Supporting Information

Directed Evolution of Replication-Competent dsDNA Bacteriophage towards New Host Specificity

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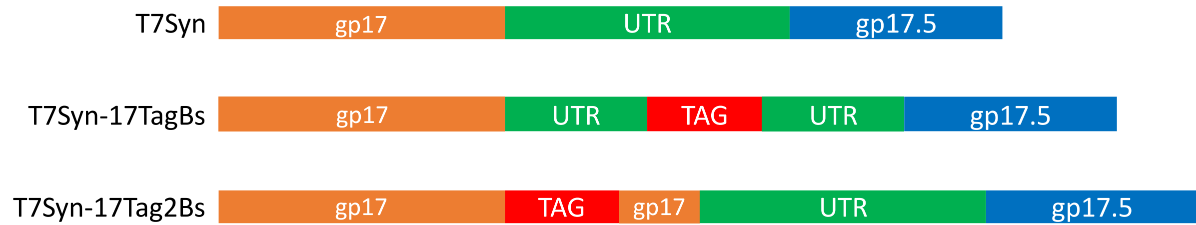
#	Name	Construct	Derived from	Modification	Remark
1	T7Syn	T7Gp11 T7Gp12 T7Gp17	Chemical synthesis	N.A.	NGS Sequenced. GenBank MW248381
2	T7Syn-17Bs	T7Gp11 T7Gp12 B T7Gp17 B	#1	BsaI addition	NGS Sequenced.
3	T7Syn-1112Bs	B T7Gp11 T7Gp12 B T7Gp17	#1	BsaI addition	NGS Sequenced.
4	T7Syn-111217Bs	B T7Gp11 T7Gp12 B B T7Gp17 B	#1	BsaI addition	NGS Sequenced. GenBank MW248382
5	T7Syn-17TagBs	T7Gp11 T7Gp12 B T7Gp17 B	#1	BsaI addition, Mock tag insertion	Two versions made
6	T7Syn-17m5	T7Gp11 T7Gp12 17m5	#2	Mutagenized T7Gp17	Gp17: K214N, T216M, S241T, D540Y
7	T7Syn-1112Bs17m5	B T7Gp11 T7Gp12 B 17m5	#6	BsaI addition	
8	T7Syn-5m7	T7Gp11 T7Gp12 5m7	#2	Mutagenized T7Gp17m5	Gp17: 17m5+D304G,S477I
9	T7Syn-12m1	T7Gp11 12m1 17m5	#7	Mutagenized T7Gp11-12	Gp12: I616T Gp17: 17m5
10	T7Syn-K111217	K11Gp11 K11Gp12 K11Gp17	#1	K11Gp11,12,17	
11	T7Syn-TK17	T7Gp11 T7Gp12 K11Gp17	#4	Mutagenized T7Gp11-12, K11Gp17	Gp11: Y178F Gp12: P144L
12	T7Syn-K1112BsK17	B K11Gp11 K11Gp12 B K11Gp17	#1	BsaI addition	
13	T7Syn-K1112K17Bs	K11Gp11 K11Gp12 B K11Gp17 B	#1	BsaI addition	
14	T7Syn-KmL2	K11Gp11 K11Gp12 KmL2	#13	Mutagenized K11Gp17	

Figure S1. List of phages constructed in this work. Construct schematics are not to scale. Positions of *Bsa*I sites are represented by a dotted line and labeled "B". Phages that are derived from mutagenesis have their detected mutations listed under the Remarks column. Unless stated otherwise, only Gp11, 12, and 17 have been sequenced. The details on the construction of #1 can be found in Figure S8. The sequences of the primers used to derive #2-#14 can be found in Figure S9.

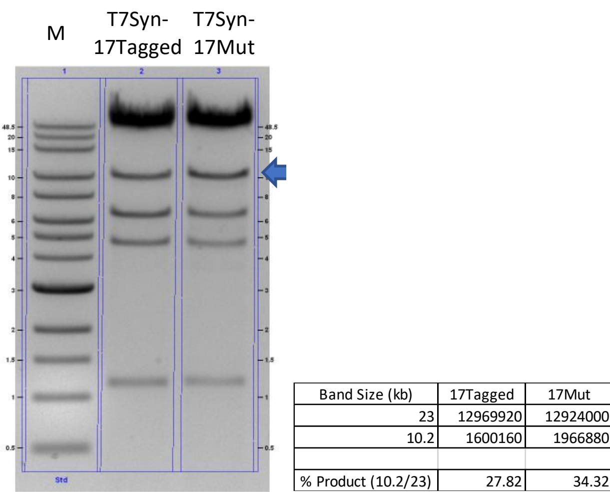


T7Syn: gp17-UTR-gp17.5
ctcggattcaagaatattgcagacagtcgttcagtacctaatgcaatcatggtggagaacgagtaattggtaaatcacaaggaaag
acgtgtagtccacggatggactctcaaggaggtacaaggtgctatcattagactttaacaacgaa

