

1 **Title: Engineering Modular Viral Scaffolds for Targeted Bacterial Population Editing**

2

3 **Authors:** Hiroki Ando^{1,2}, Sebastien Lemire^{1,2}, Diana P. Pires^{1,2,3}, Timothy K. Lu^{1,2*}

4

5 **Affiliations:**

6 ¹Department of Electrical Engineering & Computer Science and Department of Biological

7 Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA

8 02139, USA.

9 ²MIT Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge MA 02139,

10 USA.

11 ³Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710-057, Braga,

12 Portugal.

13 *Correspondence: timlu@mit.edu

14

15 **SUMMARY**

16 Bacteria are central to human health and disease, but the tools available for modulating and

17 editing bacterial communities are limited. New technologies for tuning microbial populations

18 would facilitate the targeted manipulation of the human microbiome and treatment of bacterial

19 infections. For example, antibiotics are often broad spectrum in nature and cannot be used to

20 accurately manipulate bacterial communities. Bacteriophages can provide highly specific

21 targeting of bacteria, but relying solely on natural phage isolation strategies to assemble well-

22 defined and uniform phage cocktails that are amenable to engineering can be a time-consuming

23 and labor-intensive process. Here, we present a synthetic-biology strategy to modulate phage

24 host ranges by manipulating phage genomes in *Saccharomyces cerevisiae*. We used this
25 technology to swap multiple modular phage tail components and demonstrated that *Escherichia*
26 *coli* phage scaffolds can be redirected to target pathogenic *Yersinia* and *Klebsiella* bacteria, and
27 conversely, *Klebsiella* phage scaffolds can be redirected to target *E. coli*. The synthetic phages
28 achieved multiple orders-of-magnitude killing of their new target bacteria and were used to
29 selectively remove specific bacteria from multi-species bacterial communities. We envision that
30 this approach will accelerate the study of phage biology, facilitate the tuning of phage host
31 ranges, and enable new tools for microbiome engineering and the treatment of infectious diseases.

32

33 **INTRODUCTION**

34 Bacteriophages (phages) are natural biological nanomachines that have evolved to infect host
35 bacteria with exquisite specificity and efficacy. Phages constitute the most abundant type of
36 biological particles on earth (Hendrix, 2003) and reproduce at the expense of their host bacteria.
37 Thus, phages have been explored as a means of controlling pathogenic bacteria (d'Herelle, 1931),
38 but poor understanding of the molecular relationships between bacteria and their phages can lead
39 to highly variable treatment outcomes (Brussow, 2012). With the rise of drug-resistant bacterial
40 infections and the sharp decline in antibiotic discovery and development (Fischbach and Walsh,
41 2009), phage therapy is regaining attention after years of declining interest in the Western world
42 (Carlton, 1999). Furthermore, despite the important role that the microbiome plays in regulating
43 human health and disease (Grice and Segre, 2012), strategies for precisely manipulating complex
44 microbial communities are lacking. With their ability to kill or deliver DNA into specific
45 bacteria, phages constitute a promising technology for manipulating microbiota. However, the
46 limited host range of most naturally isolated phages is a major barrier to the development and

47 approval of commercially available phage-based products. Conventional strategies for
48 identifying phages with specific host ranges rely on screening samples from nature. Naturally
49 isolated phages are often very diverse in morphology, genomic content, and life cycles, which
50 poses a challenge for the engineering, manufacturing, and regulatory approval of phages as
51 biotechnologies. For example, phage cocktails have been used to address the limited host range
52 of any single phage (Sulakvelidze et al., 2001). However, the desire to increase coverage by
53 adding more members to a phage cocktail is counterbalanced with the challenge of producing
54 and testing well-defined multi-component mixtures for regulatory approval.

55 Creating phage-based therapeutics and diagnostics is also limited by the difficulty of
56 engineering phages. Lytic phage DNA does not reside for very long inside of bacteria; this
57 makes it difficult to modify phage genomes during the phage reproductive cycle. Phage genomes
58 are also often too large to be handled *in vitro*. Phage genome engineering is thus classically
59 carried out with allele replacement methods, whereby a piece of the phage genome is cloned into
60 an appropriate bacterial vector and remodeled using classical molecular biology, and the
61 bacterium containing the resulting construct is then infected with the phage, allowing the phage
62 to recombine with the plasmid to acquire the desired mutations. This process is inefficient
63 because many phages degrade resident DNA upon entry, and time-consuming due to the absence
64 of phage selectable markers to expedite screening of output viral populations. Furthermore, there
65 are very large stretches of phage DNA that encode products toxic to bacteria, thus preventing
66 their manipulation within bacterial hosts. Finally, all existing approaches are limited in the
67 number of mutations that can be introduced simultaneously. Multiple rounds of mutations are
68 therefore often required, making the process inefficient. Here, we demonstrate a high-throughput

69 phage-engineering platform that leverages the tools of synthetic biology to overcome these
70 challenges and use this platform to engineer model phages with tunable host ranges.

71

72 **RESULTS**

73 **Yeast platform for bacteriophage genome engineering.**

74 We used an efficient yeast-based platform (Jaschke et al., 2012; Lu et al., 2013) to create phages
75 with novel host ranges based on common viral scaffolds. Inspired by gap-repair cloning in yeast
76 (Ma et al., 1987) and the pioneering work of Gibson and co-workers (Gibson, 2012; Gibson et al.,
77 2008; Gibson et al., 2009), we captured phage genomes into *Saccharomyces cerevisiae*, thus
78 enabling facile genetic manipulation of modified genomes that can be subsequently re-activated
79 or “rebooted” into functional phages after transformation of genomic DNA into bacteria (Figure
80 1A). The workflow is split into two parts. In the first part, the entirety of the viral genome to be
81 assembled in yeast is amplified by PCR in such a way that each adjacent fragment has homology
82 over at least 30 bp. The first and last fragments of the phage genome are amplified with primers
83 that carry “arms” that have homology with a yeast artificial chromosome (YAC) fragment, which
84 may be obtained by PCR or any other suitable method. Upon transformation of all viral genome
85 fragments and the YAC into yeast, gap-repair will join each fragment to the adjacent one
86 templated by the homology regions found at the end of each fragment, yielding a full phage
87 genome cloned into a replicative yeast plasmid. Yeast transformants are then enzymatically
88 disrupted in order to extract the YAC-phage DNA, which is then used for transformation into
89 bacterial host cells that can support resumption of the viral life cycle. Plaques, if obtained, are
90 then picked, amplified, and sequenced to verify proper introduction of the desired mutations. If
91 no plaques are obtained, it is still possible to amplify parts of the YAC-phage genome from the

92 yeast clones in order to verify proper DNA assembly, to eliminate the possibility of unwanted
93 mutations, and to help determine potential reasons for the failure of the synthetic phage genome
94 to produce viable offspring.

95 We targeted phages from the T7-family because their life cycle is largely host
96 independent (Qimron et al., 2010) and there is a relatively large number of family members for
97 which genomic sequences are publicly available. These include T7 (coliphage, 39,937 bp), T3
98 (coliphage, 38,208 bp), K1E (K1-capsule-specific coliphage, 45,251 bp), K1F (K1-capsule-
99 specific coliphage, 39,704 bp), K1-5 (K1- or K5-capsule-specific coliphage, 44,385 bp), SP6
100 (*Salmonella* phage, 43,769 bp), LUZ19 (*Pseudomonas* phage, 43,548 bp), gh-1 (*Pseudomonas*
101 phage, 37,359 bp), K11 (*Klebsiella* phage, 41,181 bp), and others. We first sought to confirm
102 that purified phage DNA from various phages could be transformed into bacterial hosts to
103 generate functional phages. We used *E. coli* 10G® (10G) cells (Durfee et al., 2008) as a one-
104 time phage propagation host. Except for T7 and T3, all phages used in this study (K1E, K1F, K1-
105 5, SP6, LUZ19, gh-1, and K11) cannot infect 10G (Figure 1B). We extracted the genomes of a
106 diversity of phages from purified phage particles. Each genome was electroporated into 10G
107 directly. After incubation and chloroform treatment, supernatants were mixed with overnight
108 cultures of each natural host bacteria for each phage in soft agar, poured onto agar plates, and
109 incubated, looking for plaque formation (Figure 1C and 1D). We found that all the phages tested
110 could be rebooted from purified DNA into functional phages through one-step propagation in
111 10G, even if their natural target species was not *E. coli* (Figure. 1D and Table S1). This result
112 indicates that we can use *E. coli* 10G cells as an initial host for rebooting purified genomic DNA
113 into phages that infect diverse bacteria.

114

115 **Rebooting bacteriophages from PCR products via the yeast platform.**

116 To determine whether phage genomes assembled in yeast remain viable, we first attempted to
117 capture and then reboot T7 (Figure 1E), T3, and LUZ19 phages. We used PCR to amplify the
118 YAC pRS415 and add arms homologous to the ends of the phage genomes. We co-transformed
119 the YAC amplicons with phage genomic DNA into yeast. Confirmed YAC-phage constructs
120 were extracted from yeast and transformed into *E. coli* 10G. These cells were then chloroform
121 treated and the resulting lysates were assessed for plaque-forming units (pfu) on the natural
122 bacterial hosts of the phages (see Figure 1E for capturing and rebooting T7). All 3 phage
123 genomes yielded yeast clones that could be rebooted to viable phages using this strategy.

124 Next, we captured and rebooted eight different phages that target *E. coli*, *Salmonella*,
125 *Pseudomonas*, and *Klebsiella* (T7, T3, K1E, K1F, K1-5, SP6, gh-1, and K11) by assembling
126 overlapping 3.8-12 kbp-long PCR products spanning the phage genomes with the linearized
127 YAC in yeast (Figure 2A upper panel illustrates this process with T7 as an example). All eight
128 phages were rebooted from PCR fragments via yeast platform and formed plaques on their
129 natural host bacteria (data not shown). Thus, this approach enables the efficient assembly and
130 instantiation of functional recombinant phages, and allows us to potentially create any desired
131 phage genotype in one step from PCR products.

132

133 **Swapping tail fibers enables modulation of phage host range.**

134 To engineer phages with tunable host ranges, we first selected two model phages, T7 and T3,
135 which are obligate lytic phages originally isolated as a member of the seven “Type” phages that
136 grow on *E. coli* B (Demerec and Fano, 1945). T7 and T3 have linear genomes that share high
137 homology with each other, in which the primary host determinant is the product of gene *I7*

138 (gp17), the tail fiber (Dunn and Studier, 1983; Pajunen et al., 2002). Alterations in the gp17
139 sequence have been linked to the recognition of different host receptors and shifting host ranges
140 (Molineux, 2006). Thus, we hypothesized that exchanging gene *I7* or fragments of gene *I7*
141 between T7, T3, and their relatives could be used to tune their host specificities (Figure 2B). This
142 is supported by previous data on naturally occurring hybrids between T7 and T3 whose host
143 range was mostly dictated by which gene *I7* they harbored (Lin et al., 2012).

144 We first examined the host range of T7 and T3 phage on a range of hosts to determine
145 bacterial panels that were differentially targeted by the two phages. T3 is described as incapable
146 of targeting many common laboratory *E. coli* K-12 strains (Molineux, 2006), so we performed
147 plaque formation assays with four K-12 strains and a B strain (BL21) as a control. As shown in
148 Figure 2C, T7 plaqued efficiently on all strains, while T3 did not produce plaques on BW25113
149 and MG1655 at a detectable frequency (Efficiency Of Plating (EOP) below 10^{-9}). T3 exhibited
150 ~4 orders-of-magnitude reductions in adsorption efficiency on BW25113 and MG1655
151 compared with the permissive BL21 strain (Figure 2D). These results indicate that we can
152 differentiate between T7 and T3 using BW25113 or MG1655, which are only susceptible to T7.

153 The gp17 tail fibers of T7 and T3 can be split in two domains. The N-terminal 149
154 residues are thought to be necessary for the tail fiber to bind to the rest of the capsid while the
155 remaining C-terminal region forms a kinked shaft and harbors the recognition domain for host
156 receptors at its tip (Steven et al., 1988). The N-terminal regions of T7 and T3 share 99% identity
157 at the protein level, while the C-termini exhibit 83% identity, with the last 104 amino acids of the
158 T3 protein showing only 62% of identity to the corresponding 99 amino acids of the T7 protein.
159 Therefore, we hypothesized that swapping the C-terminal domain between the two viruses would
160 result in exchanging the host ranges. We constructed synthetic phages, based on either the T7 or

161 T3 viral chassis, which carried engineered gene *I7* alleles composed of fragments from the other
162 phage. Specifically, we created six synthetic phages: T7 phage with the wild-type T7 tail fiber
163 (T7_{WT}), T7 phage with 410 amino acids of the C-terminal region of the T3 tail fiber (T7_{T3(C-gp17)}),
164 T7 phage with the entire T3 tail fiber (T7_{T3(gp17)}), T3 phage with the wild-type T3 tail fiber
165 (T3_{WT}), T3 phage with 405 amino acids of the C-terminal region of the T7 tail fiber (T3_{T7(C-gp17)}),
166 and T3 phage with the entire T7 tail fiber (T3_{T7(gp17)}). T7_{WT} and T7 phages are the same at the
167 genetic level; however, T7_{WT} phage was created by capturing the T7 genome in yeast and then
168 rebooting this phage genome in bacteria and served as a control for the faithfulness of the
169 reconstruction process, whereas T7 was obtained from ATCC. The same applies to T3_{WT} and T3.
170 Each phage was assembled in yeast via six PCR fragments and was rebooted via transformation
171 into *E. coli* 10G (example schematics in Figure 2A). No unexpected mutations were found in the
172 heterologous gp17 regions of the rebooted phages.

173 To examine the host specificities of our six engineered phages, we performed plaque
174 formation assays on a range of *E. coli*, *Klebsiella*, and *Yersinia pseudotuberculosis* (*Y. ptb*)
175 strains (Figure 3). T3_{T7(C-gp17)} and T3_{T7(gp17)} plaqued on *E. coli* BW25113 and *E. coli* MG1655 at
176 a similar EOP as T7 and T7_{WT}, while T3, T3_{WT}, T7_{T3(C-gp17)}, and T7_{T3(gp17)} had >10⁵-fold-reduced
177 EOPs on these strains. In addition, T3, T7_{T3(C-gp17)}, and T7_{T3(gp17)} plaqued on *E. coli* ECOR16
178 while T7, T3_{T7(C-gp17)}, and T3_{T7(gp17)} did not. In addition, we also synthesized a codon-optimized
179 version of the tail fiber of the T7-like enterobacteriophage 13a and created synthetic T7 phages
180 containing the entire 13a tail fiber (T7_{13a(gp17)}) or the C-terminal region of the 13a tail fiber
181 (T7_{13a(C-gp17)}) (Figure 2E, Table S2). Although T7 and T7_{WT} did not plaque on *E. coli* ECOR16,
182 both T7_{13a(gp17)} and T7_{13a(C-gp17)} were able to do so efficiently (Figure 3). These results
183 demonstrate that the C-terminal region of gp17 is a major host range determinant and that new

184 host ranges can be conferred onto T7-like phage scaffolds by engineering tail fibers. Interestingly,
185 T7_{13a(C-gp17)} efficiently infected *E. coli* BW25113 and MG1655, similar to T7 and T7_{WT}, but
186 T7_{13a(gp17)} did not, which suggests that the N-terminus of the phage 13a tail fiber can also alter
187 infectivity of the virus although the mechanism is still to be investigated. A second example of
188 this phenomenon can be found between T7_{T3(C-gp17)} (lane 3, Figure 3) and T7_{T3(gp17)} (lane 4,
189 Figure 3). While the former phage infected *Y. ptb* YPIII (albeit with a low EOP), the latter phage
190 as well as wild-type T3 did not.

