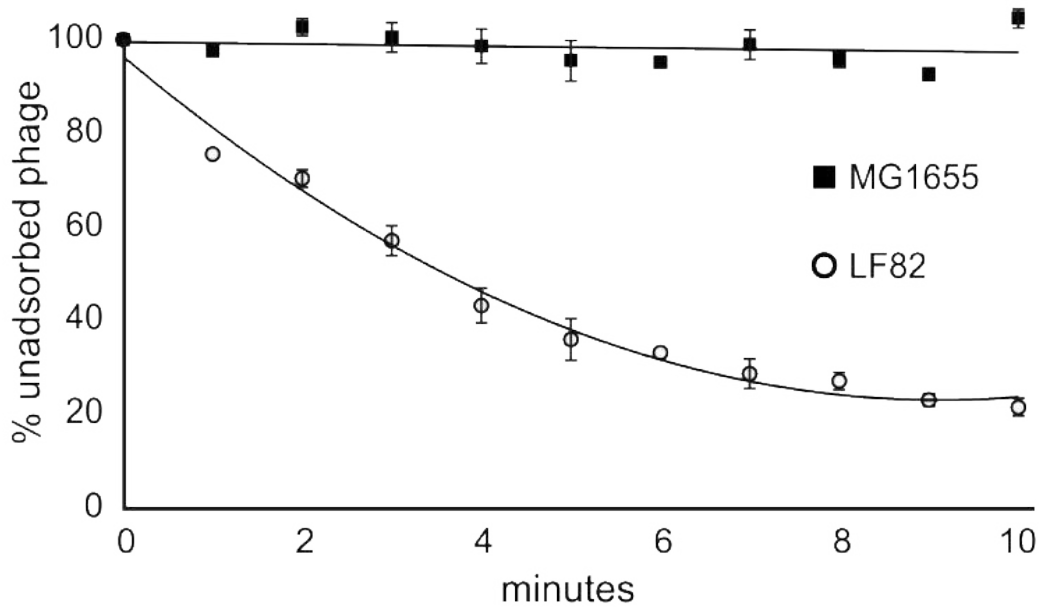


Figure S1. Adsorption assay of bacteriophage P10. Related to STAR methods. A bacteriophage solution of 2×10^5 pfu was added to exponentially growing cultures ($OD_{600}=0.3$) of strains MG1655 (black squares) or LF82 (white circles) in LB medium. Samples were collected every 60 seconds over 10 minutes to enumerate the amount of unadsorbed bacteriophage. Data points show the percentage of unadsorbed P10 as mean of three independent experiments \pm s.e.m, plus curves of best fit (black lines). A solution of 10^{10} pfu of P10 spotted onto a lawn of MG1655 did not lead to detecting any visible lysis plaque.