T7Syn-17TagBs: gp17-UTR-TAG-UTR-gp17.5
ctcggattcaagaatattgcagacagtcgttcagtacctaatgcaatcatggtggagaacgagtaattggtaaatcacaaggaaag
acgtgtagtccGGATCCTGTTCCAACCATCGGGTCTCCacggatggactctcaaggaggtacaaggtgctatcattaga
ctttaacaacgaa

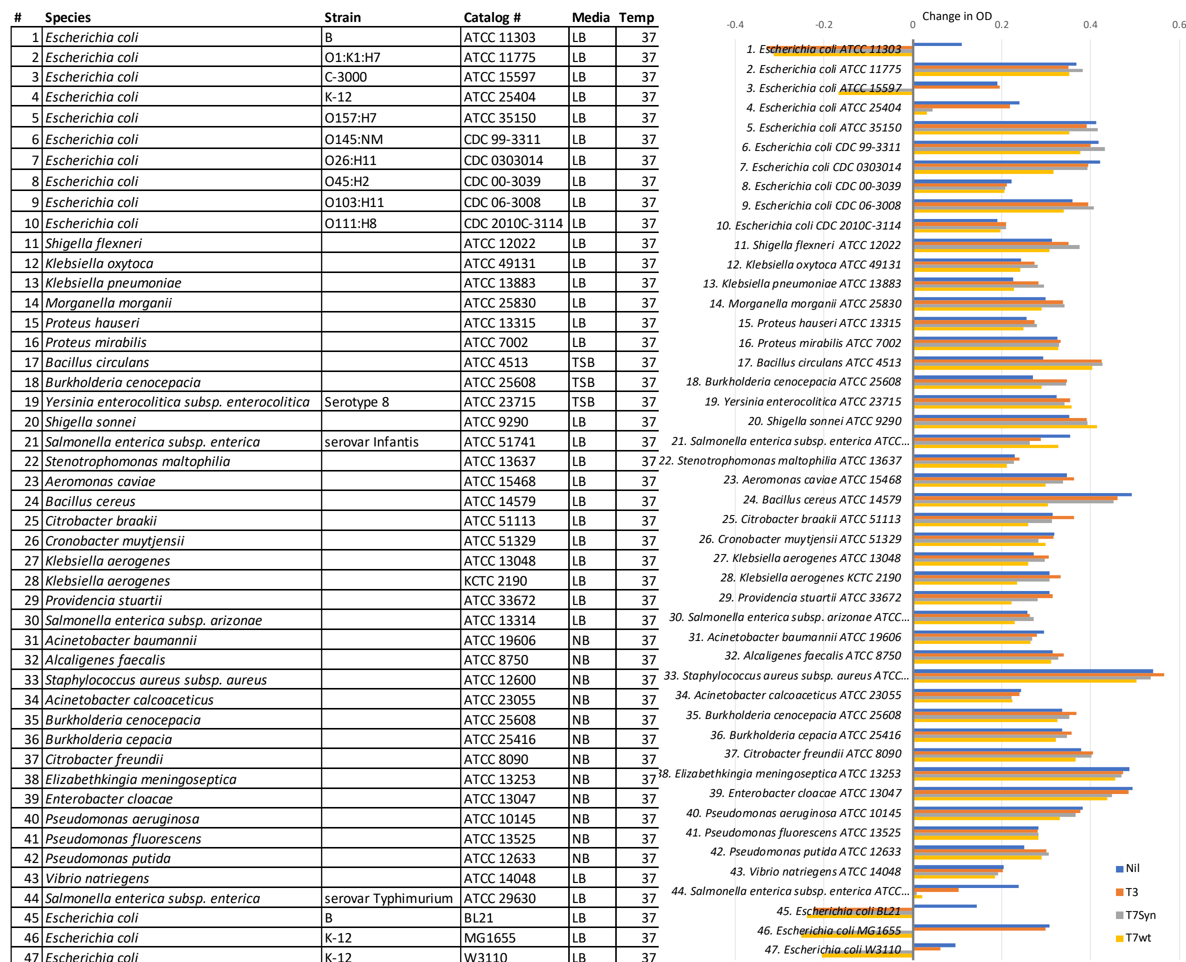
T7Syn-17Tag2Bs: gp17-TAG-UTR-gp17.5
ctcggattcaagaatattgcagacagtcgttcagtacctaatgcaatcatggtggagaacgagtaaCGGATCCTGTTCCAAC
CATCGGGTCTCCtggtggagaacgagtaattggtaaatcacaaggaaagacgtgtagtccacggatggactctcaaggaggt
acaaggtgctatcattagactttaacaacgaa

Figure S2. Extracted sequences from T7Syn, T7Syn-17TagBs, and T7Syn-17Tag2Bs. The last 66 bases of Gp17 is in orange, with the stop codon underlined. The intergenic untranslated region (UTR) between Gp17 and Gp17.5 is in green. The first 27 bases of Gp17.5 is in blue. The inserted mock tag is in CAPITALIZED RED. In the first iteration, T7Syn-17TagBs, the mock tag was inserted within the UTR. The UTR disruption likely affected downstream gene expression, which slowed the phage's propagation efficiency. In the second iteration, T7Syn-17Tag2Bs, the mock tag was inserted immediately after the Gp17 stop codon (taa). The last 17 bases of Gp17 was repeated and used as a buffer between the variable tag region and the UTR. This fully reversed the effect on the phage's propagation efficiency.