191

192 **Coliphage T3 with a *Yersinia* phage tail fiber infects both *E. coli* and *Y. pseudotuberculosis*.**

193 We further demonstrated that gene swapping between phages could overcome species barriers by
194 designing synthetic phage based on T7 or T3 scaffolds that can infect bacteria other than *E. coli*.
195 We started with coliphage T3 and *Yersinia* phage R (38,284 bp), since their gp17's share 99.5%
196 identity at the protein level and differ by only 3 nucleotides in gene 17. We hypothesized that
197 these differences could be responsible for their divergent host ranges. Indeed, we were unable to
198 detect productive T3 infection of *Y. ptb* strains IP2666 and YPIII, which are known hosts for
199 phage R (Rashid et al., 2012). Because we did not have access to phage R, we introduced the
200 desired mutations in T3 gene 17 by PCR so that it would encode the same tail fiber as phage R
201 (Figure 4A). The mutated gene 17 was then swapped into the genome of T3. Synthetic T3 phage
202 with the R tail fiber (T3_{R(gp17)}) was able to infect *Y. ptb* IP2666 and YPIII. Interestingly, T3_{R(gp17)}
203 maintained the capacity to infect *E. coli* BL21 (Figure 4B), demonstrating that the introduced
204 mutations conferred a host range expansion and not just a host range shift. In addition to
205 plaquing assays, we further characterized the ability of T3_{WT} versus T3_{R(gp17)} to kill *Y. ptb*

206 IP2666 over time. After 1.5 h of treatment, T3_{R(gp17)} killed 99.999% of IP2666 while T3 had no
207 effect on the bacteria (Figure 4C).

208

209 **Redirection of host range between coliphage and *Klebsiella* phage by swapping whole tail**
210 **components.**

211 We further explored our ability to overcome species barriers by engineering phages with lower
212 similarity with one another. K11 is a *Klebsiella* phage that belongs to the T7-like family (Dietz et
213 al., 1990). K11 has a similarly sized genome to T7 and shares gene synteny with T7. The average
214 homology between K11 and T7 is 59% among the genes that have homologs between the two
215 viruses. For comparison, T7 and T3 share 72% identity at the genomic level between
216 homologous genes. While T7 is a coliphage and does not infect *Klebsiella*, K11 infects
217 *Klebsiella*, such as *Klebsiella* sp. 390, but not *E. coli* (Figure 3) (Bessler et al., 1973). Their
218 respective host range determinants, gp17, are very different and do not share any homology
219 outside of the N-terminal 150 amino acids, which is only 47% identical between the two proteins.
220 Specifically, the T7 gp17 encodes tail fibers while K11 gp17, which is 322 amino acids longer
221 than the T7 gp17, directs the synthesis of a tail spike, an enzymatic host range determinant that
222 actively breaks down the capsule of *Klebsiella* to allow K11 phage to gain access to unknown
223 secondary receptors located beneath the capsule (Bessler et al., 1973).

224 To create a T7 phage with a K11 tail fiber and a K11 phage with a T7 tail fiber, we first
225 swapped the entire gene 17, but this yielded no viable phages. We then tried to construct
226 composite tail fibers composed of gene 17 fragments from both phages hybridized at various
227 points along the length of the gene, but this was also unsuccessful at generating functional
228 synthetic phages. We speculated that one possible reason for these failures could be that the K11

229 genome cannot create productive phages within *E. coli* 10G. However, the natural K11 genome
230 produced functional virions when it was electroporated into 10G cells, which were subsequently
231 lysed via chloroform and plated onto a suitable host (Figure 1D and Table S1).

232 Alternatively, the gene *I7* product from K11 may require a function or factor that is
233 absent from T7. Cuervo *et al.* reported that the tail of T7 phage, which assembles independently
234 of the head, is assembled from a dodecamer of gp11 (the adaptor) and an hexamer of gp12 (the
235 nozzle) (Figure 5A) onto which 6 trimers of gp17 attach (Cuervo et al., 2013). T7's six tail fibers
236 attach at the interface between the adaptor and nozzle, thus making contacts with both proteins.
237 The adaptor ring is responsible for the attachment of the preformed tail to the prohead via
238 interactions with the portal composed of 12 subunits of gp8. The homology between the gp8 of
239 T7 and K11 (80% identity at the amino acid level) is much higher than the homology between
240 the gp11 and gp12 proteins of T7 and K11 (60 and 61% identity, respectively), which led us to
241 suspect that replacing all three tail genes of T7 with their K11 equivalents (gp11, gp12, and
242 gp17) could be necessary to create functional virions (Figure 5B). Indeed, both T7 with K11 tail
243 components (T7_{K11(gp11-12-17)}) and K11 with T7 tail components (K11_{T7(gp11-12-17)}) were
244 successfully engineered into functional phages that exhibited tail-dependent host ranges.
245 Specifically, T7_{K11(gp11-12-17)} infected *Klebsiella* sp. 390 and did not target *E. coli*, while
246 K11_{T7(gp11-12-17)} infected *E. coli*, but did not plaque on *Klebsiella* (Figure 5C and Figure 3). The
247 yeast-based phage engineering platform enabled the facile construction of these phages via one-
248 step genome construction even though gene *I1* and *I2* are physically separated from gene *I7*, a
249 feat that no other phage engineering method can currently achieve. To further validate the ability
250 of synthetic T7_{K11(gp11-12-17)} to target *Klebsiella*, we performed a time-course experiment that

251 showed that T7_{K11(gp11-12-17)} killed 99.955% of *Klebsiella* sp. 390 after 1 hour of treatment (Figure
252 5D), but was about 100-fold less effective than K11_{WT} (Figure 5C and Figure S1).

253

254 **Synthetic phage cocktails efficiently remove target bacteria from mixed bacterial**
255 **populations.**

256 Our results demonstrate that common phage scaffolds can be retargeted against new bacteria
257 hosts by engineering single or multiple tail components. This capability enables the construction
258 of defined phage cocktails that only differ in their host-range determinants and can be used to
259 edit the composition of microbial consortia and/or treat bacterial infections. To demonstrate
260 microbial population editing, we used our synthetic phages to specifically remove targeted host
261 bacteria from a mixed population containing the probiotic *E. coli* strain Nissle 1917, *Klebsiella*
262 sp. 390, and *Y. ptb* IP2666. The amount of each bacterial member in this mixed population was
263 quantified using their differing sensitivities to chemical antimicrobials (Figure S2). *Klebsiella* sp.
264 390 is naturally resistant to 25 µg/ml carbenicillin while *Y. ptb* IP2666 is naturally resistant to
265 1 µg/ml triclosan (Figure S2A). *E. coli* Nissle 1917 however is sensitive to both. These
266 concentrations of antimicrobials completely killed susceptible strains but did not visibly affect
267 the growth of resistant strains (Figure S2B). After 1 h treatment of the multi-species population
268 with T7_{K11(gp11-12-17)} or T3_{R(gp17)}, >99.9% or >98% of their target bacteria, *Klebsiella* sp. 390 or *Y.*
269 *ptb* IP2666, respectively, were removed without affecting the remaining bacterial species (Figure
270 6 and Table S3). Furthermore, a phage cocktail consisting of two phages with the same chassis
271 but different host ranges, T7_{WT} and T7_{K11(gp11-12-17)}, resulted in >99% killing of *Klebsiella* sp. 390
272 and >99.9% killing of *Y. ptb* IP2666 after 1 hour, thus enriching for probiotic *E. coli* Nissle 1917
273 (Figure 6 and Table S3). These results demonstrate the high efficiency and selectivity of our

274 engineered phages in microbial consortia, and the potential for combining well-defined phage
275 cocktails with probiotics.

276

277 **DISCUSSION**

278 In this study, we utilized an efficient yet simple yeast-based platform for phage engineering to
279 modulate phage host ranges for several members of the T7 phage family. Traditional phage
280 engineering strategies, such as *in vitro* manipulation, allele-exchange within bacterial hosts, and
281 phage crossing via co-infection of bacteria (Beier et al., 1977; Garcia et al., 2003; Lin et al.,
282 2011) have been used to modulate phage host range (Tetart et al., 1998; Trojet et al., 2011;
283 Yoichi et al., 2005), but these strategies are inefficient and unable to achieve multiple genetic
284 modifications in a single step. Screening for a desired mutation after classical crossing or
285 recombination experiments can require PCR, restriction digestion, or plaque hybridization on
286 hundreds of individual plaques, which are all costly and time-consuming methods. Conversely,
287 our strategy rarely requires the screening of more than a few yeast clones. Specifically, we found
288 that at >25% of our yeast clones contained properly assembled phage genomes (composed of up
289 to 11 DNA fragments) that could be used to generate functional plaques after transformation into
290 bacteria. Previously, a scheme for engineering phage T4 through electroporation of PCR
291 products was devised (Pouillot et al., 2010), but it is based on a particular feature of the genetic
292 regulation of T4 and cannot easily be applied to other phage families. Recently, the 5.4 kb
293 filamentous coliphage ϕ X174 was assembled in yeast in order to stably store the genome and aid
294 in phage refactoring (Jaschke et al., 2012). In this approach, the majority of the genome
295 assembly was performed *in vitro* and the YAC cloning was mostly used to store the resulting
296 genome, whereas the majority of the genome engineering in our approach stems from the actual

297 gap-repair cloning process in yeast. In addition, the phages we have cloned using this method are
298 in the 38-45 kbp range and we have indications that it can also be used for much larger phage
299 genomes (e.g., up to 100 kbp, data not shown).

300 More recently, type I-E CRISPR-Cas counter-selection has been shown to be a useful
301 tool to edit the genome of phage T7 (Kiro et al., 2014). The *S. pyogenes* CRISPR-Cas9 system
302 was also shown to be functional on the heavily modified genomes of a few members of the T-
303 even family, suggesting that it could be used to modify their genomes, although the authors did
304 not report any such attempts (Yaung et al., 2014). We have successfully used the *Streptococcus*
305 *pyogenes* CRISPR-Cas9 system to select for mutants in phage T7 but with variable efficiencies
306 (data not shown). Thus, CRISPR-Cas systems can help to overcome some challenges associated
307 with engineering phage genomes in bacterial hosts for therapeutic applications (Bikard et al.,
308 2014; Citorik et al., 2014; Goldberg et al., 2014; Kiro et al., 2014). In contrast, leveraging yeast
309 to modify phages enables the decoupling of phage genome engineering from phage fitness and
310 viability, obviates the need for selective or screenable markers in phage genomes, reduces the
311 risks of phage contamination during the engineering process, and permits facile one-step genetic
312 manipulations. For example, the ability to simultaneously engineer multiple loci in a phage
313 genome was crucial for constructing K11_{T7(gp11-12-17)} and T7_{K11(gp11-12-17)}. However, a challenge of
314 yeast-based phage engineering (which is shared by *in vitro* strategies) is the need to reboot
315 modified phage genomes into functional phages. Here, we used high-efficiency DNA
316 transformation to deliver phage DNA into bacterial hosts, but future work may be facilitated by
317 *in vitro* transcription-translation systems capable of supporting functional phage synthesis (Shin
318 et al., 2012).

319 In summary, we demonstrated that synthetic phages based on common viral scaffolds can
320 be designed to target a range of different bacterial hosts. Furthermore, we showed that a cocktail
321 containing multiple engineered phages could effectively remove select bacterial targets in mixed
322 microbial populations. We anticipate that the systematic and high-throughput engineering of
323 viral genomes will enable new applications and enhanced understanding of bacterial viruses. For
324 example, the engineering of common viral scaffolds could help simplify the discovery and
325 manufacturing of novel bacteriophages and reduce the regulatory burden required for the use of
326 phage cocktails as human therapeutics. Furthermore, abundant phage sequences contained within
327 metagenomic databases could be synthesized and bootstrapped into functional phage particles for study
328 and use. Finally, the systematic deconstruction and manipulation of these viral nanomachines
329 will enable a greater understanding of phage biology and may provide insights that are useful for
330 bioinspired nanotechnologies.

331

332 **EXPERIMENTAL PROCEDURES**

333 **Strains, vector, and primers.** Phages T7 (ATCC BAA-1025-B2, NC_001604) and T3 (ATCC
334 110303-B3, AJ318471) were laboratory stocks. Phages K1E (NC_007637), K1F (NC_007456),
335 K1-5 (NC_008152), SP6 (NC_004831), and K11 (EU734173) were kindly provided by Ian
336 Molineux (The University of Texas at Austin). Phage LUZ19 (NC_010326) was kindly provided
337 by Rob Lavigne (KU Leuven). Phage gh-1 (ATCC 12633-B1, NC_004665) was obtained from
338 ATCC. Synthetic phages are listed in Table S4. *Saccharomyces cerevisiae* BY4741 (*MATa*
339 *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was obtained from Thermo Scientific. *Escherichia coli* strains
340 BL21 [B, F⁻ *ompT hsdS_B* (*r_B⁻ m_B⁻*) *gal dcm*], DH5α [K-12, F⁻ λ⁻ Φ80d *lacZΔM15 Δ(lacZYA-*
341 *argF)*U169 *deoR recA1 endA1 hsdR17* (*r_K⁻ m_K⁺*) *phoA supE44 thi-1 gyrA96 relA1*], BW25113

342 [K-12, F⁻ λ⁻ Δ(*araD-araB*)567 Δ*lacZ*4787(::*rrnB*-3) *rph*-1 Δ(*rhaD-rhaB*)568 *hsdR*514], MG1655
343 (K-12, F⁻ λ⁻ *ilvG*⁻ *rfb*-50 *rph*-1), and Nissle 1917 were obtained from laboratory stocks. *E. cloni*
344 10G [K-12, F⁻ λ⁻ Δ(*ara leu*)7697 *araD*139 Δ*lacX*74 *galU galK* Φ80d *lacZ*ΔM15 *recA1 endA1*
345 *nupG rpsL* (Str^R) Δ(*mrr-hsdRMS-mcrBC*) *tonA*] was obtained from Lucigen. 10G is a DH10B
346 derivative and is suitable for maintaining large DNA constructs (Durfee et al., 2008). Bacterial
347 strains IJ284 *Klebsiella* sp. 390 (O3:K11), IJ1668 K-12 hybrid; K1 capsule, and IJ612
348 *Salmonella typhimurium* LT2 were kindly provided by Ian Molineux. Virulence-plasmid-less
349 *Yersinia pseudotuberculosis* IP2666 and YPIII were kindly provided by Joan Mecsas (Tufts
350 University). *Pseudomonas aeruginosa* PAO1 was obtained from a laboratory stock. *E. coli*
351 libraries, such as the ECOR group and DECA set, were sourced from the Thomas S. Whittam
352 STEC Center (Michigan State University). *Pseudomonas putida* C1S (ATCC 23287) was
353 obtained from ATCC. The pRS415 yeast centromere vector with *LEU2* marker (ATCC 87520)
354 was obtained from a laboratory stock. Primers used in this study are listed in Table S5.