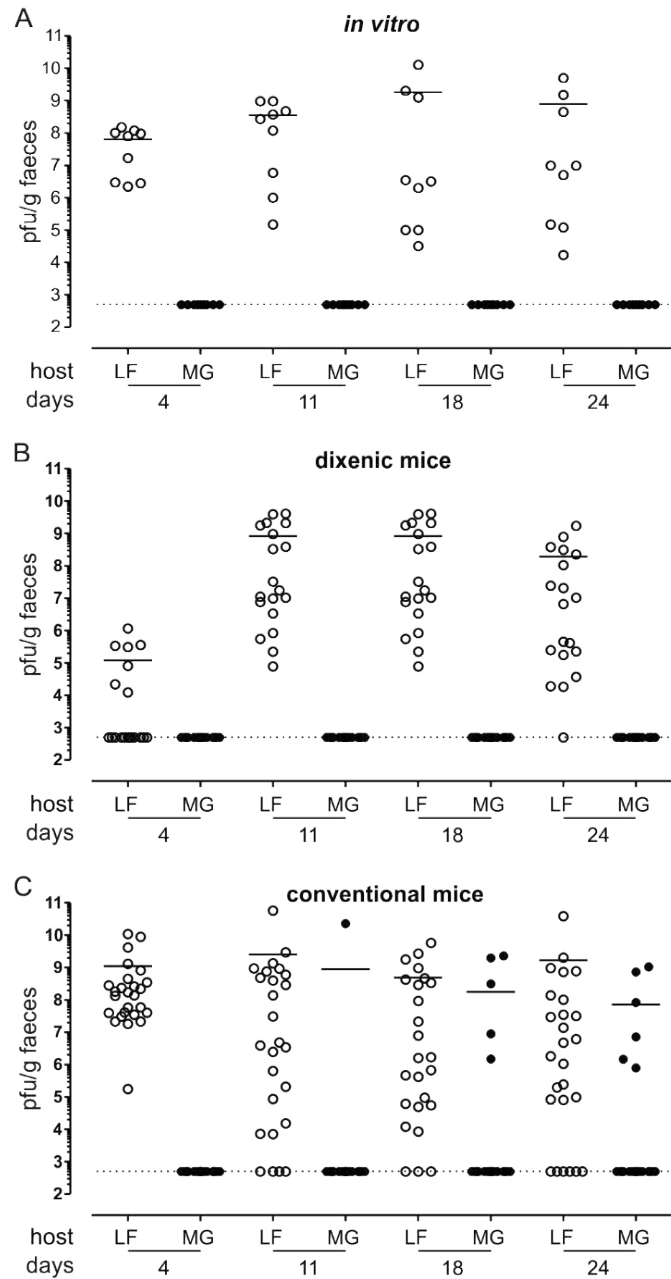


Figure S2. Intestinal microbiota is required for bacteriophage P10 adaptation. Related to Figure 1. Densities of bacteriophage P10 were recorded over 24 days of exposure to strains LF82 and MG1655 *in vitro* (n=9) (A), in dixenic mice (n=19, 10 groups) (B) and in conventional mice (n=26, 10 groups) (C). Bacteriophage titers were measured by plaque assay on LB agar plates overlaid with the hosts LF82 (LF) (white circles) and MG1655 (MG) (black circles). Twenty percent of the samples from conventional mice showed bacteriophage adaptation. A dotted line represents the threshold of detection. Scales are logarithmic on base 10.