Band Size (kb)	17Tagged	17Mut
23	12969920	12924000
10.2	1600160	1966880
% Product (10.2/23)	27.82	34.32

Figure S3. Assessment of Golden Gate reaction efficiency. Gel electrophoresis of two completed Golden Gate reactions, digested with multiple enzymes to excise a 10.2 kb signature band (blue arrow) that would result only from the correctly ligated product. % product generated is estimated by dividing the scaled intensity of the signature band by the scaled intensity of a common band (23 kb in this case) that is present in all phage constructs. Single-insert reaction efficiency typically range from 20 to 35%.



#	Species	Strain	Catalog #	Media	Temp
1	*Escherichia coli*	B	ATCC 11303	LB	37
2	*Escherichia coli*	O1:K1:H7	ATCC 11775	LB	37
3	*Escherichia coli*	C-3000	ATCC 15597	LB	37
4	*Escherichia coli*	K-12	ATCC 25404	LB	37
5	*Escherichia coli*	O157:H7	ATCC 35150	LB	37
6	*Escherichia coli*	O145:NM	CDC 99-3311	LB	37
7	*Escherichia coli*	O26:H11	CDC 0303014	LB	37
8	*Escherichia coli*	O45:H2	CDC 00-3039	LB	37
9	*Escherichia coli*	O103:H11	CDC 06-3008	LB	37
10	*Escherichia coli*	O111:H8	CDC 2010C-3114	LB	37
11	*Shigella flexneri*		ATCC 12022	LB	37
12	*Klebsiella oxytoca*		ATCC 49131	LB	37
13	*Klebsiella pneumoniae*		ATCC 13883	LB	37
14	*Morganella morganii*		ATCC 25830	LB	37
15	*Proteus hauseri*		ATCC 13315	LB	37
16	*Proteus mirabilis*		ATCC 7002	LB	37
17	*Bacillus circulans*		ATCC 4513	TSB	37
18	*Burkholderia cenocepacia*		ATCC 25608	TSB	37
19	*Yersinia enterocolitica subsp. enterocolitica*	Serotype 8	ATCC 23715	TSB	37
20	*Shigella sonnei*		ATCC 9290	LB	37
21	*Salmonella enterica subsp. enterica*	serovar Infantis	ATCC 51741	LB	37
22	*Stenotrophomonas maltophilia*		ATCC 13637	LB	37
23	*Aeromonas caviae*		ATCC 15468	LB	37
24	*Bacillus cereus*		ATCC 14579	LB	37
25	*Citrobacter braakii*		ATCC 51113	LB	37
26	*Cronobacter muytjensii*		ATCC 51329	LB	37
27	*Klebsiella aerogenes*		ATCC 13048	LB	37
28	*Klebsiella aerogenes*		KCTC 2190	LB	37
29	*Providencia stuartii*		ATCC 33672	LB	37
30	*Salmonella enterica subsp. arizonae*		ATCC 13314	LB	37
31	*Acinetobacter baumannii*		ATCC 19606	NB	37
32	*Alcaligenes faecalis*		ATCC 8750	NB	37
33	*Staphylococcus aureus subsp. aureus*		ATCC 12600	NB	37
34	*Acinetobacter calcoaceticus*		ATCC 23055	NB	37
35	*Burkholderia cenocepacia*		ATCC 25608	NB	37
36	*Burkholderia cepacia*		ATCC 25416	NB	37
37	*Citrobacter freundii*		ATCC 8090	NB	37
38	*Elizabethkingia meningoseptica*		ATCC 13253	NB	37
39	*Enterobacter cloacae*		ATCC 13047	NB	37
40	*Pseudomonas aeruginosa*		ATCC 10145	NB	37
41	*Pseudomonas fluorescens*		ATCC 13525	NB	37
42	*Pseudomonas putida*		ATCC 12633	NB	37
43	*Vibrio natriegens*		ATCC 14048	LB	37
44	*Salmonella enterica subsp. enterica*	serovar Typhimurium	ATCC 29630	LB	37
45	*Escherichia coli*	B	BL21	LB	37
46	*Escherichia coli*	K-12	MG1655	LB	37
47	*Escherichia coli*	K-12	W3110	LB	37

Figure S4. Phage susceptibility testing. List of strains tested against 3 phages – T7wt, T7Syn, and T3 – using a liquid lysis endpoint assay. Two hundred µL of log-phase cultures of different bacterial strains were inoculated with ~10^8 phages at 0.5OD, and incubated at 37ºC with shaking. After an hour, the ODs were measured and the change in OD tabulated. A negative change in OD suggest lysis, whereas a less positive change compared to no-phage control suggests growth inhibition.