355

356 **Synthesis of codon-optimized 13a gene 17.** The gene was synthesized by Gen9. The sequence
357 is shown in Table S2.

358

359 **Culture conditions.** *S. cerevisiae* BY4741 was cultured in YPD [1% Bacto Yeast Extract (BD),
360 2% Bacto Peptone (BD), 2% dextrose (VWR)] at 30°C. *Y. pseudotuberculosis* strains and *P.*
361 *putida* C1S were cultured in LB (BD) at 30°C. All other strains were cultured in LB at 37°C.

362

363 **Preparation of linearized pRS415 amplicon.** We linearized the pRS415 by using PCR
364 amplification with specific primer sets (Table S5) and KAPA HiFi DNA Polymerase (Kapa

365 Biosystems). For capturing phage genomes, we added sequences to the pRS415 vector that were
366 homologous to the 5' and 3' termini of phages. To prevent the appearance of false-positive
367 colonies, we excised and purified the pRS415 amplicon from an agarose gel after electrophoresis
368 with QIAquick Gel Extraction Kit (Qiagen).

369

370 **Preparation of phage genomes.** Lysates were made by infecting 200 ml of logarithmically
371 growing cells with the appropriate phage at a MOI of 0.1-0.01 and incubating the cultures until
372 clearance. Cells were lysed and lysates were sterilized by adding 200 μ l chloroform (Sigma).
373 Lysates were centrifuged at 8,000 g for 5 min and then filtered through 0.22 μ m filters (VWR) to
374 remove cell debris. We added 216 μ l of buffer L1 [20 mg/ml RNase A (Sigma), 6 mg/ml DNase
375 I (NEB), 0.2 mg/ml BSA (NEB), 10 mM EDTA (Teknova), 100 mM Tris-HCl (VWR), 300 mM
376 NaCl (VWR), pH 7.5] and incubated at 37°C for 1 h with gentle shaking. Then we added 30 ml
377 of ice cold buffer L2 [30% polyethylene glycol (PEG) 6000 (Sigma), 3 M NaCl] and stored the
378 samples overnight in 4°C. Samples were centrifuged at 10,000 g for 30 min at 4°C. Phage pellets
379 were suspended in 9 ml buffer L3 (100 mM Tris-HCl, 100 mM NaCl, 25 mM EDTA, pH7.5).
380 Then, we added 9 ml buffer L4 [4% SDS (VWR)] and incubated the samples at 70°C for 20 min.
381 After cooling down on ice, 9 ml buffer L5 [2.55 M potassium acetate, pH4.8 (Teknova)] were
382 added, and the samples were centrifuged at 10,000 g for 30 min at 4°C. Phage genomes in the
383 supernatant were purified by using the Qiagen-tip 100 system (Qiagen) according to the
384 manufacturer's instructions.

385

386 **Preparation of PCR products for assembling phage genomes.** All PCR products were
387 prepared with specific primer sets (Table S5) and KAPA HiFi DNA Polymerase. Five to ten 3.8-

388 12.0 kbp PCR products including the YAC were used per reaction. Homology arms between the
389 YAC and the phage genomes were added to the first and last phage genome fragments in order to
390 decrease background recombination between the YAC and phage genomic DNA used as
391 templates for PCR, and to eliminate the need for the time-consuming step of gel extraction for
392 each PCR fragment.

393

394 **Preparation of yeast competent cells.** *S. cerevisiae* BY4741 was grown in 5 ml YPD at 30°C
395 for 24 h. Overnight cultures were added into 50 ml YPD, and incubated at 30°C for 4 h. Cells
396 were harvested by centrifugation at 3,000 g and washed with 25 ml water and then with 1 ml of
397 100 mM lithium acetate (LiAc) (Alfa Aesar), and suspended in 400 µl of 100 mM LiAc. Fifty
398 microliters were used for each transformation.

399

400 **Yeast transformation.** All DNA samples and a linearized pRS415 were collected in a tube (0.5
401 - 4.0 µg for each DNA sample and 100 ng linearized pRS415 in 50 µl water), and mixed with the
402 transformation mixture [50 µl yeast competent cell, 240 µl 50% PEG3350 (Sigma), 36 µl 1 M
403 LiAc, 25 µl 2 mg/ml salmon sperm DNA (Sigma)]. The mixture was incubated at 30°C for 30
404 min, then at 42°C for 20 min or at 42°C for 45 min, centrifuged at 8,000 g for 15 sec, and
405 suspended in 200 µl water. Transformants were selected on complete synthetic defined medium
406 without leucine (SD-Leu) [0.67% YNB+Nitrogen (Sunrise Science Products), 0.069% CSM-Leu
407 (Sunrise Science Products), 2% dextrose] agar plates at 30°C for 3 days.

408

409 **Extraction of captured phage genomes.** Individual yeast transformants were picked into SD-
410 Leu liquid medium and incubated at 30°C for 24 h. DNA was extracted from these cells using

411 the YeaStar Genomic DNA Kit (Zymo Research) or Yeast Genomic DNA Purification Kit
412 (Amresco) according to the manufacturer's instructions.

413

414 **Rebooting of phages.** The *E. coli* 10G strain was used as a host bacterium for the initial
415 propagation of phages. To reboot T7 and T3 phages, 3 μ l of extracted DNA were electroporated
416 into 20 - 25 μ l cells in a 2 mm gap electroporation cuvette (Molecular BioProducts) at 2,500 V,
417 25 μ F, 200 Ω using a Gene Pulser Xcell (Bio-Rad). Cells were mixed with 3 ml LB soft agar
418 (LB + 0.6% agar) warmed at 55°C, poured onto LB plate, and incubated for 4 h at 37°C. To
419 reboot other phages, after electroporation, cells were incubated at 37°C for 1-2 h in 1 ml LB
420 medium. Then, we added drops of chloroform to kill the cells and release phages. After
421 centrifugation at 12,000 g for 1 min, supernatants were mixed with 300 μ l overnight cultures of
422 host bacteria for the phages and 3 ml LB soft agar, poured onto LB plate, and incubated for 4 -
423 24 h at 30 or 37°C.

424

425 **One-time phage propagation assays.** We used *E. coli* 10G from Lucigen as a one-time phage
426 propagation host (Durfee et al., 2008). To validate the ability of the 10G strain as a one-time
427 phage propagation plant, we electroporated 10 – 200 ng of purified phage genomes into the
428 bacteria. After incubation for 1 - 2 h, which should be sufficient time for phages to have
429 completed a full growth cycle, we added chloroform to kill the cells and release phages that may
430 have failed to lyse cells. Then, supernatants were mixed with overnight cultures of each natural
431 host bacteria for each phage in soft agar, poured onto agar plates, incubated for 4 - 24 h at 30 to
432 37°C, and analyzed for plaque formation.

433

434 **Determination of Plaque Forming Units (PFUs).** We mixed serially diluted phages in 0.95%
435 saline, 300 μ l overnight culture of host bacteria, and 3 ml LB soft agar, and poured the mixture
436 onto LB plates. After 4 - 24 h incubation at 30 or 37°C, phage plaques were counted, and
437 PFU/ml values were calculated.

438
439 **Plaque formation assays.** We mixed 300 μ l bacterial overnight cultures and 3 ml LB soft agar,
440 and poured the mixtures onto LB plate. After 5 min at room temperature (RT), 2.5 μ l of 10-fold
441 serially diluted phages in 0.95% saline were spotted onto LB soft agar and incubated at 30 or
442 37°C.

443
444 **Adsorption assay.** We mixed 100 μ l of $\sim 10^8$ CFU/ml *E. coli* strains and T3 phage (MOI = 0.5),
445 and incubated at RT for 10 min. Then, we added 700 μ l of 0.95% saline and drops of chloroform
446 to kill the cells and prevent the production of progeny phages. After centrifugation at 11,000 g
447 for 1 min, supernatants were serially diluted and mixed with 300 μ l of *E. coli* BL21 overnight
448 cultures and 3 ml LB soft agar, and the mixtures were poured onto LB plates. After 4 h
449 incubation at 37°C, phage plaques were counted, and adsorption efficiencies were calculated.

450 Adsorption efficiency (%) = $[1 - (\text{PFU of unadsorbed phage} / \text{original PFU in the BL21 and phage}$
451 $\text{mixture})] \times 100$

452
453 **Bacterial killing assays.** Overnight cultures of *Y. pseudotuberculosis* IP2666 and *Klebsiella* sp.
454 390 were diluted 1:200 into LB and grown to log-phase ($\approx 10^8$ CFU/ml), i.e., for 5 h at 30°C and
455 for 3 h at 37°C, respectively. Bacterial cultures were mixed with phage lysates (MOI ~ 0.1) and
456 incubated at 30 or 37°C. At each time point, bacteria were collected, washed twice with 0.95%

457 saline, serially diluted, plated onto LB, and incubated at 30 or 37°C. Colonies were enumerated
458 to calculate CFU/ml.

459
460 **Microbiome editing assays.** Overnight cultures of *E. coli* Nissle 1917, *Klebsiella* sp. 390, and *Y.*
461 *pseudotuberculosis* IP2666 were diluted 1:200 into LB and grown to log-phase ($\sim 10^8$ CFU/ml),
462 i.e., for 3 h at 37°C, for 3 h at 37°C, and for 5 h at 30°C, respectively. Cultures were mixed and
463 treated with phage lysates (MOI ~ 0.1) and incubated at 30°C. At each time point, bacteria were
464 collected, washed twice with 0.95% saline, serially diluted, plated onto LB, LB containing 25
465 $\mu\text{g/ml}$ carbenicillin (VWR), and LB containing 1 $\mu\text{g/ml}$ triclosan (VWR), and incubated at 30°C.
466 Colonies were enumerated to calculate CFU/ml.

467
468 **Statistical analysis.** For all data points in all experiments, three samples were collected. The
469 data are presented as the mean, and the error bars represent the SEM. In the “**Bacterial killing**
470 **assays**” and the “**Microbiome editing assays**”, all CFU data were \log_{10} -transformed before
471 analysis.

472

473 **AUTHOR CONTRIBUTIONS**

474 H.A. and T.K.L. designed the study. H.A., S.L., and D.P.P. performed experiments. All authors
475 analyzed the data and discussed results. H.A., S.L., and T.K.L. wrote the manuscript. H.A., S.L.,
476 and T.K.L. have filed a provisional application on this work.

477

478 **ACKNOWLEDGMENTS**

479 Strains IJ284 *Klebsiella* sp. 390, IJ1668 K-12 hybrid; K1 capsule, IJ612 *S. typhimurium* LT2,
480 K1E, K1F, K1-5, SP6, and K11 were kindly provided by Ian Molineux (The University of Texas
481 at Austin). LUZ19 was kindly provided by Rob Lavigne (KU Leuven). *Yersinia*
482 *pseudotuberculosis* IP2666 and YPIII were kindly provided by Joan Mecsas (Tufts University).
483 We thank Oliver Purcell and Jennifer Henry for critical reading of the manuscript. This work was
484 supported by grants from the Defense Threat Reduction Agency (HDTRA1-14-1-0007), the
485 National Institutes of Health (1DP2OD008435, 1P50GM098792, 1R01EB017755), and the U. S.
486 Army Research Laboratory and the U. S. Army Research Office through the Institute for Soldier
487 Nanotechnologies, under contract number W911NF-13-D-0001. H.A. was supported by
488 fellowships from the Japan Society for the Promotion of Science, and the Naito Foundation.
489 D.P.P. was supported by the Portuguese Foundation for Science and Technology through the
490 grant SFRH/BD/76440/2011.

491 **REFERENCES**

- 492 Beier, H., Golomb, M., and Chamberlin, M. (1977). Isolation of recombinants between T7 and
493 T3 bacteriophages and their use in vitro transcriptional mapping. *Journal of virology* 21, 753-
494 765.
- 495 Bessler, W., Freund-Molbert, E., Knufermann, H., Rduolph, C., Thurow, H., and Stirm, S.
496 (1973). A bacteriophage-induced depolymerase active on *Klebsiella* K11 capsular
497 polysaccharide. *Virology* 56, 134-151.
- 498 Bikard, D., Euler, C.W., Jiang, W., Nussenzweig, P.M., Goldberg, G.W., Duportet, X., Fischetti,
499 V.A., and Marraffini, L.A. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-
500 specific antimicrobials. *Nature biotechnology* 32, 1146-1150.
- 501 Brussow, H. (2012). What is needed for phage therapy to become a reality in Western medicine?
502 *Virology* 434, 138-142.
- 503 Carlton, R.M. (1999). Phage therapy: past history and future prospects. *Archivum immunologiae*
504 *et therapiae experimentalis* 47, 267-274.
- 505 Citorik, R.J., Mimee, M., and Lu, T.K. (2014). Sequence-specific antimicrobials using efficiently
506 delivered RNA-guided nucleases. *Nature biotechnology* 32, 1141-1145.
- 507 Cuervo, A., Pulido-Cid, M., Chagoyen, M., Arranz, R., Gonzalez-Garcia, V.A., Garcia-Doval,
508 C., Caston, J.R., Valpuesta, J.M., van Raaij, M.J., Martin-Benito, J., *et al.* (2013). Structural
509 characterization of the bacteriophage T7 tail machinery. *The Journal of biological chemistry* 288,
510 26290-26299.
- 511 d'Herelle, F. (1931). Bacteriophage as a Treatment in Acute Medical and Surgical Infections.
512 *Bulletin of the New York Academy of Medicine* 7, 329-348.

513 Demerec, M., and Fano, U. (1945). Bacteriophage-Resistant Mutants in *Escherichia Coli*.
514 *Genetics* 30, 119-136.

515 Dietz, A., Weisser, H.J., Kossel, H., and Hausmann, R. (1990). The gene for *Klebsiella*
516 bacteriophage K11 RNA polymerase: sequence and comparison with the homologous genes of
517 phages T7, T3, and SP6. *Molecular & general genetics* : MGG 221, 283-286.

518 Dunn, J.J., and Studier, F.W. (1983). Complete nucleotide sequence of bacteriophage T7 DNA
519 and the locations of T7 genetic elements. *Journal of molecular biology* 166, 477-535.

520 Durfee, T., Nelson, R., Baldwin, S., Plunkett, G., 3rd, Burland, V., Mau, B., Petrosino, J.F., Qin,
521 X., Muzny, D.M., Ayele, M., *et al.* (2008). The complete genome sequence of *Escherichia coli*
522 DH10B: insights into the biology of a laboratory workhorse. *Journal of bacteriology* 190, 2597-
523 2606.