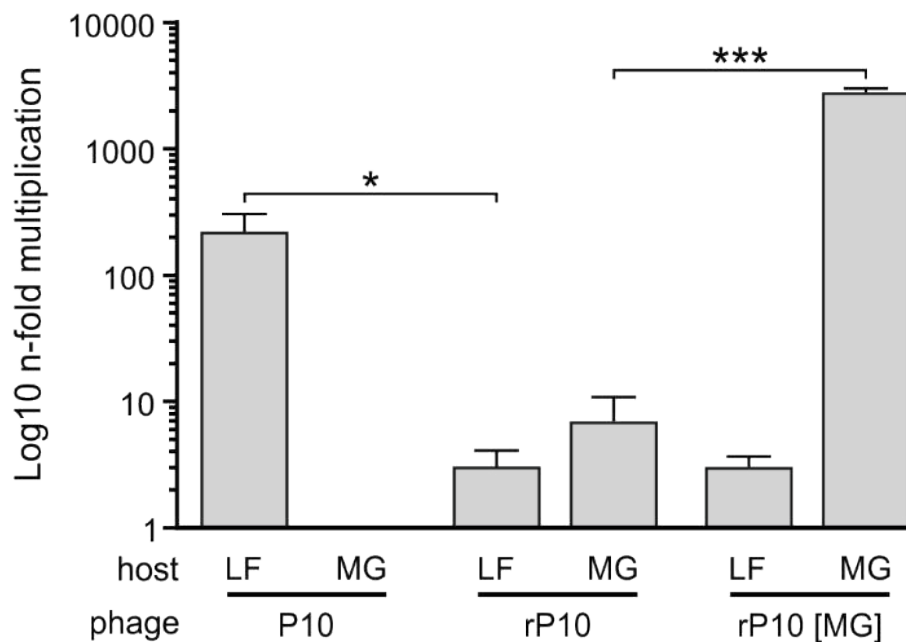























































Figure S3: The host jump is associated to a fitness cost. Related to Figure 1. Recombinant bacteriophage P10 (rP10) carrying mutation Y284H in the Gp91 tail fiber protein, acquired the ability to infect strain MG1655 (MG) (host jump) but is significantly less efficient in infecting the original host, strain LF82 (LF). Bacteriophage rP10 was let replicating on its new host during 4 replicative cycles of 4 hours at M.O.I. 0.001 generating rP10 [MG]. When compared to rP10, this variant showed significant increase in infectivity towards strain MG1655, to levels higher than those reported for wild type P10 on strain LF82. Bacteria in logarithmic phase of growth and bacteriophages were inoculated at M.O.I. = 0.01 and incubated at 37°C for 4 hours. Bars represent the mean fold replication coefficient of three independent replicates \pm s.e.m. Scales are logarithmic on base 10. * $p < 0.05$; *** $p < 0.0001$; unpaired, one-tailed t test.

Table S1. Host spectrum of P10 and different ad_P10 against 105 bacterial strains (see below for full references), Related to Figure 2.

Strain	Bacteriophage Infection		Reference
	P10	ad_P10 (n=16)	
MG1655 (<i>E. coli</i> K-12)			Laboratory collection
LF82			Miquel <i>et al.</i> , 2010 (PMID:20862302)
NRG857c			Small <i>et al.</i> , 2013 (PMID:23748852)
LF31			Darfeuille-Michaud <i>et al.</i> , 2004 (PMID:15300573)
LF50			Darfeuille-Michaud <i>et al.</i> , 2004 (PMID:15300573)
LF73			Darfeuille-Michaud <i>et al.</i> , 2004 (PMID:15300573)
LF110			Darfeuille-Michaud <i>et al.</i> , 2004 (PMID:15300573)
LF06011			AIEC collection from University of Lille, France, via N. Barnich
LF06023			AIEC collection from University of Lille, France, via N. Barnich
LF06024			AIEC collection from University of Lille, France, via N. Barnich
LF06026			AIEC collection from University of Lille, France, via N. Barnich
LF06027			AIEC collection from University of Lille, France, via N. Barnich
LF06028			AIEC collection from University of Lille, France, via N. Barnich
LF06029			AIEC collection from University of Lille, France, via N. Barnich
LF06030			AIEC collection from University of Lille, France, via N. Barnich
LF06031			AIEC collection from University of Lille, France, via N. Barnich
LF06033			AIEC collection from University of Lille, France, via N. Barnich
LF06072			AIEC collection from University of Lille, France, via N. Barnich
LF06073			AIEC collection from University of Lille, France, via N. Barnich
LF06075			AIEC collection from University of Lille, France, via N. Barnich
LF06076			AIEC collection from University of Lille, France, via N. Barnich
LF06087			AIEC collection from University of Lille, France, via N. Barnich
LF06088			AIEC collection from University of Lille, France, via N. Barnich
LF06089			AIEC collection from University of Lille, France, via N. Barnich
LF06150			AIEC collection from University of Lille, France, via N. Barnich
LF06151			AIEC collection from University of Lille, France, via N. Barnich
LF06152			AIEC collection from University of Lille, France, via N. Barnich
LF06166			AIEC collection from University of Lille, France, via N. Barnich
LF06167			AIEC collection from University of Lille, France, via N. Barnich
LF06168			AIEC collection from University of Lille, France, via N. Barnich
LF06169			AIEC collection from University of Lille, France, via N. Barnich
LF06170			AIEC collection from University of Lille, France, via N. Barnich
LF06171			AIEC collection from University of Lille, France, via N. Barnich
LF06172			AIEC collection from University of Lille, France, via N. Barnich
LF06173			AIEC collection from University of Lille, France, via N. Barnich
LF06254			AIEC collection from University of Lille, France, via N. Barnich
LF06256			AIEC collection from University of Lille, France, via N. Barnich