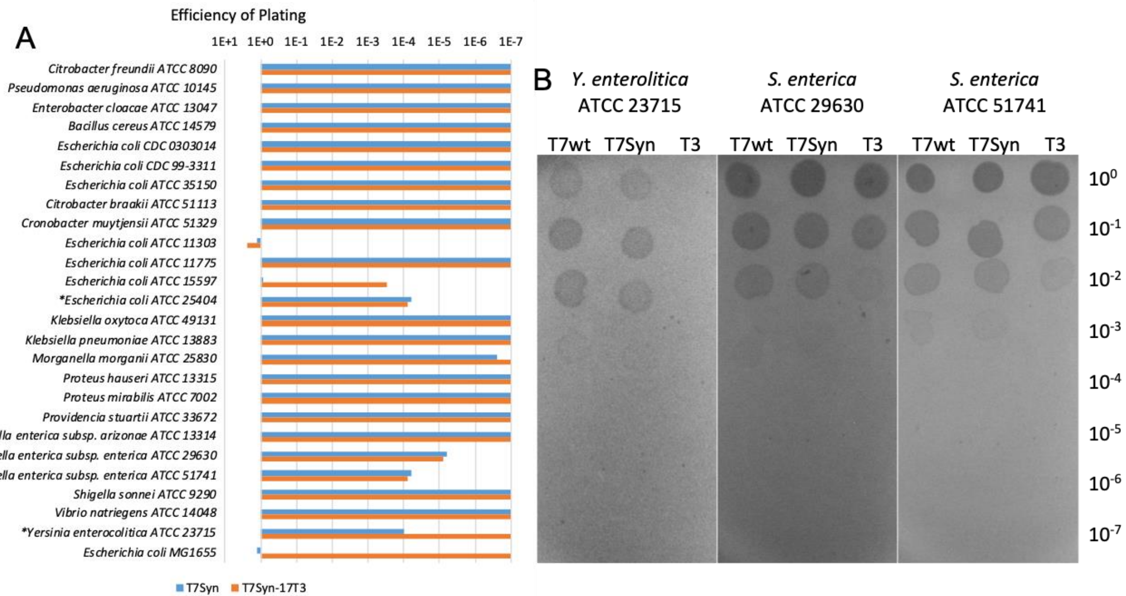


Figure S5. Phage susceptibility testing. A subset of strains was tested by plating, i.e., serial dilutions of 3 phage lysates were deposited onto a thin layer of soft agar containing the strain of interest. After drying, the plates were incubated at 37℃ for up to five hours, or when the plaques became clearly visible. (A) The efficiency of plating was calculated by dividing the observed phage titer on the target strain compared to the observed phage titer on BL21. Bars reaching 1E-7 indicate no observable plaque or zone of inhibition on the target strain. T7wt was not shown as it was invariably identical to that of T7Syn. Asterisk (*) indicates that no individual plaques were visible for these strains, however zones of inhibition were visible at high titer. (B) Three of the strains showing zones of inhibition.

#	Backbone	Mutagenized insert	Pool
M1	T7Syn-17Bs	T7Gp17mut	T1
M2	T7Syn-1112Bs	T7Gp1112mut	
M3	T7Syn-17Bs	T7Gp17m5mut	T2
M4	T7Syn-1112Bs17m5	T7Gp1112mut	
M5	T7Syn-K1112BsK17	K11Gp1112mut	K1
M6	T7Syn-K1112K17Bs	K11Gp17mut	

#	Species	Strain	Catalog #	Media	Temp	Library screened	Result
1	*Acinetobacter baumannii*		ATCC 19606	NB	37	T1	Negative
2	*Pseudomonas putida*		ATCC 12633	NB	26	T1	Negative
3	*Salmonella enterica subsp. enterica*	serovar Typhimurium	ATCC 29630	LB	37	T1	Negative
4	*Salmonella enterica subsp. enterica*	serovar Infantis	ATCC 51741	LB	37	T1	Negative
5	*Citrobacter braakii*		ATCC 51113	LB	37	T1	Negative
6	*Citrobacter freundii*		ATCC 8090	NB	37	T1	Negative
7	*Escherichia coli*	O1:K1:H7	ATCC 11775	LB	37	T1	Negative
8	*Escherichia coli*	O145		LB	37	T1	Negative
9	*Escherichia coli*	O157		LB	37	T1	Negative
10	*Shigella sonnei*		ATCC 9290	LB	37	T1	Negative
11	*Shigella flexneri*		ATCC 12022	NB	37	T1	Negative
12	*Klebsiella aerogenes*		ATCC 13048	LB	37	K1	Negative
13	*Klebsiella pneumoniae*		ATCC 13883	LB	37	K1	Negative
14	*Klebsiella oxytoca*		ATCC 49131	LB	37	K1	Negative
15	*Klebsiella sp. 390*	O3:K11	IJ284	LB	37	K1	T7Syn-KmL2
16	*Burkholderia cepacia*		ATCC 25416	NB	30	T1, K1	Negative
17	*Burkholderia cenocepacia*		ATCC 25608	NB	37	T1, K1	Negative
18	*Pseudomonas aeruginosa*		ATCC 10145	NB	37	T1, K1	Negative
19	*Enterobacter cloacae*		ATCC 13047	NB	30	T1, K1	Negative
20	*Proteus hauseri*		ATCC 13315	LB	37	T1, K1	Negative
21	*Proteus mirabilis*		ATCC 7002	LB	37	T1, K1	Negative
22	*Yersinia enterocolitica subsp. enterocolitica*	Serotype 8	ATCC 23715	TSB	37	T1	T7Syn-17m5
23	*Yersinia enterocolitica subsp. enterocolitica*	Serotype 8	ATCC 23715	TSB	37	T2	T7Syn-5m7, T7Syn-12m1