524 Fischbach, M.A., and Walsh, C.T. (2009). Antibiotics for emerging pathogens. *Science* 325,
525 1089-1093.

526 Garcia, E., Elliott, J.M., Ramanculov, E., Chain, P.S., Chu, M.C., and Molineux, I.J. (2003). The
527 genome sequence of *Yersinia pestis* bacteriophage phiA1122 reveals an intimate history with the
528 coliphage T3 and T7 genomes. *Journal of bacteriology* 185, 5248-5262.

529 Gibson, D.G. (2012). Oligonucleotide assembly in yeast to produce synthetic DNA fragments.
530 *Methods in molecular biology* 852, 11-21.

531 Gibson, D.G., Benders, G.A., Axelrod, K.C., Zaveri, J., Algire, M.A., Moodie, M., Montague,
532 M.G., Venter, J.C., Smith, H.O., and Hutchison, C.A., 3rd (2008). One-step assembly in yeast of
533 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium* genome.
534 *Proceedings of the National Academy of Sciences of the United States of America* 105, 20404-
535 20409.

536 Gibson, D.G., Young, L., Chuang, R.Y., Venter, J.C., Hutchison, C.A., 3rd, and Smith, H.O.
537 (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature methods*
538 *6*, 343-345.

539 Goldberg, G.W., Jiang, W., Bikard, D., and Marraffini, L.A. (2014). Conditional tolerance of
540 temperate phages via transcription-dependent CRISPR-Cas targeting. *Nature* *514*, 633-637.

541 Grice, E.A., and Segre, J.A. (2012). The human microbiome: our second genome. *Annual review*
542 *of genomics and human genetics* *13*, 151-170.

543 Hendrix, R.W. (2003). Bacteriophage genomics. *Current opinion in microbiology* *6*, 506-511.

544 Jaschke, P.R., Lieberman, E.K., Rodriguez, J., Sierra, A., and Endy, D. (2012). A fully
545 decompressed synthetic bacteriophage oX174 genome assembled and archived in yeast.
546 *Virology* *434*, 278-284.

547 Kiro, R., Shitrit, D., and Qimron, U. (2014). Efficient engineering of a bacteriophage genome
548 using the type I-E CRISPR-Cas system. *RNA biology* *11*, 42-44.

549 Lin, A., Jimenez, J., Derr, J., Vera, P., Manapat, M.L., Esvelt, K.M., Villanueva, L., Liu, D.R.,
550 and Chen, I.A. (2011). Inhibition of bacterial conjugation by phage M13 and its protein g3p:
551 quantitative analysis and model. *PloS one* *6*, e19991.

552 Lin, T.Y., Lo, Y.H., Tseng, P.W., Chang, S.F., Lin, Y.T., and Chen, T.S. (2012). A T3 and T7
553 recombinant phage acquires efficient adsorption and a broader host range. *PloS one* *7*, e30954.

554 Lu, T.K., Koeris, M.S., Chevalier, B.S., Holder, J.W., McKenzie, G.J., and Brownell, D.R.
555 (2013). Recombinant phage and methods. Patent *WO 2013049121 A2*.

556 Ma, H., Kunes, S., Schatz, P.J., and Botstein, D. (1987). Plasmid construction by homologous
557 recombination in yeast. *Gene* *58*, 201-216.

- 558 Molineux, I.J. (2006). The T7 Group. In *The Bacteriophages*, R. Calendar, ed. (New York:
559 Oxford Univ. Press), pp. 277-301.
- 560 Pajunen, M.I., Elizondo, M.R., Skurnik, M., Kieleczawa, J., and Molineux, I.J. (2002). Complete
561 nucleotide sequence and likely recombinatorial origin of bacteriophage T3. *Journal of molecular*
562 *biology* *319*, 1115-1132.
- 563 Pouillot, F., Blois, H., and Iris, F. (2010). Genetically engineered virulent phage banks in the
564 detection and control of emergent pathogenic bacteria. *Biosecurity and bioterrorism : biodefense*
565 *strategy, practice, and science* *8*, 155-169.
- 566 Qimron, U., Tabor, S., and CC., R. (2010). New details about bacteriophage T7-host interactions.
567 In *Microbe magazine*.
- 568 Rashid, M.H., Revazishvili, T., Dean, T., Butani, A., Verratti, K., Bishop-Lilly, K.A.,
569 Sozhamannan, S., Sulakvelidze, A., and Rajanna, C. (2012). A *Yersinia pestis*-specific, lytic
570 phage preparation significantly reduces viable *Y. pestis* on various hard surfaces experimentally
571 contaminated with the bacterium. *Bacteriophage* *2*, 168-177.
- 572 Shin, J., Jardine, P., and Noireaux, V. (2012). Genome replication, synthesis, and assembly of
573 the bacteriophage T7 in a single cell-free reaction. *ACS synthetic biology* *1*, 408-413.
- 574 Steven, A.C., Trus, B.L., Maizel, J.V., Unser, M., Parry, D.A., Wall, J.S., Hainfeld, J.F., and
575 Studier, F.W. (1988). Molecular substructure of a viral receptor-recognition protein. The gp17
576 tail-fiber of bacteriophage T7. *Journal of molecular biology* *200*, 351-365.
- 577 Sulakvelidze, A., Alavidze, Z., and Morris, J.G., Jr. (2001). Bacteriophage therapy. *Antimicrob*
578 *Agents Chemother* *45*, 649-659.

579 Tetart, F., Desplats, C., and Krisch, H.M. (1998). Genome plasticity in the distal tail fiber locus
580 of the T-even bacteriophage: recombination between conserved motifs swaps adhesin specificity.
581 *Journal of molecular biology* 282, 543-556.

582 Trojet, S.N., Caumont-Sarcos, A., Perrody, E., Comeau, A.M., and Krisch, H.M. (2011). The
583 gp38 adhesins of the T4 superfamily: a complex modular determinant of the phage's host
584 specificity. *Genome biology and evolution* 3, 674-686.

585 Yaung, S.J., Esvelt, K.M., and Church, G.M. (2014). CRISPR/Cas9-mediated phage resistance is
586 not impeded by the DNA modifications of phage T4. *PloS one* 9, e98811.

587 Yoichi, M., Abe, M., Miyanaga, K., Unno, H., and Tanji, Y. (2005). Alteration of tail fiber
588 protein gp38 enables T2 phage to infect *Escherichia coli* O157:H7. *Journal of biotechnology*
589 115, 101-107.

590

591

592 **FIGURE LEGENDS**

593 **Figure 1. Yeast platform for phage engineering.** (A) Schematic illustrating the workflow to
594 capture and reboot phages using our yeast platform. An entire phage genome or PCR products
595 spanning an entire phage genome are transformed into yeast cells along with a linearized yeast
596 replicon fragment from the yeast artificial chromosome, pRS415. In yeast, the phage genome is
597 assembled and captured in the YAC by gap-repair cloning. The resulting YAC-phage DNA is
598 extracted and transformed into host bacteria. Active phages are produced from the YAC-phage
599 DNA and generate plaques on a lawn of host bacteria. (B) To determine the phage sensitivity of
600 the *E. coli* 10G strain, high-titer phage lysates ($>10^9$ PFU/ml) were spotted onto 10G lawns. The
601 10G strain was sensitive only to infection by T7 and T3 phages. (C) Rebooting T7 and T3
602 phages from purified phage genomes. We electroporated 10 ng of genomic DNA into 10G,
603 mixed chloroform-treated lysates with *E. coli* BL21 and LB soft agar, poured the mixtures onto
604 LB plates, and incubated at 37°C. (D) One-time phage propagation assays. Rebooting phages
605 from purified phage genomes via 10G. We electroporated 10-200 ng of phage genomic DNA
606 into 10G and incubated the cells at 37°C for 1 h. We then treated the bacteria with chloroform.
607 After centrifugation, supernatants were mixed with host bacteria and LB soft agar, poured onto
608 LB plates, and incubated at 30 or 37°C. All tested phage genomes, including non-*E. coli* phages,
609 could be rebooted in *E. coli* 10G cells. Host bacteria: IJ1668 K-12 hybrid; K1 capsule for K1E,
610 K1F, and K1-5 phages, IJ612 *Salmonella typhimurium* LT2 for SP6 phage, *Pseudomonas*
611 *aeruginosa* PAO1 for LUZ19 phage, *Pseudomonas putida* C1S for gh-1 phage, and *Klebsiella*
612 sp. 390 for K11 phage. (E) An example of capturing and rebooting a phage through the yeast
613 platform. An excised YAC pRS415 amplicon and the T7 genome were co-transformed in yeast
614 cells. The T7 genome was captured in the YAC by gap-repair cloning. The YAC-T7 DNA was

615 extracted and used for transformation. Progeny phages were produced from YAC-T7 DNA in the
616 *E. coli* 10G strain and generated plaques on *E. coli* BL21.

617

618 **Figure 2. Creation of synthetic phages with engineered host range. (A)** We prepared multiple

619 PCR fragments encoding the wild-type T7 phage genome (T7_{WT}), T7 phage with the entire T3

620 phage tail fiber (T7_{T3(gp17)}), and T7 phage with a hybrid T7-T3 tail fiber (T7_{T3(C-gp17)}). All

621 fragments were co-transformed and assembled in yeast along with YAC DNA. M, 1 kb DNA

622 size marker (NEB). Y, YAC amplicon. **(B)** Phage A with its primary host determinant, gene *a*,

623 infects bacteria A, but cannot infect bacteria B. Phage B with its primary host determinant, gene

624 *b*, infects bacteria B, but cannot infect bacteria A. We hypothesized that by swapping these host

625 determinants between phage A and B, engineered phage A with gene *b* and engineered phage B

626 with gene *a* should infect bacteria B and A, respectively. **(C)** Host ranges of T7 and T3 phages.

627 Each bacterial overnight culture and LB soft agar were mixed, and poured onto LB plates. 2.5 μ L

628 of 10-fold serially diluted T7 and T3 phages were spotted onto the bacterial lawns and incubated

629 at 37°C. T3 phage did not plaque efficiently on *E. coli* BW25113 and MG1655, whereas T7

630 phage plaqued efficiently on all tested *E. coli* strains. **(D)** Adsorption assay. Bacteria and T3

631 phage were mixed at an MOI \sim 0.5 and incubated for 10 min. Growth of adsorbed progeny was

632 stopped by the addition of chloroform. After centrifugation, supernatants were serially diluted

633 and mixed with *E. coli* BL21 and LB soft agar, and poured onto LB plates. After incubation at

634 37°C, phage plaques were counted, and adsorption efficiencies were calculated. The data are

635 presented as the mean of three independent experiments, and the error bars represent the SEM.

636 Small error bars are obscured by bar charts. **(E)** Creation of synthetic T7 phage with phage 13a

637 tail fiber (encoded by gene *I7*). We synthesized 13a's gene *I7* and assembled it with the rest of

638 the T7 genome via overlapping PCR products in yeast. The YAC-phage DNA was extracted and
639 used for transformation. M, 1 kb DNA size marker (NEB). Y, YAC amplicon.

640

641 **Figure 3. Plaque formation assays with natural, reconstructed wild-type, and synthetic**

642 **phages.** Bacterial lawns were spotted with 2.5 μ L of 10-fold serially diluted phages and

643 incubated at 30 or 37°C. Synthetic phages showed tail-fiber- or tail-component-dependent host

644 ranges and the ability to cross between species.

645

646 **Figure 4. Creation of synthetic T3 phage with *Yersinia* phage R tail fiber.** (A) We introduced

647 mutations in T3 gene *17* PCR to convert it into phage R gene *17* and assembled the resulting

648 product with the rest of the T3 genome and YAC DNA in yeast. The YAC-phage DNA was

649 extracted and used for transformation into *E. coli*. M, 1 kb DNA size marker (NEB). Y, YAC

650 amplicon. (B) Plaquing assay with T3_{WT} and T3_{R(gp17)} on *E. coli* BL21, *Y. ptb* IP2666, and *Y. ptb*

651 YPIII demonstrates that T3_{R(gp17)} has the ability to infect both *E. coli* and *Y. ptb*. Ten-fold serial

652 dilutions of phage lysates were spotted on bacterial lawns and incubated for 4 h at 37°C for *E.*

653 *coli* BL21 or 24 h at 30°C for *Y. ptb* strains. These pictures were cut out from Figure 3. Bottom

654 panels show images of individual plaques. NP, no plaque. (C) Killing curves of *Y. ptb* IP2666

655 treated with T3_{R(gp17)}. $\sim 10^8$ CFU/ml bacteria and $\sim 10^7$ PFU/ml phage were used (MOI ~ 0.1). The

656 data are presented as the mean of three independent experiments, and the error bars represent the

657 SEM. Small error bars are obscured by symbols. The detection limit was 2.0×10^3 CFU/ml.

658

659 **Figure 5. Creation of synthetic T7 phage with *Klebsiella* phage K11 tail components as well**

660 **as K11 phage with T7 tail components.** (A) The tail complex of T7 phage is composed of two

661 components, a tubular structure and tail fibers. The tubular structure consists of an upper
662 dodecameric ring made of adaptor protein gp11 and a pyramidal hexameric complex of the
663 nozzle protein gp12. The tail fiber protein gp17 interacts with the interface between gp11 and
664 gp12 (Cuervo et al., 2013). **(B)** Schematics illustrating the construction of synthetic hybrids
665 between phages T7 and K11. Whole genomes were amplified as overlapping PCR amplicons as
666 shown on the gel. Appropriate fragments were co-transformed and assembled in yeast. YAC-
667 phage genomes were extracted and used for transformation. We swapped K11 genes *I1*, *I2* and
668 *I7* into T7 to create T7_{K11(gp11-12-17)} and T7 genes *I1*, *I2* and *I7* into K11 to create K11_{T7(gp11-12-17)}.
669 M, 1 kb DNA size marker (NEB). Y, YAC amplicon. **(C)** Plaquing of T7_{K11(gp11-12-17)} and
670 K11_{T7(gp11-12-17)} on *E. coli* BL21 and *Klebsiella* sp. 390. Ten-fold serial dilutions of phage lysates
671 were spotted on bacterial lawns and incubated for 4 h at 37°C. These pictures were cut from
672 Figure 3. Bottom panels show images of individual plaques. NP, no plaque. **(D)** Killing curves of
673 *Klebsiella* sp. 390 treated with T7_{K11(gp11-12-17)}. $\sim 10^8$ CFU/ml bacteria and $\sim 10^7$ PFU/ml phage
674 were used (MOI ~ 0.1). The data are presented as the mean of three independent experiments, and
675 the error bars represent the SEM. Small error bars are obscured by symbols. The detection limit
676 was 2.0×10^3 CFU/ml.