LF07126			AIEC collection from University of Lille, France, via N. Barnich
LF07127			AIEC collection from University of Lille, France, via N. Barnich
LF07128			AIEC collection from University of Lille, France, via N. Barnich
LF07134			AIEC collection from University of Lille, France, via N. Barnich
LF07135			AIEC collection from University of Lille, France, via N. Barnich
LF07136			AIEC collection from University of Lille, France, via N. Barnich
LF07137			AIEC collection from University of Lille, France, via N. Barnich
<i>E. coli</i> 536			Brzuszkiewicz E <i>et al.</i> , 2006 (PMID:16912116)
<i>E. coli</i> 55989			Mossoro <i>et al.</i> , 2002 (PMID:12149388)
<i>E. coli</i> AL505			Galtier <i>et al.</i> , 2016 (PMID:26971586)
<i>E. coli</i> BE			Laboratory collection, gift from H. Krisch
<i>E. coli</i> BW25113			Datsenko and Wanner, 2000 (PMID:10829079)
<i>E. coli</i> CR63			Laboratory collection, gift from H. Krisch
<i>E. coli</i> E22			Camguilhem and Milon, 1989 (PMID:2656746)
<i>E. coli</i> LM33			Dufour <i>et al.</i> , 2016 (PMID:27387322)
<i>E. coli</i> MI/5			Laboratory collection, gift from E. Oswald
<i>E. coli</i> Nissle			Laboratory collection, gift from E. Oswald
<i>E. coli</i> O42			Laboratory collection, gift from C. Le Bouguéneq
<i>E. coli</i> OP50			Laboratory collection, gift from J. Ewbank
<i>E. coli</i> S15			Laboratory collection, gift from E. Oswald
<i>E. coli</i> Sp15			Laboratory collection, gift from E. Oswald
<i>Citrobacter rodentium</i> ICC180			Wiles <i>et al.</i> , 2004 (PMID:15339271)
<i>Erwinia caratovora</i> 15 (CFBP2141)			Collection Française de Bactéries Phytopathogènes, INRA (Angers, France)
<i>Rouxiiella chamberiensis</i> 130333T			Le Flèche-Mateos <i>et al.</i> , 2015 (PMID:25747423)
<i>Serratia marcescens</i> Db11			Flyg <i>et al.</i> , 1981 (PMID:7012273)
<i>Serratia marcescens</i> SM365			Laboratory collection, gift from V. Braun via C. Wandersman

References for Table S1

1. E. Brzuszkiewicz *et al.*, Proc Natl Acad Sci U S A 103, 12879 (Aug 22, 2006).
2. R. Camguilhem, A. Milon, J Clin Microbiol 27, 743 (Apr, 1989).
3. A. Darfeuille-Michaud *et al.*, Gastroenterology 127, 412 (Aug, 2004).
4. K. A. Datsenko, B. L. Wanner, Proc Natl Acad Sci U S A 97, 6640 (Jun 6, 2000).
5. N. Dufour *et al.*, J Antimicrob Chemother, (Jul 7, 2016).
6. C. Flyg, K. Kenne, H. G. Boman, J Gen Microbiol 120, 173 (Sep, 1980).
7. M. Galtier *et al.*, Environ Microbiol 18, 2237 (Jul, 2016).
8. A. Le Fleche-Mateos *et al.*, Int J Syst Evol Microbiol 65, 1812 (Jun, 2015).
9. S. Miquel *et al.*, PLoS One 5, (Sep 17, 2010).
10. C. Mossoro *et al.*, J Clin Microbiol 40, 3086 (Aug, 2002).
11. C. L. Small, S. A. Reid-Yu, J. B. McPhee, B. K. Coombes, Nat Commun 4, 1957 (2013).