Figure S6. Screening of mutant libraries. Six different mutant libraries (M1-M6) were created by different combinations of backbones and mutagenized inserts. Related mutant libraries were pooled together after TXTL reaction to form three mutant pools (T1, T2, K1). The pools were then used to screen a number of strains listed above. At least 10^5 phage variants from each pool were used to screen each strain, however the number is an underestimate for the reason highlighted in the main text. In the first round of screening, 12 strains (#1-11, #22) were used to screen pool T1.

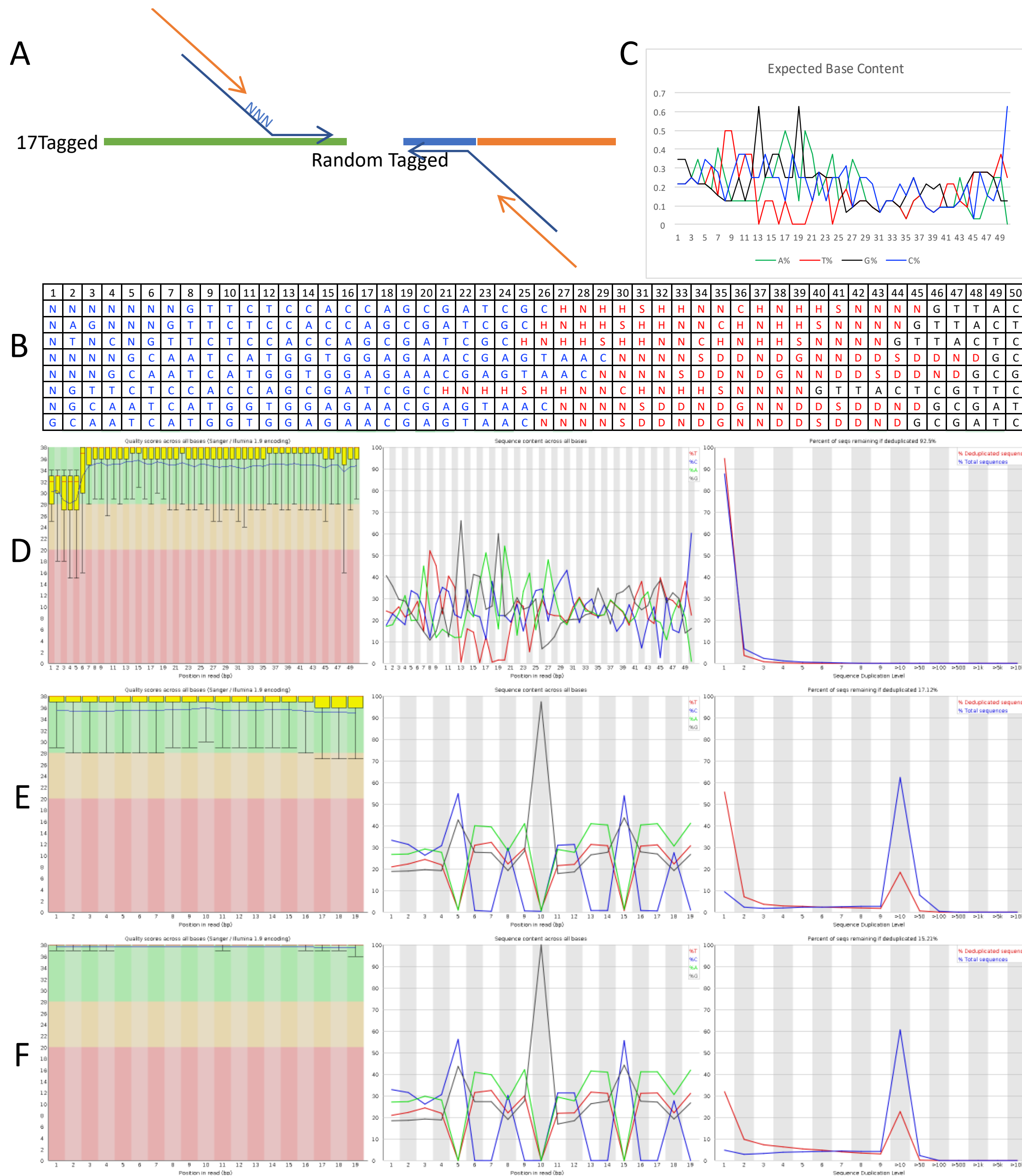


Figure S7. Verification of library size by tag sequencing. (A) PCR amplification scheme. First set of 8 primer pairs, in blue, adds varying numbers of N to the amplicon. Second set of 1 primer pair, in orange, attaches the appropriate Illumina adapters. The amplicons produced were between 130 to 140 bp. (B) The expected sequencing reads from the 8 amplicons. Primer originated sequences are in blue, tag originated sequences are in red, remaining amplicon originated sequences are in black. (C) The expected base distribution of the amplicon pool. All 4 bases are represented in every one of the first 12 read positions. (D-F) Quality, base distribution, and read duplication reports. (D) Raw reads, 1.66×10^8 reads. (E) After extracting 19bp tags from raw reads and filtering for reads that fit the expected tag format NNNNSDDNDGNNDDSDDND, 1.45×10^8 reads. (F) Stringent quality filter, Q>28 at every base within tag, 8.63×10^7 reads passed. There are 1.24×10^7 unique sequences.