677

678 **Figure 6. Microbiome editing assay.** A synthetic microbial community composed of *E. coli*
679 Nissle 1917, *Klebsiella* sp. 390, and *Y. ptb* IP2666 was treated with various individual synthetic
680 phages and a pairwise combination of phages. After adding $\sim 10^7$ PFU/ml of each phage, the
681 resulting samples were incubated at 30°C with shaking for 1 h. At each time point, bacteria were
682 collected, washed in saline, serially diluted, and plated onto selective plates for viable cell counts
683 after a 24 h incubation at 30°C. The data are presented as the mean of three independent

684 experiments and the total numbers of cells (CFU/ml) are shown. The sizes of the pie charts
685 reflect the total number of cells. Note that the chart does not allow the display of fractions
686 smaller than ~1%. The detailed data and the SEM are shown in Table S3. The detection limit
687 was 2.0×10^3 CFU/ml.

Figure 1

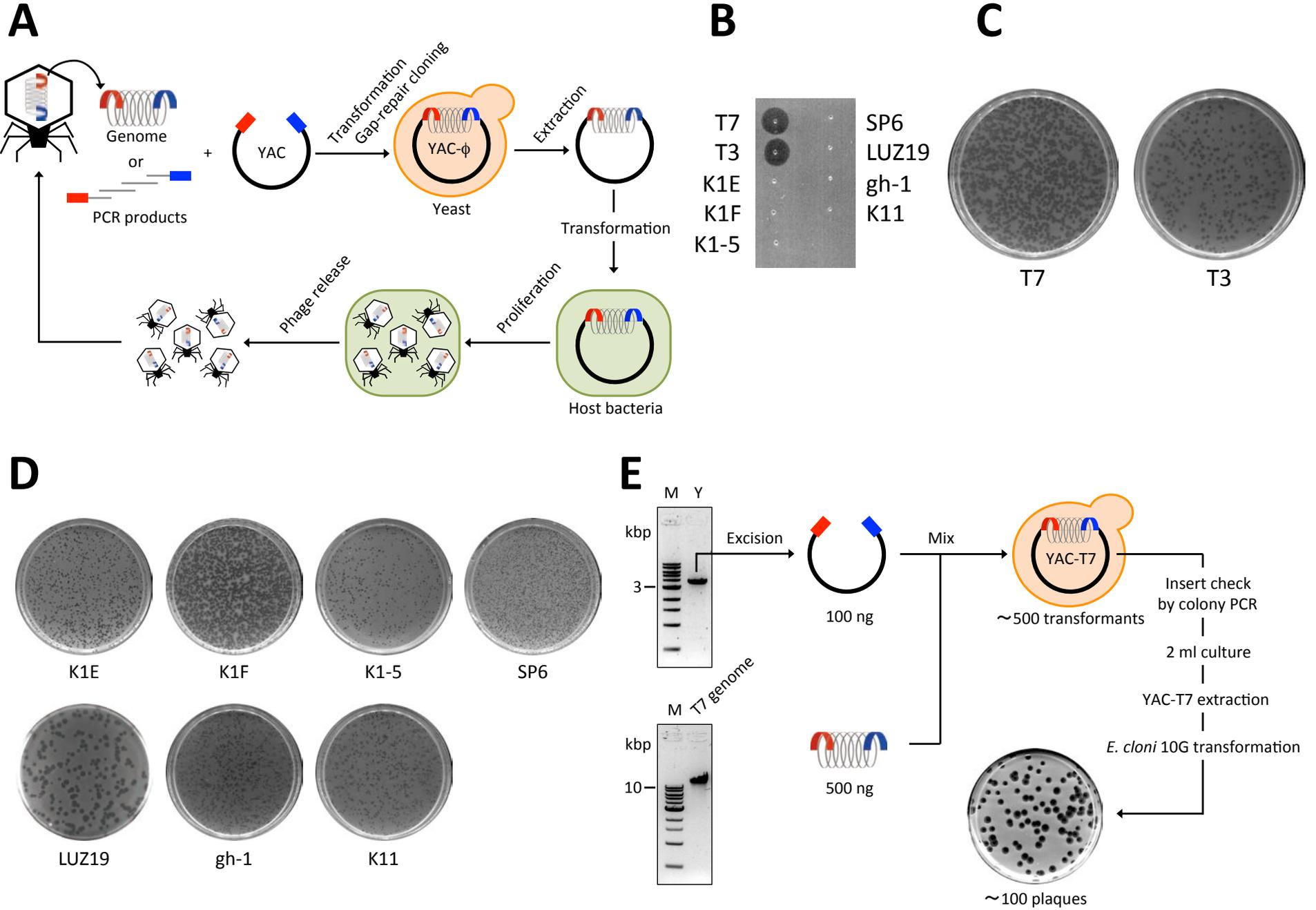


Figure 2

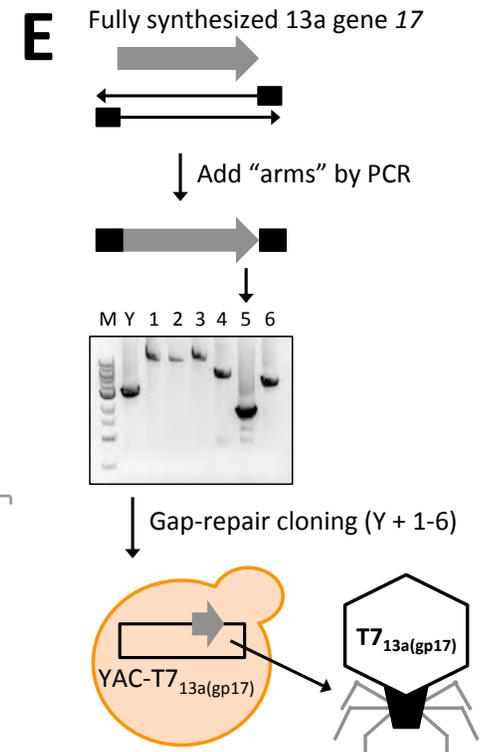
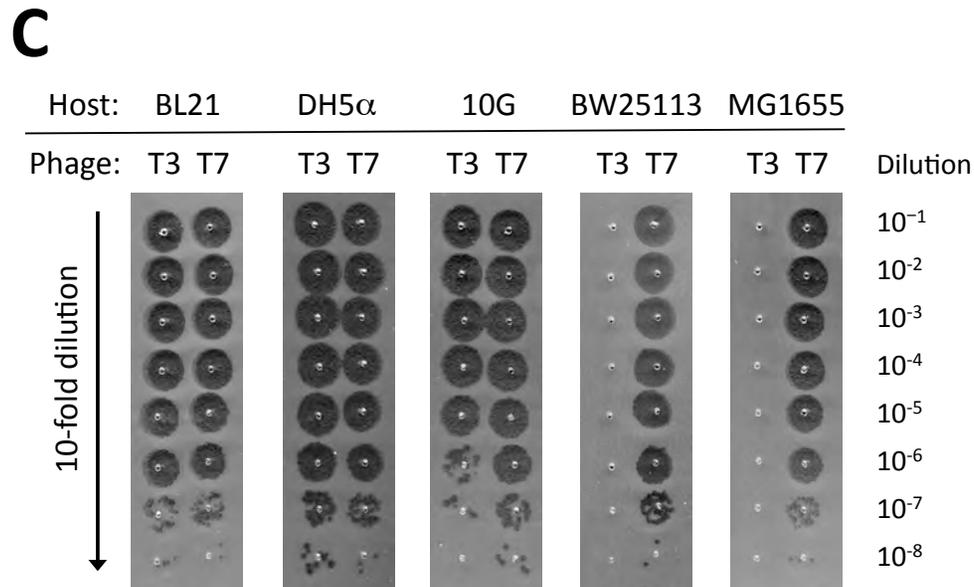
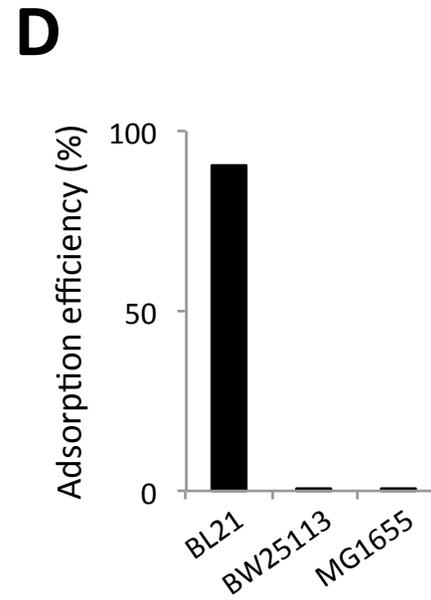
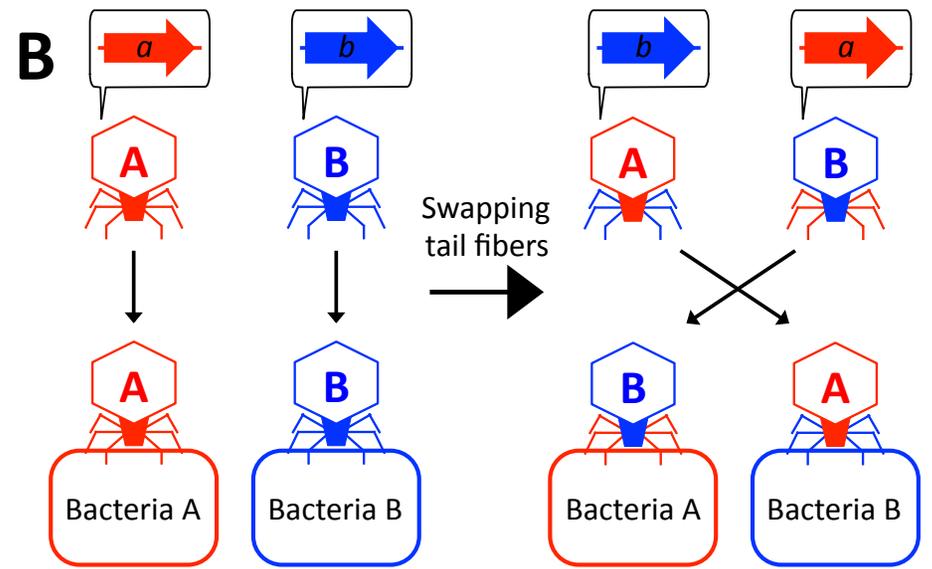
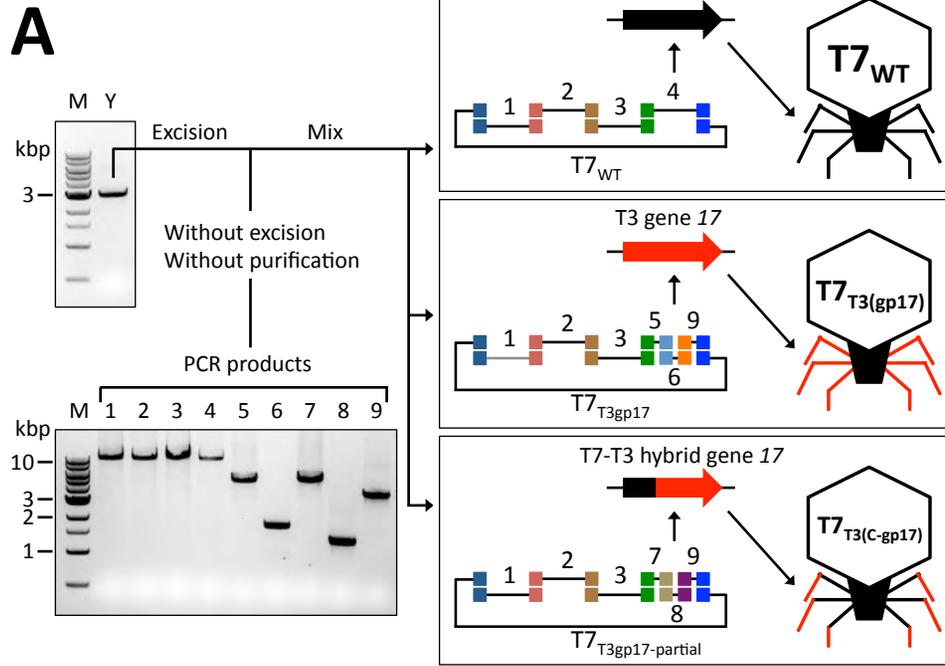