Table S2. Positive Blastn results (>80% identity, >40 nt alignment) of annotated bacteriophage tail fiber genes of the same organism, Related to Figure 3.

Organism	Gene 1 location	Gene 2 location	% identity	length	Gene 1 start	Gene 1 end	Gene 2 start	Gene 2 end	e-value
Clostridium phage phiCD6356 (NC_015262)	19898-20509	18602-19225	91.11	90	1	89	1	89	3.00E-29
Cronobacter phage ENT90 (HQ110084)	27721-28122	1021-2247	100	402	1	402	826	1227	0
Cyanophage NATL2A-133 (GU071104)	6419-10879	4469-6058	90.24	82	657	737	777	857	3.00E-23
Enterobacteriophage UAB_Phi87 (NC_027360)	65019-66185	62620-64968	95.21	167	781	947	2023	2189	5.00E-75
Halomonas phage phiHAP-1 (NC_010342)	19516-19929	16930-19515	100	414	1	414	2173	2586	0
Organic Lake virophage (HQ704801) ^a	20266-21570	12557-14884	89.66	358	1	353	1702	2054	1.00E-116
P2-like Enterobacteria phage Fels-2 (NC_010463)	9707-11281	7623-8702	98.77	163	824	986	95	257	4.00E-87
Rhodococcus phage ReqiPoco6 (GU580942)	4020-5780	1771-3999	100	44	16	59	1	44	2.00E-20
Salmonella phage Felix 01 (NC_005282)	51117-53465	49900-51066	91.06	179	2011	2187	769	945	7.00E-62
Salmonella phage HB-2014 (NC_027329)	52722-55064	51509-52675	88.46	130	2030	2156	794	920	2.00E-34
Synechococcus phage S-CRM01 (NC_015569) ^a	28846-29976	25264-27000	83.64	165	316	468	844	1005	8.00E-24
uvMED-CGR-C55-MedDCM-OCT-S33-C61 (AP013424) ^b	32717-33379	2-1333	100	48	1	48	1285	1332	1.00E-23
uvMED-GF-U-MedDCM-OCT-S42-C64 (AP014376) ^b	12691-14499	10890-12674	93.44	61	245	305	131	191	3.00E-21
Vibriophage phi-pp2 (JN849462)	165607-168882	162206-165535	91.38	58	1012	1069	1801	1858	2.00E-16

^a multiple alignment results, only the longest alignment length is shown for clarity

^b uncultured Mediterranean phage

Table S3. Frequency of single nucleotide polymorphisms (SNPs) over 1% in P10 populations *in vitro* (IV), dixenic mice (DM) or conventional mice (CM), in the presence of a single strain (SS), LF82, or double strains (DS), LF82 and MG1655; the adapted (_ad) population from conventional mice was sequenced separately, Related to Figure 1.