Table S8. The domestication of T7 bacteriophage.

We have used a 14-fragment assembly scheme to construct T7Syn. To ensure the accuracy of the fragments, the fragments were cloned into vectors and confirmed by sequencing before being used in the assembly. Chemical gene synthesis was done by General Biosystems (Morrisville, NC, USA), and Fragment 8-14 were delivered in pUC57 completed and verified. Fragment 1-7 were found to be toxic in *E. coli* and were either not delivered or delivered in smaller pieces. We cloned the toxic fragments into pRS425 via yeast homologous recombination (HR) and propagated the plasmid in yeast. Fragment 2 did not contain any *Bsa*I or *Esp*3I site. Fragment 7 was delivered with a single base deletion, which we fixed by yeast HR.

Fragment	Size	GG Junction	Method	Pieces	Toxic
1	2863	CGAA	Pieces > Yeast HR	2	√
2	2857	CGCT	PCR > Yeast HR	1	√
3	2910	CGGT	Pieces > Yeast HR	3	√
4	2938	ATGG	Pieces > Yeast HR	3	√
5	2899	GACT	Pieces > Yeast HR	4	√
6	2942	ACAG	Pieces > Yeast HR	3	√
7	2850	GCTG	Fix Mut > Yeast HR	1	√
8	2926	GCAC	Gene Synthesis	1	X
9	2921	TCCG	Gene Synthesis	1	X
10	2926	CTGG	Gene Synthesis	1	X
11	2805	TGCT	Gene Synthesis	1	X
12	2607	GGAC	Gene Synthesis	1	X
13	2621	GTTG	Gene Synthesis	1	X
14	2885	-	Gene Synthesis	1	X

The following primers were used to amplify the GG-compatible fragments from the 14 plasmids:

F primer for PCR of T7 insert in pRS425	GGCGAATTGGGTACCGG
R primer for PCR of T7 insert in pRS425	CAAAAGCTGGAGCTCCACC
F primer for PCR of T7 insert in pUC57	GTAACGCCAGGGTTTTCCC
R primer for PCR of T7 insert in pUC57	CAAGCTTGCATGCAGGC

Table S9. The domestication of K11 Gp11, Gp12, Gp17. The following primers were used to generate *Bsa*I- and *Esp*3I-free fragments of the genes. The fragments were assembled into pUC vector through Golden Gate assembly, into two plasmids, and subsequently used as templates for the construction of T7-K11 hybrid phages. The plasmid pAmp-K11Gp1112NoBs carries the domesticated K11Gp11 and K11Gp12, while the plasmid pAmp-K11Gp17NoBs carries the domesticated K11Gp17.

K11Gp1112NoBs F1 For	CTGGTCTCACGTCGCTGAAAGGAGGAACTATATGAACATGCAAGATGCTTACTTTGGGTC
K11Gp1112NoBs F1 Rev	CAGGTCTCTCCGTTTCTGTGGACTTGTCGTACACCCAAC
K11Gp1112NoBs F2 For	CTGGTCTCAACGGATACCTTCTCTGGGGC
K11Gp1112NoBs F2 Rev	CAGGTCTCTCCTCCATTTATTAATGTTAACGACCGATCAGGCCCTGCACGTATGCGTCG
K11Gp1112NoBs F3 For	CTGGTCTCAGAGGTATTATTATGGCTCTCGTATCACAATCAATCAAG
K11Gp1112NoBs F3 Rev	CAGGTCTCTGGACTTGATGAACACCATAGGTGGTCGCTTCTGCAGTCCCTCAGTCTCGGAGGACC
K11Gp1112NoBs F4 For	CTGGTCTCAGTCCCTTGGTCCTAGGGGCTACTTGGGGGAAGATCCGTACATTCACCTCATCAACCG
K11Gp1112NoBs F4 Rev	CAGGTCTCTAGGTGACGCATAGTAGATGTTCCTGCC
K11Gp1112NoBs F5 For	CTGGTCTCAACCTCGAAGCTCCTTTACGTCC
K11Gp1112NoBs F5 Rev	CAGGTCTCTCGTTTCGATGTCGTACGCAGTCTCTG
K11Gp1112NoBs F6 For	CTGGTCTCAAACGAACCAGACGGTAGTGAACGTCAAG
K11Gp1112NoBs F6 Rev	CAGGTCTCTACCGACAGGGAGAATATTTAATTAAATGCCGTTGGCTCGTCTG
K11Gp17NoBs F1 For	CTGGTCTCACGTCTCTCTTAGATTTACTTTAAGGAGGTCAAATGGACCAAGACATTAAAACAGTCATTCAG
K11Gp17NoBs F1 Rev	CAGGTCTCTACACCCAGTTGATTCCCTTGAGGCTCGTGGTGTCATCTGCAAAGTTCTC
K11Gp17NoBs F2 For	CTGGTCTCAGTGTATAACAATGGGTCGGCCAATGGTGGCGAAACGTCCATCCTGATTACCCGCGAGG
K11Gp17NoBs F2 Rev	CAGGTCTCTGCGACGTTTCAAAGTGGCATTTGGTTGCCG
K11Gp17NoBs F3 For	CTGGTCTCATCGCTTGTAAACCTGTACATTCCGGGCAC
K11Gp17NoBs F3 Rev	CAGGTCTCTACCGTACACGTCTTTCCTTGTGATTTACCAATTATAAAACAAATGATGCTAATCTCGAGATGTTCACAAC