Figure 3

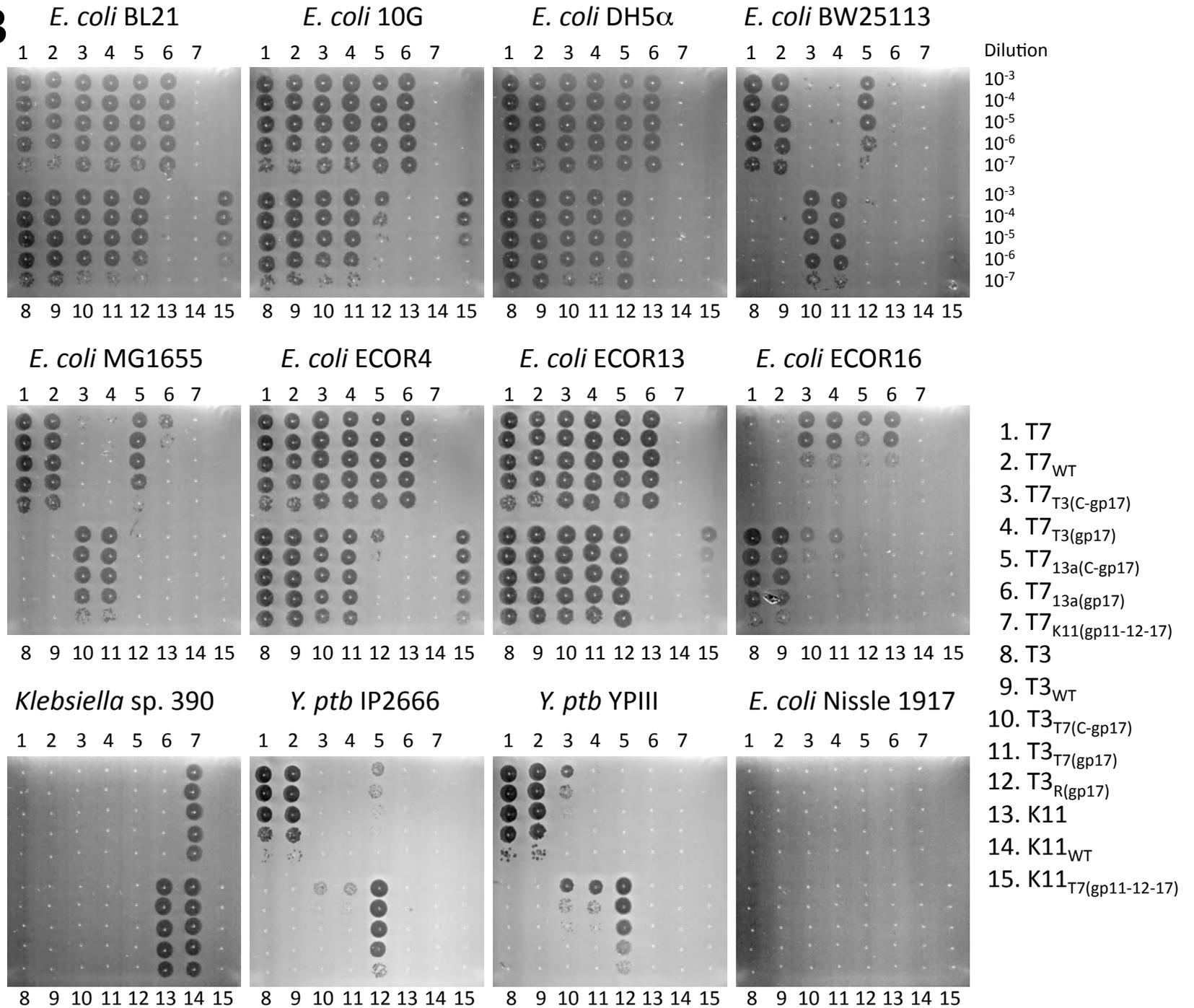


Figure 4

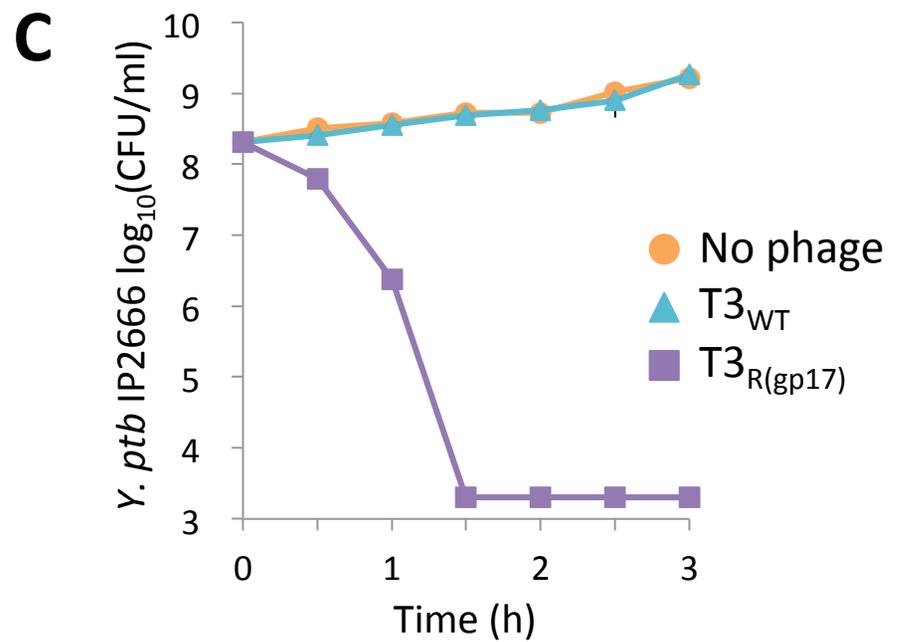
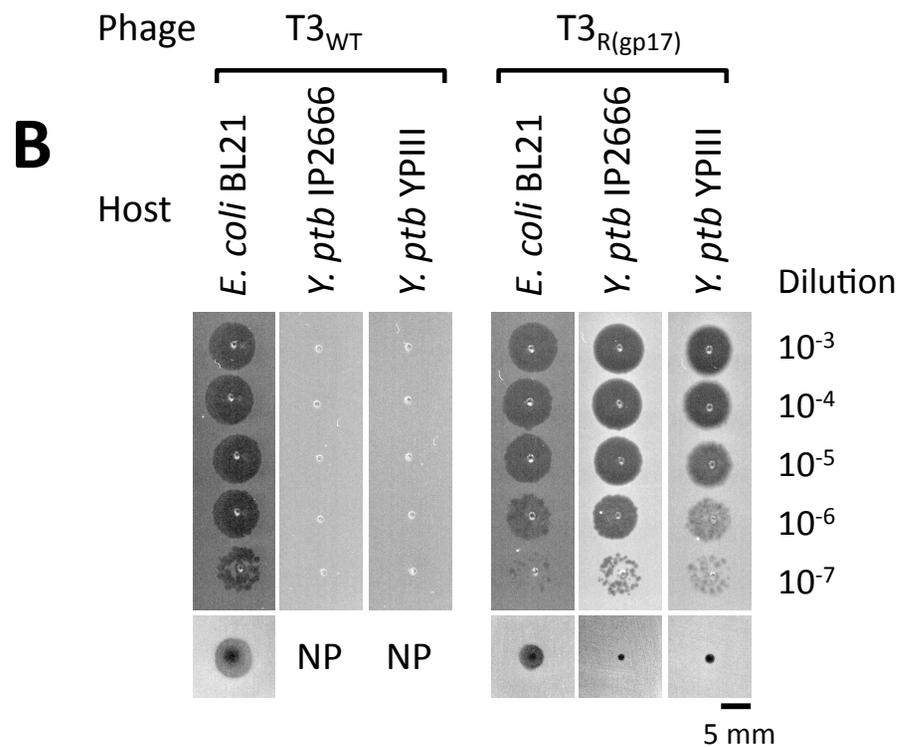
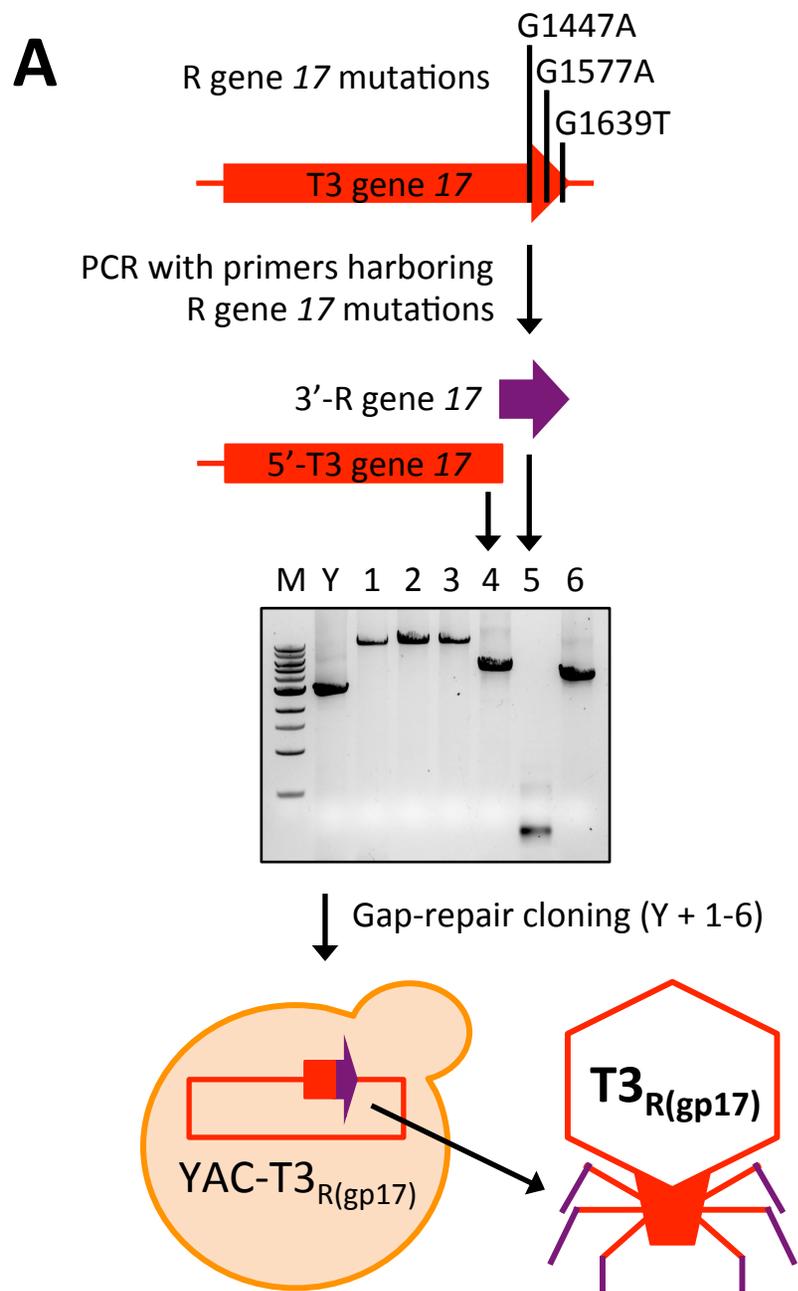


Figure 5

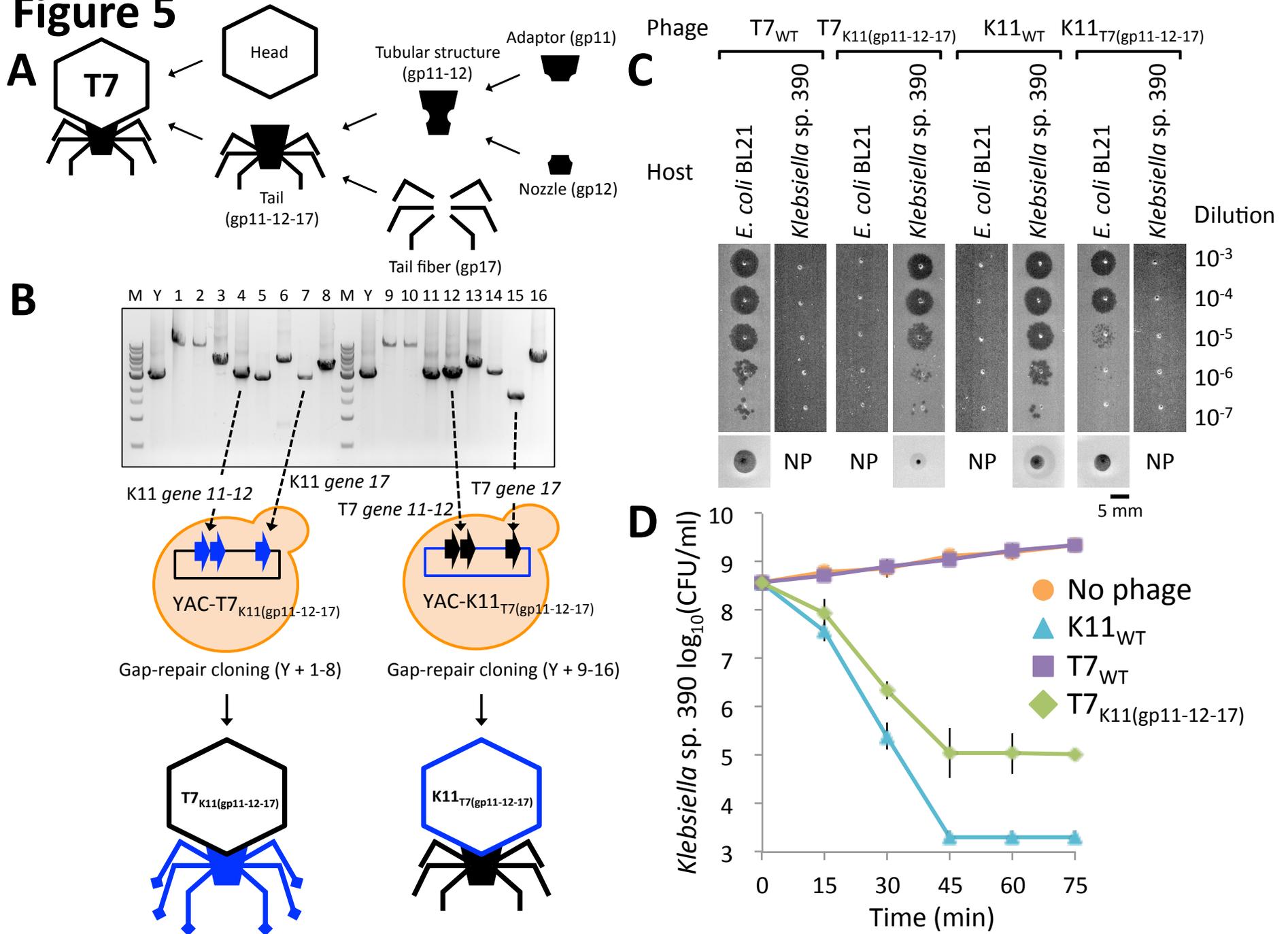
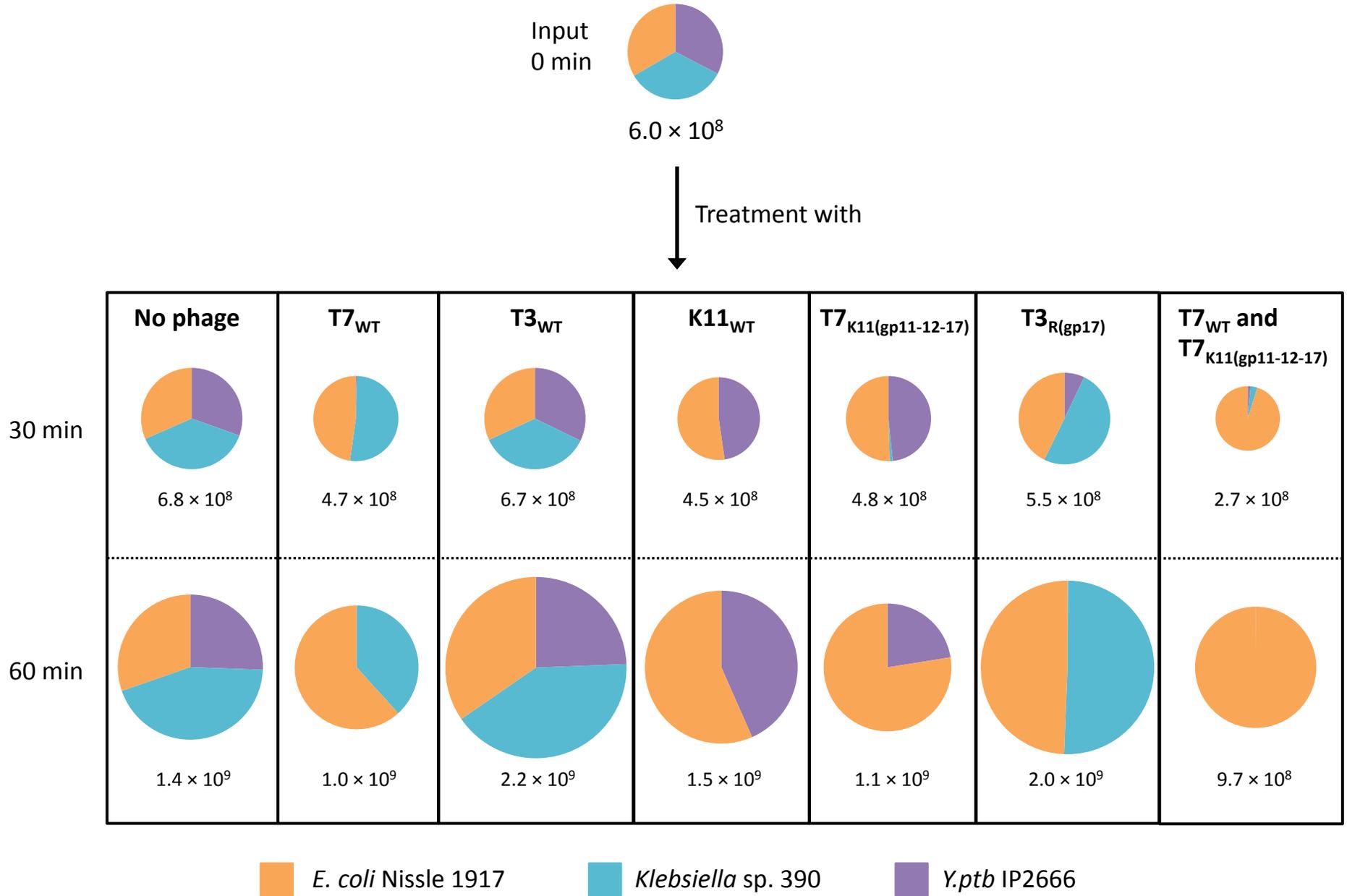


Figure 6



1 **Supplemental Information**

2

3 **Figure S1. Plaque formation assays with T7_{K11(gp11-12-17)} and K11_{T7(gp11-12-17)}, related to**

4 **Figure 5.** To confirm correct EOPs of T7_{K11(gp11-12-17)} and K11_{T7(gp11-12-17)} phages, 2.5 µL of 10-

5 fold serially diluted phages were spotted onto bacterial lawns and incubated at 37°C. K11_{T7(gp11-}

6 ₁₂₋₁₇₎ adopted the host range of T7_{WT} while T7_{K11(gp11-12-17)} adopted the host range of K11, thus

7 demonstrating that tail component swapping can lead to acquisition of novel host ranges.