Position	Reference	Mutation	Type	aa_reference	aa_mutation	aa_position	codon_ref	codon_mut	gene_name	start_gene	end_gene	strand_gene	Product	IV 1S	IV 2S	DM 2S	CM 1S	CM 2S	CM 2S_ad
1624	C	T	NON_SYNONYMOUS_CODING	A	V	89	gCt	gTt	LF82_P10_gp4	1359	1862	+	Phage protein					51.6	
5394	G	T	NON_SYNONYMOUS_CODING	H	N	62	Cat	Aat	LF82_P10_gp9	5329	5577	-	Phage protein				24.4		
10842	G	A	NON_SYNONYMOUS_CODING	T	I	66	aCa	aTa	LF82_P10_gp22	10745	11038	-	Phage protein				9.9		
19451	C	A	STOP_GAINED							17838	20204	-	Phage rIIA lysis inhibitor	4					
19706	C	G	NON_SYNONYMOUS_CODING	G	R	167	Ggt	Cgt		17838	20204	-	Phage rIIA lysis inhibitor	22.5	5				
19816	A	G	NON_SYNONYMOUS_CODING	F	S	130	tTc	tCc		17838	20204	-	Phage rIIA lysis inhibitor	2.8	1.8				50
19912	T	C	NON_SYNONYMOUS_CODING	Y	C	98	tAc	tGc	LF82_P10_gp37	17838	20204	-	Phage rIIA lysis inhibitor		1.6	100	98.9	65.4	
19957	C	A	NON_SYNONYMOUS_CODING	R	L	83	cGt	cTt		17838	20204	-	Phage rIIA lysis inhibitor	3.7				32.7	50
19988	G	A	NON_SYNONYMOUS_CODING	P	S	73	Cct	Tct		17838	20204	-	Phage rIIA lysis inhibitor	59.9	88.5				
20045	C	T	NON_SYNONYMOUS_CODING	A	T	54	Gca	Aca		17838	20204	-	Phage rIIA lysis inhibitor	3.3					
20755	T	C	Intergenic																11.3
20756	A	G	Intergenic																11.4
24550	C	T	NON_SYNONYMOUS_CODING	G	D	38	gGc	gAc	LF82_P10_gp44	24342	24662	-	Phage protein				1.9		
29513	T	C	NON_SYNONYMOUS_CODING	K	R	322	aAg	aGg	LF82_P10_gp55	29404	30477	-	Ribonucleotide reductase of class Ia						22.7
31180	C	T	NON_SYNONYMOUS_CODING	M	I	614	atG	atA		30787	33021	-	Ribonucleotide reductase of class Ia			57.2			
31422	G	A	NON_SYNONYMOUS_CODING	H	Y	534	Cat	Tat	LF82_P10_gp57	30787	33021	-	Ribonucleotide reductase of class Ia	8.9					
32150	G	A	NON_SYNONYMOUS_CODING	A	V	291	gCt	gTt		30787	33021	-	Ribonucleotide reductase of class Ia						6.7
34036	C	T	SYNONYMOUS_CODING						LF82_P10_gp60	33703	34458	-	Phage protein	8.3					
37994	A	G	NON_SYNONYMOUS_CODING	I	T	539	aTt	aCt		37624	39609	-	DNA primase/helicase	8.4	12.2				
38405	A	G	NON_SYNONYMOUS_CODING	I	T	402	aTc	aCc		37624	39609	-	DNA primase/helicase	7.3					
38550	T	C	NON_SYNONYMOUS_CODING	T	A	354	Aca	Gca	LF82_P10_gp67	37624	39609	-	DNA primase/helicase		6.8				
38558	C	T	NON_SYNONYMOUS_CODING	G	D	351	gGt	gAt		37624	39609	-	DNA primase/helicase	1.5					
39093	A	G	NON_SYNONYMOUS_CODING	F	L	173	Ttc	Ctc		37624	39609	-	DNA primase/helicase	11					
41152	C	T	NON_SYNONYMOUS_CODING	D	N	93	Gac	Aac	LF82_P10_gp70	40616	41428	-	Phage minor tail protein				2.9		50
41323	T	C	NON_SYNONYMOUS_CODING	T	A	36	Aca	Gca		40616	41428	-	Phage minor tail protein				24.4		
44472	G	A	NON_SYNONYMOUS_CODING	G	E	788	gGa	gAa	LF82_P10_gp72	42110	44809	+	DNA polymerase	4.1					
44473	A	C	SYNONYMOUS_CODING							42110	44809	+	DNA polymerase	3.9					
48938	T	G	NON_SYNONYMOUS_CODING	S	A	68	Tcc	Gcc	LF82_P10_gp82	48737	49036	+	Phage protein						1.1
51713	G	A	NON_SYNONYMOUS_CODING	A	V	109	gCc	gTc		51667	52038	-	Phage protein					30.8	
51764	G	A	NON_SYNONYMOUS_CODING	T	I	92	aCt	aTt		51667	52038	-	Phage protein				1.5		
51780	C	T	NON_SYNONYMOUS_CODING	A	T	87	Gca	Aca		51667	52038	-	Phage protein				1.7		50
51828	C	A	NON_SYNONYMOUS_CODING	G	W	71	Ggg	Tgg	LF82_P10_gp88	51667	52038	-	Phage protein						21.5
51860	T	C	NON_SYNONYMOUS_CODING	D	G	60	gAt	gGt		51667	52038	-	Phage protein						2
51870	C	T	NON_SYNONYMOUS_CODING	A	T	57	Gct	Act		51667	52038	-	Phage protein				14.6		
51957	T	C	NON_SYNONYMOUS_CODING	T	A	28	Act	Gct		51667	52038	-	Phage protein			100			