Table S10. List of PCR primers used to generate the fragments for T7Syn variant constructions.

#	Description	Primer Name	Sequence
1	T7Syn Fragment 1	T7 L1Es For	TCTCACAGTGTACGGACCTAAAGTTCC
		T7 L1Es Rev	GTTTCGTCTCTAGAGGTATCTTAGCTCGGCGCTC
2	T7Syn Fragment 2	T7 L2Es For	TCAACGTCTCACTCTTATTGGTAACACAGTTCCTTTGGCAC
		T7 L2Es Rev	GTTTCGTCTCTCGCTCTTTACCAGCACCAACAATC
3	T7Syn Fragment 3	T7 L3Es For	TCAACGTCTCAAGCGCGGTAAGGAACTCAAGAAG
		T7 L3Es Rev	GTTTCGTCTCTCAGCAGCTTCTGGGCGAAG
4	T7Syn Fragment 4	T7 L4Es For	TCAACGTCTCAGCTGGTGCAGTGGTTTTCAAAGTG
		T7 L4Es Rev	GTTTCGTCTCTTCTGTTGGTAAGTCCTCATAAGGGACAC
5	T7Syn Fragment 5	T7 L5Es For	TCAACGTCTCACAGAGAGAGGTGCCGTGGTG
		T7 L5Es Rev	AGGGACACAGAGAGACACTCAAGG
6	17Bs Left	T7 L5Es For	TCAACGTCTCACAGAGAGAGGTGCCGTGGTG
		T7 gp17 5' Rev	GTTTCGTCTCTGCCTCATTACGGAACTGTAAGGCTTC
7	17Bs Mid	T7 gp17 Bs-adapt For	AAGACGTCTCAAGGCTGAGACCTTCAGAAACCAAGCGGAGGGC
		T7 gp17 Bs-adapt Rev	GAATCGTCTCTCCGTTGAGACCGGACTACACGTCTTTCCTTGTGATTTACC
8	17Bs Right	T7 gp17 3' For	TCAACGTCTCAACGGATGGACTCTCAAGGAGG
		T7 L5Es Rev	AGGGACACAGAGAGACACTCAAGG
9	1112Bs Left	T7 L4Es For	TCAACGTCTCAGCTGGTGCAGTGGTTTTCAAAGTG
		T7 gp11 5' Rev	GAATCGTCTCTCCTCAAGACCCGTTTAGAGGC
10	1112Bs Mid	T7 gp11 Bs-adapt For	TCAACGTCTCAGAGGAGAGACCGGTTTTTTGCTGAAAGGAGGAACTATATG
		T7 gp12 Bs-adapt v2 Rev	GAATCGTCTCTAGCCAGAGACCACCACAGGGAGAATATTTAATTAAATACCG
11	1112Bs Right	T7 gp12 3' v2 For	TCAACGTCTCAGGCTCGAAATTAATACGACTCACTATAGGGAGAAC
		T7 L4Es Rev	GTTTCGTCTCTTCTGTTGGTAAGTCCTCATAAGGGACAC
12	17mut	T7 gp17 Bs-mut For	CAAAGGTCTCTAGGCTGAGACTTTCAGAAACCAAGC
		T7 gp17 Bs-mut Rev	GAATGGTCTCTCCGTGGACTACACGTCTTTCCTTG
13	1112mut	T7 gp11 mut For	CAAAGGTCTCAGAGGGGTTTTTTGCTGAAAGGAGGAACTATATG
		T7 gp12 mut v2 Rev	GAATGGTCTCGAGCCACCACAGGGAGAATATTTAATTAAATAC
14	17TagBs	T7 gp17 Bs-adapt For	AAGACGTCTCAAGGCTGAGACCTTCAGAAACCAAGCGGAGGGC
		T7 gp17 TAG-Bs-adapt Rev	GAATCGTCTCTCCGTCGAGACCGGATCCAGCATGCGGACTACACGTCTTTCCTTGTGATTTACC
15	17Tag2Bs	T7 gp17 Bs-adapt For	AAGACGTCTCAAGGCTGAGACCTTCAGAAACCAAGCGGAGGGC