8

9 **Figure S2. Antimicrobial susceptibilities of *E. coli* Nissle 1917, *Klebsiella* sp. 390, and *Y.***

10 ***pseudotuberculosis* IP2666, related to Figure 6. (A)** Five microliters of each overnight cultures

11 ($>10^9$ CFU/ml) for each bacteria were streaked on LB plates with or without antibiotics. Plates

12 were incubated at 30°C for 24 h. *Klebsiella* sp. 390 was naturally resistant to 25 mg/ml

13 carbenicillin and *Y. ptb* IP2666 was naturally resistant to 1 mg/ml triclosan, while *E. coli* Nissle

14 1917 was sensitive to both. **(B)** Diluted log-phase cultures were plated onto LB with or without

15 antibiotics. After incubation at 30°C for 18-24 h, colonies were enumerated.

16

17 **Table S1. One-time phage propagation assay, related to Figure 1.**

18

19 **Table S2. Sequence of codon-optimized 13a gene 17, related to Figure 2E.**

20

21 **Table S3. Microbiome editing assay, related to Figure 6.**

22

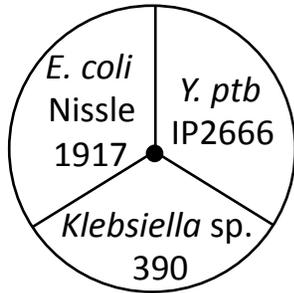
23 **Table S4. Synthetic phages created in this study, related to Experimental Procedures.**

24

25 **Table S5. Oligonucleotide primers used in this study, related to Experimental Procedures.**

Figure S2

A



Control
LB

Carbenicillin
25 μ g/ml

Triclosan
1 μ g/ml



B

E. coli Nissle 1917



Klebsiella sp. 390



Y. ptb IP2666



Table S1

One-time phage propagation assay.

Phage	Plaque formation on <i>E. coli</i> 10G	Propagation in <i>E. coli</i> 10G	Host bacteria
T7	Yes	Yes	<i>E. coli</i> BL21
T3	Yes	Yes	<i>E. coli</i> BL21
K1E	No	Yes	<i>E. coli</i> IJ1668
K1F	No	Yes	<i>E. coli</i> IJ1668
K1-5	No	Yes	<i>E. coli</i> IJ1668
SP6	No	Yes	<i>S. typhimurium</i> IJ612
LUZ19	No	Yes	<i>P. aeruginosa</i> PAO1
gh-1	No	Yes	<i>P. putida</i> C1S
K11	No	Yes	<i>Klebsiella</i> sp. 390

Table S2

Sequence of codon-optimized 13a gene 17.

ATGGCGAATGTGATTAAGACCGTTCTGACGTATCAGTTAGATGGATCCAATAGCGATTTTAATATTCCATTTGAAT
ACCTGGCGCGCAAATTTGTCGCCGTGACGCTGATTGGGGTTGATCGCAAGGTATTAACCATTAACACCGACTAT
CGCTTTGCTACGCGCACGACCATCTCTCTACTAAGGCGTGGGGACCGGCGGATGGTTATACTACCATCGAGTT
GCGCCGCGTTACGTGACAACTGACCGCCTCGTGGACTTTACCGACGGTAGCATTCTGCGTGCGTACGATCTTA
ACGTGGCCCAGATTCAGACAATCCACGTCGCGAGAAGAAGCACGTGACCTGACTGCCGACACCATTGGCGTAAA
CAATGACGGCCATCTGGATGCGCGCGGTGCGCGTATTGTCAATTTGGCGAACGCCGTTGATGATCGTGATGCGG
TGCCGCTCGGCCAACTCAAACTATGAATCAGAACAGTTGGCAGGCTCGCAACGAGGCACTGCAATTCCGCAA
TGAAGCCGAAACTTTTTCGCAATCAGGCGGAAGGTTTTAAAAACGAGAGCGGTACTAACGCCACTAACACGAA
ACAGTGGCGCGACGAGACAAAAGGCTTCCGCGACGAAGCGGAACAATTTAAGAACACCGCGGGTCAAGTATG
ATACATCCGCGGGTAACAGCGCGAGCGCTGCCCATCAGAGCGAAGTAAACGCAGAAAACAGTGCGACCGCGT
CCGCTAATAGCGCCCACCTCGCCGAGCAACAGGCCGATCGCGCGGAGCGTGAAGCTGATAAACTCGGCAACTT
TAATGGTCTTGCTGGTGCATCGACAAGTTCGACGGCACAAACGTGTATTGGAAAGGTAACATCCATGCAAAC
GGTCGCCTCTACATCACGACCAATGGCTTCGACTGCGGTCAATATCAGCAGTTCTTTGGTGGCGATACGAATCG
CTACAGCGTGATGGAATGGGGTGACGATAACGGGTGGCTGATGTATGTGCAGCGCCGTGAATGGACCACCGCA
ATCGGCGGCAACATTCAGTTGGTTGTCAACGGTCAGATCATCACCCAGGGCGGTGCCATGACGGGGCAACTGA
AACTTCAGAATGGTCACGTTCTGCAATTGGAATCCGCCAGTGACAAAGCGCATTATATTTTATCAAAGGATGGC
AACCGTAATAATTGGTATATTGGTCGTGGATCGGATAACAATAACGACTGCACGTTTCACTCCTACGTTACGGT
ACCACGTTAACCTTGAAACAGGATTATGCAGTGGTCAACAAACACTTTCATGTGGGACAGGCGGTCTGTGCAA
CTGATGGCAATATCCAGGGCACCAAATGGGGTGGTAAATGGCTGGATGTTTATTTAAATGATACATACGTTAAGA
AAACGATGGCTTGGACACAAGTGTGGGCTGCGGACTCGGGTAAATACCTCCCGGGTGGGAGTCAAACCTGATA
CTCTGCCGACGATCTGCGTTTCCGCAACATCTGGATTCGTACGCGTAACAACCTATTGGAACTTTTTTCGCACGG
GCCCCGATGGGATCTATTTCTGTGCGCTGAAGGAGGTTGGCTGAAATTTACAGATCCACTCAAACGGTTCGCGT
CTTCAAAAACATCTCTGATCGCGACGCACCCCCGACCGCAATCGCTGTGGAAGACGTTTAA

Table S3

Microbiome editing assay.

Treatment	Time after treatment	log ₁₀ (mean ± SEM CFU/ml)		
		<i>E. coli</i> Nissle 1917	<i>Klebsiella</i> sp. 390	<i>Y. ptb</i> IP2666
No phage	0 min	8.300 ± 0.036	8.307 ± 0.086	8.290 ± 0.155
	30 min	8.327 ± 0.037	8.407 ± 0.084	8.312 ± 0.125
	60 min	8.598 ± 0.148	8.761 ± 0.123	8.524 ± 0.180
T7 _{WT}	30 min	8.355 ± 0.018	8.390 ± 0.043	6.341 ± 0.032
	60 min	8.768 ± 0.114	8.560 ± 0.400	3.301 ± 0
T3 _{WT}	30 min	8.327 ± 0.037	8.379 ± 0.030	8.331 ± 0.100
	60 min	8.861 ± 0.059	8.935 ± 0.046	8.709 ± 0.192
K11 _{WT}	30 min	8.368 ± 0.018	5.386 ± 0.154	8.327 ± 0.037
	60 min	8.935 ± 0.046	3.301 ± 0	8.820 ± 0.059
T7 _{K11gp(11-12-17)}	30 min	8.379 ± 0.030	6.740 ± 0.230	8.361 ± 0.080
	60 min	8.861 ± 0.059	5.560 ± 0.197	8.324 ± 0.643
T3 _{R(gp17)}	30 min	8.367 ± 0.034	8.436 ± 0.015	7.587 ± 0.163
	60 min	8.984 ± 0.115	8.994 ± 0.072	6.327 ± 0.460
T7 and T7 _{K11(gp11-12-17)}	30 min	8.414 ± 0.027	6.968 ± 0.046	6.588 ± 0.223
	60 min	8.987 ± 0.063	5.683 ± 0.350	3.301 ± 0

Table S4

Synthetic phages.

Phage	Genotype	Description
T7 _{WT}	T7 wild-type	Synthesized from PCR products
T3 _{WT}	T3 wild-type	Synthesized from PCR products
K1E _{WT}	K1E wild-type	Synthesized from PCR products
K1F _{WT}	K1F wild-type	Synthesized from PCR products
K1-5 _{WT}	K1-5 wild-type	Synthesized from PCR products
SP6 _{WT}	SP6 wild-type	Synthesized from PCR products
gh-1 _{WT}	gh-1 wild-type	Synthesized from PCR products
K11 _{WT}	K11 wild-type	Synthesized from PCR products
T7 _{T3(C-gp17)}	T7 _{WT} gene 17 (1-447)-T3 gene 17 (448-1677)	T7 with T7-T3 hybrid tail fiber
T7 _{T3(gp17)}	T7 _{WT} Δ gene 17, T3 gene 17	T7 with T3 tail fiber
T7 _{13a(C-gp17)}	T7 _{WT} gene 17 (1-450)-13a gene 17 (451-1677)	T7 with T7-13a hybrid tail fiber
T7 _{13a(gp17)}	T7 _{WT} Δ gene 17, 13a gene 17	T7 with 13a tail fiber
T7 _{K11(gp11-12-17)}	T7 _{WT} Δ gene (11 12 17), K11 gene (11 12 17)	T7 with K11 tail
T3 _{T7(C-gp17)}	T3 _{WT} gene 17(1-447)-T7 gene 17(448-1662)	T3 with T3-T7 hybrid tail fiber
T3 _{T7(gp17)}	T3 _{WT} Δ gene 17, T7 gene 17	T3 with T7 tail fiber
T3 _{R(gp17)}	T3 _{WT} Δ gene 17, R gene 17	T3 with R tail fiber
K11 _{T7(gp11-12-17)}	K11 _{WT} Δ gene (11 12 17), T7 gene (11-12 17)	K11 with T7 tail

Table S5. Oligonucleotide primers.