52428	C	T	NON_SYNONYMOUS_CODING	G	D	756	gGt	gAt		52313	54694	-	Phage tail fibers				5.4	
52456	T	C	NON_SYNONYMOUS_CODING	N	D	747	Aac	Gac		52313	54694	-	Phage tail fibers		1.8			
52638	G	T	NON_SYNONYMOUS_CODING	A	D	686	gCt	gAt		52313	54694	-	Phage tail fibers				10	
52641	G	T	NON_SYNONYMOUS_CODING	A	D	685	gCt	gAt		52313	54694	-	Phage tail fibers				24.3	
53226	G	T	STOP_GAINED						LF82_P10_gp90	52313	54694	-	Phage tail fibers					50
53341	C	T	NON_SYNONYMOUS_CODING	D	N	452	Gat	Aat		52313	54694	-	Phage tail fibers			8.7		
53410	C	T	NON_SYNONYMOUS_CODING	E	K	429	Gaa	Aaa		52313	54694	-	Phage tail fibers		34.4	26.8		
54388	T	C	NON_SYNONYMOUS_CODING	K	E	103	Aaa	Gaa		52313	54694	-	Phage tail fibers					18.9
54447	G	T	NON_SYNONYMOUS_CODING	S	Y	83	tCt	tAt		52313	54694	-	Phage tail fibers			5.9		
54897	G	A	SYNONYMOUS_CODING							54741	55928	-	Phage tail fiber protein					1.4
54900	G	A	SYNONYMOUS_CODING							54741	55928	-	Phage tail fiber protein					1.3
54901	C	G	NON_SYNONYMOUS_CODING	S	T	343	aGc	aCc		54741	55928	-	Phage tail fiber protein					1.4
54912	A	G	SYNONYMOUS_CODING							54741	55928	-	Phage tail fiber protein					32.2
55016	A	T	NON_SYNONYMOUS_CODING	S	T	305	Tcg	Acg		54741	55928	-	Phage tail fiber protein					50
55031	T	C	NON_SYNONYMOUS_CODING	T	A	300	Aca	Gca	LF82_P10_gp91	54741	55928	-	Phage tail fiber protein					56
55079	A	G	NON_SYNONYMOUS_CODING	Y	H	284	Tat	Cat		54741	55928	-	Phage tail fiber protein					100
55108	G	T	NON_SYNONYMOUS_CODING	S	Y	274	tCt	tAt		54741	55928	-	Phage tail fiber protein					7
55118	A	T	NON_SYNONYMOUS_CODING	S	T	271	Tca	Aca		54741	55928	-	Phage tail fiber protein					1.8
55119	C	A	SYNONYMOUS_CODING							54741	55928	-	Phage tail fiber protein					1.7
55160	T	C	NON_SYNONYMOUS_CODING	T	A	257	Act	Gct		54741	55928	-	Phage tail fiber protein					1.6
56175	A	G	NON_SYNONYMOUS_CODING	V	A	20	gTa	gCa	LF82_P10_gp92	55931	56233	-	Phage protein					45.2
56315	C	T	NON_SYNONYMOUS_CODING	G	D	259	gGt	gAt		56233	57090	-	Phage protein				21	
56441	G	A	NON_SYNONYMOUS_CODING	A	V	217	gCt	gTt		56233	57090	-	Phage protein		33.5			
56447	G	A	NON_SYNONYMOUS_CODING	A	V	215	gCa	gTa	LF82_P10_gp93	56233	57090	-	Phage protein				2.7	