		T7 gp17 TAGv3-Bs-adapt Rev	GAATCGTCTCTCCGTGGACTACACGTCTTTCCTTGTGATTTACCAATTACTCGTTCTCCACCAGGAGACCCGATGGTTGGAACAGGATCCGTTACTCGTTCTCCACCATGATTGCATTAG
16	17Tagged	T7 gp17 Bs-mut For	CAAAGGTCTCTAGGCTGAGACTTTCAGAAACCAAGC
		T7 gp17 Bs-TAGv3 Rev	GAATGGTCTCTACCAGCGATCGCHNHHSHHNNCHNHHSNNNNGTTACTCGTTCTCCACCATGATTGCATTAG
17	T7K11 Fragment 3	T7 L3Es For	TCAACGTCTCAAGCGCGGTAAGGAACTCAAGAAG
		T7_L3aEs Rev	GTTTCGTCTCTCCTCAAGACCCGTTTAGAGGCC
18	K11Gp1112	K11Gp11_M1 Es For	TCAACGTCTCAGAGGGGTTTTTTGCTGAAAGGAGGAACTATATGAACATGC
		K11Gp12_End Es Rev	GTTCGTCTCTAGCCACCACAGGGAGAATATTTAATTAAATGCCGTTGG
19	K11Gp1112Bs	K11Gp11_M1Bs Ad Es For	TCAACGTCTCAGAGGGGAGACCTTTTTTGCTGAAAGGAGGAACTATATGAACATGC
		K11Gp12_EndBs Ad Es Rev	GTTCGTCTCTAGCCAGAGACCACAGGGAGAATATTTAATTAAATGCCGTTGG
20	T7K11 Fragment 4	T7_1316Es For	TCAACGTCTCAGGCTCGAAATTAATACGACTCACTATAGGGAGAACAATACG
		T7_1316Es Rev	GTTTCGTCTCTCAGGGAGAAGATTATTTCGCCTCTCC
21	K11Gp17	K11Gp17_M1 Es For	TCAACGTCTCACCTGTAGTCTCTTAGATTTACTTTAAGGAGGTCAAATGGAC
		K11Gp17_End Es Rev	GTTCGTCTCTCCGTGGACTACACGTCTTTCCTTGTGATTTACCAATTATAAAAC
22	K11Gp17Bs	K11Gp17_M1Bs Ad Es For	TCAACGTCTCACCTGTGAGACCTCTCTTAGATTTACTTTAAGGAGGTCAAATGGAC
		K11Gp17_EndBs Ad Es Rev	GTTCGTCTCTCCGTGGAGACCTACACGTCTTTCCTTGTGATTTACCAATTATAAAAC
23	T7K11 Fragment 5	T7_17.5EndEs For	TCAACGTCTCAACGGATGGACTCTCAAGGAGG
		T7 L5Es Rev	AGGGACACAGAGAGACACTCAAGG
24	TK17	K11Gp17_T169 Bs For	TCAAGGTCTCAAGGCTAAGGACATTCAGGATTACATCGAGAAC
		K11Gp17_End Bs Rev	GTTGGTCTCTCCGTGGACTACACGTCTTTCCTTGTGATTTACCAATTATAAAAC
25	K11Gp17mut	T7gp17full mut For	CAAGGTCTCACCTGTAGTCTCTTAGATTTACTTTAAGGAGGTCAAATG
		K11Gp17_End Bs Rev	GTTGGTCTCTCCGTGGACTACACGTCTTTCCTTGTGATTTACCAATTATAAAAC

Table S11. List of fragments that were used to assemble each T7Syn variant. Fragment numbers and identities were listed in Table S10.

#	Name	Parent	Modification	Constructed From Fragment
P1	T7Syn	N.A.	N.A.	N.A.
P2	T7Syn-17Bs	P1	BsaI addition	1, 2, 3, 4, 6, 7, 8
P3	T7Syn-1112Bs	P1	BsaI addition	1, 2, 3, 9, 10, 11, 5
P4	T7Syn-111217Bs	P1	BsaI addition	1, 2, 3, 9, 10, 11, 6, 7, 8
P5	T7Syn-17TagBs	P1	BsaI addition, Mock tag insertion	1, 2, 3, 4, 6, 14, 8 1, 2, 3, 4, 6, 15, 8 (Better)
P6	T7Syn-17m5	P2	Mutagenized T7Gp17	
P7	T7Syn-1112Bs17m5	P6	BsaI addition	1, 2, 3, 9, 10, 11, 5 (P6 template)
P8	T7Syn-5m7	P2	Mutagenized T7Gp17m5	P2, 12 (P6 template)
P9	T7Syn-12m1	P7	Mutagenized T7Gp11-12	P7, 13
P10	T7Syn-K111217	P1	K11Gp11,12,17	1, 2, 17, 18, 20, 21, 23
P11	T7Syn-TK17	P4		P4, 13, 24
P12	T7Syn-K1112BsK17	P1	BsaI addition	1, 2, 17, 19, 20, 21, 23
P13	T7Syn-K1112K17Bs	P1	BsaI addition	1, 2, 17, 18, 20, 22, 23
P14	T7Syn-KmL2	P13	Mutagenized K11Gp17	P13, 25