56447	G	T	NON_SYNONYMOUS_CODING	A	E	215	gCa	gAa		56233	57090	-	Phage protein		5			
56448	C	T	NON_SYNONYMOUS_CODING	A	T	215	Gca	Aca		56233	57090	-	Phage protein		60	5		
57140	G	T	NON_SYNONYMOUS_CODING	Q	K	475	Caa	Aaa		57093	58562	-	putative bacteriophage protein		61.4			
57497	C	T	NON_SYNONYMOUS_CODING	A	T	356	Gct	Act	LF82_P10_gp94	57093	58562	-	putative bacteriophage protein				6.1	
57938	T	C	NON_SYNONYMOUS_CODING	R	G	209	Aga	Gga		57093	58562	-	putative bacteriophage protein					50
58939	C	T	NON_SYNONYMOUS_CODING	A	T	15	Gcc	Acc	LF82_P10_gp95	58562	58981	-	Phage protein				3.8	
65602	C	T	NON_SYNONYMOUS_CODING	A	T	293	Gct	Act	LF82_P10_gp104	65126	66478	-	Phage protein	4.9				
66147	T	C	NON_SYNONYMOUS_CODING	Y	C	111	tAc	tGc		65126	66478	-	Phage protein					1
68207	T	C	NON_SYNONYMOUS_CODING	Q	R	59	cAa	cGa	LF82_P10_gp108	67933	68382	-	Phage protein					27.7
68391	C	T	Intergenic															5.6
69005	G	A	NON_SYNONYMOUS_CODING	P	L	171	cCt	cTt	LF82_P10_gp109	68404	69516	-	major capsid protein					
76190	C	T	NON_SYNONYMOUS_CODING	M	I	126	atG	atA	LF82_P10_gp117	75833	76567	-	Phage protein				3	
77858	C	A	Intergenic														4.7	
80775	G	A	Intergenic														5	
83476	G	T	Intergenic											100				
86471	C	A	NON_SYNONYMOUS_CODING	F	L	56	ttC	ttA	LF82_P10_gp129	86304	86702	+	Phage protein	5.3				
86992	C	T	STOP_GAINED							86695	87090	+	Phage protein		100			
87004	G	T	STOP_GAINED							86695	87090	+	Phage protein			37.7		
87049	T	C	NON_SYNONYMOUS_CODING	S	P	119	Tca	Cca	LF82_P10_gp130	86695	87090	+	Phage protein	4.7				

Table S4. Genomic mutations in rP10 after four cycles of replication on strain MG1655 (the introduced mutation Y284H is highlighted in yellow), Related to Figure 1 and Figure S3.

position	mutation	putative function
42,490	(AG <u>A</u> →AG <u>C</u>); R127S	DNA polymerase
46,169	(AG <u>C</u> →AG <u>T</u>); S45S	unknown
51,957	(AC <u>T</u> →GC <u>T</u>); T28A	unknown
52,892	1 bp deletion	Phage tail fiber
55,030	(A <u>C</u> A→A <u>A</u> A); T300K	Phage tail fiber protein
55,079	(<u>I</u> AT→ <u>C</u> AT); Y284H	Phage tail fiber protein
56,722	(CC <u>T</u> →CC <u>C</u>); P123P	unknown
65,021	(TT <u>G</u> →TT <u>A</u>); L30L	unknown
67,828	(<u>C</u> AT→ <u>I</u> AT); H36Y	unknown