Primer	5' → 3' sequence	Description
3'T7-pRS415-F-4 5'T7-pRS415-R-2	GTGTTACCTTGAGTGTCTCTGTGTCCCTGTCTCATGAGCGGATACATATTTGAATGT GGGGAACTTTAGTCCGTACACTGTGAGACCTTGTTCATGTGTGTTCAAAAACGTTATA	To amplify YAC for capturing T7 genomic DNA
3'T3-pRS415-F-4 5'T3-pRS415-R-2	CAGTATGATAGTACATCTCTATGTGTCCCTGTCTCATGAGCGGATACATATTTGAATGT GGGGTACTTTGGGTTCTTGAACATATGAGACCTTGTTCATGTGTGTTCAAAAACGTTATA	To amplify YAC for capturing T3 genomic DNA
LUZ19_AS8_Y2_Fw LUZ19_AS8_Y2_Rev	TCCTGTGGGTGGTGGTGGGGAGTGCTATGTCTCATGAGCGGATACATATTTGAATGT GGAAGGTGGGCTGATCAAGATCGGAGGCGCTTGTTCATGTGTGTTCAAAAACGTTATA	To amplify YAC for capturing LUZ19 genomic DNA
pRS415-F-4 pRS415-R-2	TTGCTCATGAGCGGATACATATTTGAATGT CCCTGTTCATGTGTGTTCAAAAACGTTATA	To amplify YAC for capturing PCR products of T7, T3, gh-1, K11, and synthetic phages
PST255 PST256	CCTGACTCTCTTTCATGTGTGTTCAAA ATAAACAAATAGGGGTTCCGCACATTTC	To amplify YAC for capturing PCR products of K1E, K1F, K1-5, and SP6
pRS415-R-2-1-30-F 9971-10000-R 9960-9989-F 19930-19959-R 19920-19949-F 29890-29919-R 29880-29909-F pRS415-F-4-39908-39937-R	TATAACGTTTTTGAACACACATGAACAAGG CTCACAGTGTACGGACCTAAAGTCCCCC ATTACGGGATGACAGTAGAACCTTCCG TGCAGCAATACCGGAAAGGTTGCTACTGT ATATGTCTCTCATAGATGTGCTATGTGG ACTTGTGACTCCACATAGGCACATCTATGA GAATAACCTGAGGGTCAATACCCGCTTGT GACATGATGCAACGAGGCTATTGACCTT ACATTCAAATATGTATCCGCTCATGAGACAGGGACACAGAGACACTCAAGTAAAC	For T7 _{WT} Primer pairs: pRS415-R-2-1-30-F and 9971-10000-R 9960-9989-F and 19930-19959-R 19920-19949-F and 29890-29919-R 29880-29909-F and pRS415-F-4-39908-39937-R
pRS415-R-2-T3-1-30-F T3-9971-10000-R T3-9961-9990-F T3-19931-19960-R T3-19921-19950-F T3-29891-29920-R T3-29881-29910-F pRS415-F-4-T3-38179-38208-R	TATAACGTTTTTGAACACACATGAACAAGG CTCATAGTTCAAGAACCAAAGTACCCCC ACGGAACCTCTCTTGGGTTCTTTGACGC CCAGTGGCTGGGCTCAAGAACCCAAAGAAG GGAAGTCGGTTTCATCGCTAAGCAGCATTTGC TGGCGATGATGCAATCGTCTTACGGATGA GATGCAACGTTACGCGCAGCATCTCCGCA TTGTAGTTGGTCCGAAAGTCTGCTGCTGA ACATTCAAATATGTATCCGCTCATGAGACAGGGACACATAGAGATGTACTATCACTG	For T3 _{WT} Primer pairs: pRS415-R-2-T3-1-30-F and T3-9971-10000-R T3-9961-9990-F and T3-19931-19960-R T3-19921-19950-F and T3-29891-29920-R T3-29881-29910-F and pRS415-F-4-T3-38179-38208-R
K1E-1 K1E-2 K1E-3 K1E-4 K1E-5 K1E-6 K1E-7 K1E-8	TTTGAACACACATGAACAAGGAAGTACAGG TCTGCCCTCGCCCTCGCCGATTTTCCCC TAGTATATGCTAATTCATTTGTGACTTAT CAGGTCTAAGAAAGAGGCTCTTTTGTGA AGTGGAGAACTTCTCCATAGCTTAGGTAT ATTTGGAGTATGGAGTAAAGCGCTAAGA GCTTATTGCTCAAGGCTCTCATCAGCAT CTGAGGATGAATTGAACCTCCGATTCAGA GAAATGTGCGGGAAACCCCTATTGTTTATAGCCACACCTCACACCTGTCAAACAC	For K1E _{WT} Primer pairs: K1E-1 and K1E-2 K1E-3 and K1E-4 K1E-5 and K1E-6 K1E-7 and K1E-8
K1F-1 K1F-2 K1F-3 K1F-4 K1F-5 K1F-6 K1F-7 K1F-8	TTTGAACACACATGAACAAGGAAGTACAGG TCTCACAGTCAAGAACCTCAAGTCTCCCC ACGATGAACGCTGCGCCACACCAATCTTG GACTGTGAAGCTGAAGTCTGACAACTCGA AAGTTGTGTTCTCTATAGTAGATTTA CAGGAGCTGGTGAAGCAGTGGCTCAAGCC GTCAGCGAACTTAAAGCTTGGTACTAAA CGTATGCCAATCCGTGAAGGAGGAGACCC GAAATGTGCGGGAAACCCCTATTGTTTATAGACTCAGAGACAGCAATAGTCAACCAC	For K1F _{WT} Primer pairs: K1F-1 and K1F-2 K1F-3 and K1F-4 K1F-5 and K1F-6 K1F-7 and K1F-8
K1-5*1 K1-5*2 K1-5*3 K1-5*4 K1-5*5 K1-5*6 K1-5*7 K1-5*8 K1-5*9 K1-5*10 K1-5*11 K1-5*12 K1-5*13 K1-5*14 K1-5*15 K1-5*16 K1-5*17 K1-5*18	GTTTTTGAACACACATGAACAAGGAAGTACAGG TGCCCTCGCCCTCGCCGTTTGT GGAGAGTCAAGGGCTTAAAGTTTACTGCT TGCTATGTACGCGATGCAAGTGGTGGGAA CAGGCTCACGCATCTCATATGGTCAAGA TGGACTTCTCACCACATGAGGATTCCTCT GCTTTGTGAGCTGCTCAGGAAGCAAGCA TAACCTCGCTGCTGCTGCTGAGTCTGCTG TGTGCATTTGTTGCTCATTCATGAGGCT TGTGCATCTTAAAGAGACCCACCACT AAGAAGCTGAGTGGCTATCTGCTGCGCAGT TCTAAGGATGAGATCAGACTAAGCTAGCC GCCTTAGCTGTAACCTCTCTCCGCAATA TAAACCGAAAGTGTGAGACTTAAAGTAAAG TATTGCCGCCAGCTTACATCTGTTAA TTGACGGGTTTTATCAGAAGGACTTCA GCTATCTCTATTACTTCAACCTCCCT TTGAGCGGCTTACTAGCCAATCTTCAT GAAATGTGCGGGAAACCCCTATTGTTTATAGCCACACCCCTCACACCTGTCAAATCC	For K1-5 _{WT} Primer pairs: K1-5*1 and K1-5*2 K1-5*3 and K1-5*4 K1-5*5 and K1-5*6 K1-5*7 and K1-5*8 K1-5*9 and K1-5*10 K1-5*11 and K1-5*12 K1-5*13 and K1-5*14 K1-5*15 and K1-5*16 K1-5*17 and K1-5*18
SP6-1 SP6-2 SP6-3 SP6-4 SP6-5 SP6-6 SP6-7 SP6-8 SP6-9 SP6-10 SP6-11 SP6-12 SP6-13 SP6-14 SP6-15 SP6-16 SP6-17 SP6-18	TTTGAACACACATGAACAAGGAAGTACAGG TCTCGGCTCGCCCTCGCCGATGTC CGTCTGATGACTGTAGTGTAGTGGGA ATTTGGATGATGAAGGAGGCGGACGAAT TTCCCGTGTAGTATAGCCTTCCATATA CGGCTTCTTTTTGAGAAAGCATTCCCGGA AAGATAATAACTTTGAGGTAATCTTTCATC AGATTATGTGTATGGTGTGATGCAAAAT CTGGAACCTTAGCTGCCTCAATGCGAGGTG CATTCAAGCAGTAGTCTGGCACAAGG CTTGTGTCAAAGATTCAAGTACTTGAC AGGAGGAGTATTTCTATAATGAAGAAGG CCACATACGCATCTGATTGCTCAAAGTT GCAGTTAAAGGCGGATGAAGCGAAGAAAG TCAATCTCCAATAGTCTACGCTGGCCTT GCAATACGATTTGTTAGTGTGATGATGACC TAACTCTACTACTACAGCCCTCCCT TTGAGCGGCTTACTACACAGTCTTCCAC GAAATGTGCGGGAAACCCCTATTGTTTATAGCCACACCCCTCACACCTGTCAAATCC	For SP6 _{WT} Primer pairs: SP6-1 and SP6-2 SP6-3 and SP6-4 SP6-5 and SP6-6 SP6-7 and SP6-8 SP6-9 and SP6-10 SP6-11 and SP6-12 SP6-13 and SP6-14 SP6-15 and SP6-16 SP6-17 and SP6-18
pRS415-R-2-gh-1_1-30-F gh-1_9971-10000-R gh-1_9960-9989-F gh-1_19930-19959-R gh-1_19920-19949-F gh-1_29890-29919-R gh-1_29880-29909-F pRS415-F-4-gh-1_37330-37359-R	TATAACGTTTTTGAACACACATGAACAAGG CTCAAGGAAACAGCCGAGGATGTC CTCGCTCACAGATGACTCGCCATCACCTC CGAGTATGAAAGGCTGATGGGAGTCACTC GGAGCTCTGGGCTCATCCAAAGAACTCT TGGCTGATGAGAGTCTTTGGGATGACCC CGAACGTTGAAGTCTGAGTGTGCGAGAT GGCATACGCGATCTCGCACTCAGGACTT ACATTCAAATATGTATCCGCTCATGAGACAGACAGATAGGGCCCTTGTAGTTCCCTAT	For gh-1 _{WT} Primer pairs: pRS415-R-2-gh-1_1-30-F and gh-1_9971-10000-R gh-1_9960-9989-F and gh-1_19930-19959-R gh-1_19920-19949-F and gh-1_29890-29919-R gh-1_29880-29909-F and pRS415-F-4-gh-1_37330-37359-R
pRS415-R-2-K11-1-30-F K11-9971-10000-R	TATAACGTTTTTGAACACACATGAACAAGG CTCACAGTTTACACTTTTGTGTTTCCCCC ATTAGAAGTCACTGCTTCTTGGCTTCGC	For K11 _{WT}

K11-9900-9929-F K11-19961-19990-R K11-19950-19979-F K11-29950-29979-R K11-29880-29909-F pRS415-F-4-K11-41152-41181-R	AGCGGACGAATCTCGCAGCCGTAACCTCA TCATCACCTCGAGGGCTTAAGGGCTGAC ATTGCCGATGGTACCCCTTAAGGCCCTC CATCGTGTCTTGAACACATCGTACCCATC CGGGGACGCTGCTGAGGCTCAGATTCAAGAA ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACATCAACATATAGTGTG	Primer pairs: pRS415-R-2, K11-1-30-F and K11-9971-10000-R K11-9900-9929-F and K11-19961-19990-R K11-19950-19979-F and K11-29950-29979-R K11-29880-29909-F and pRS415-F-4-K11-41152-41181-R
29880-29909-F 35042-35071-R T7(447)-T3(448)-gp17-F T3-T7-gp17-R T3-T7-gp17-F pRS415-F-4-39908-39937-R	GACATGATGGACAAGCAGGTTATGACCTT AACAGCATCGGGTCAACCCAGCGTTCGC GCGAACCGCTGGATGACCGGATGTTCCGTTGGTCACTTAAGACCATGAACCAG GACTACACGCTTCTTCTGTGATTACCAATACACGCTCTCCACGGCTATTGCTGTGG CCAACAGCAATAGCCGTAGAGACGCTGTAATGGTAAATCACAGGAAGACGCTGTAGTC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACACTCAAGTAAAC	For T7 _(H) (gp17) Primer pairs: 29880-29909-F and 35042-35071-R T7(447)-T3(448)-gp17-F and T7-T3-gp17-R T3-T7-gp17-F and pRS415-F-4-39908-39937-R
29880-29909-F Up-gp17-R gp17-F-1 T7-T3-gp17-R T3-T7-gp17-F pRS415-F-4-39908-39937-R	GACATGATGGACAAGCAGGTTATGACCTT TTGACCTCCTTAAAGTAAATCTAAGAGACT AGTCTCTTAGATTACTTTAAGGAGGTCAAATGGCTAACGTAATTAACACCGTTTTGACT GACTACACGCTTCTTCTGTGATTACCAATACACGCTCTCCACAGCGATTGGCTGTGG CCAACAGCAATAGCCGTAGAGACGCTGTAATGGTAAATCACAGGAAGACGCTGTAGTC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACACTCAAGTAAAC	For T7 _(H) (gp17) Primer pairs: 29880-29909-F and Up-gp17-R gp17-F-1 and T7-T3-gp17-R T3-T7-gp17-F and pRS415-F-4-39908-39937-R
29880-29909-F 35045-35074-R T7(450)-13a(451)-gp17-F T7-13a-gp17-R 13a-T7-gp17-F pRS415-F-4-39908-39937-R	GACATGATGGACAAGCAGGTTATGACCTT CGGAACAGCATCGGGTCAACCCAGCGCTT AACCGCTGGATGACCGGATGTTCCGCTCGCCCAACTCAAATATGATGATCAGAAC GACTACACGCTTCTTCTGTGATTACCAATTAACGCTCTCCACAGCGATTGGCTGTGG CCGACCGCAATCGCTGTGGAAAGACGTTAATGGTAAATCACAGGAAGACGCTGTAGTC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACACTCAAGTAAAC	For T7 _(H) (gp17) Primer pairs: 29880-29909-F and 35045-35074-R T7(450)-13a(451)-gp17-F and T7-13a-gp17-R 13a-T7-gp17-F and pRS415-F-4-39908-39937-R
29880-29909-F Up-gp17-R 13a-gp17-F-1 T7-13a-gp17-R 13a-T7-gp17-F pRS415-F-4-39908-39937-R	GACATGATGGACAAGCAGGTTATGACCTT TTGACCTCCTTAAAGTAAATCTAAGAGACT AGTCTCTTAGATTACTTTAAGGAGGTCAAATGGCGAATGTTAAGACCGTTCTGACG GACTACACGCTTCTTCTGTGATTACCAATTAACGCTCTCCACAGCGATTGGCTGTGG CCGACCGCAATCGCTGTGGAAAGACGTTAATGGTAAATCACAGGAAGACGCTGTAGTC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACACTCAAGTAAAC	For T7 _(H) (gp17) Primer pairs: 29880-29909-F and Up-gp17-R 13a-gp17-F-1 and T7-13a-gp17-R 13a-T7-gp17-F and pRS415-F-4-39908-39937-R
19920-19949-F T7-24198-24227-R T7-24198-24227+K11gp11-F T7-27227-27256+K11gp12-R T7-27227-27256-F 29890-29919-R 29880-29909-F Up-gp17-R K11-gp17-F-1 T7-K11-gp17-R K11-T7-gp17-F pRS415-F-4-39908-39937-R	ACTTGTGACTCCACATAGCCACTATGATG ATAGTTCCTCTTTCAGCAAAAACCCCTC GAGGGGTTTTTTGCTGAAGGAGGAACTATATGAACATGCAAGATGCTTACTTTGGGCT ATTTGAGCCACCACAGGGAGAATATTTAATTAATGCGTGGCTGCTGCTGTAGTT TTAAATATTCCCTGTGGTCTCGAAAT GAATAACCTGAGGGTCAATACCTGCTTGT GACATGATGGACAAGCAGGTTATGACCTT TTGACCTCCTTAAAGTAAATCTAAGAGACT AGTCTCTTAGATTACTTTAAGGAGGTCAAATGGCAACGACATTAAACAGCTATTGACTG GACTACACGCTTCTTCTGTGATTACCAATATAACAAAGATGATGATGCTGAGAT ATCTCGAGATTGACATCTTTGTTTATAATGGTAAATCACAGGAAGACGCTGTAGTC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACACTCAAGTAAAC	For T7 _(K11gp11-12-17) Primer pairs: 19920-19949-F and T7-24198-24227-R T7-24198-24227+K11gp11-F and T7-27227-27256+K11gp12-R T7-27227-27256-F and 29890-29919-R 29880-29909-F and Up-gp17-R K11-gp17-F-1 and T7-K11-gp17-R K11-T7-gp17-F and pRS415-F-4-39908-39937-R
T3-29881-29910-F T3-33249-33278-R T3(447)-T7(448)-gp17-F T3-T7-gp17-R T7-T3-gp17-F pRS415-F-4-T3-38179-38208-R	TTGTAGTTGGTCCGAAAGTCTGCGCTGA AACAGCTGCGGGTCAACCCAGCGTTCGC GCGAACCGCTGGATGACCGGACGCTGTTCCGTTGGTCACTTAAGACCATGAACCAG GTGGACTTAAAGTAGTCTTCTTGTGATCTTACTCGTCTCCACCATGATTGATTAGG CCTAATGCAATCATGGTGGAGAACGAGTAATAAGCATCAAAGAACTACTTTAAGTCCAC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACATAGAGATGACTACTCATACTG	For T3 _(H) (gp17) Primer pairs: T3-29881-29910-F and T3-33249-33278-R T3(447)-T7(448)-gp17-F and T3-T7-gp17-R T7-T3-gp17-F and pRS415-F-4-T3-38179-38208-R
T3-29881-29910-F Up-gp17-R gp17-F-1 T3-T7-gp17-R T7-T3-gp17-F pRS415-F-4-T3-38179-38208-R	TTGTAGTTGGTCCGAAAGTCTGCGCTGA TTGACCTCCTTAAAGTAAATCTAAGAGACT AGTCTCTTAGATTACTTTAAGGAGGTCAAATGGCTAACGTAATTAACACCGTTTTGACT GTGGACTTAAAGTAGTCTTCTTGTGATCTTACTCGTCTCCACCATGATTGATTAGG CCTAATGCAATCATGGTGGAGAACGAGTAATAAGCATCAAAGAACTACTTTAAGTCCAC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACATAGAGATGACTACTCATACTG	For T3 _(H) (gp17) Primer pairs: T3-29881-29910-F and Up-gp17-R gp17-F-1 and T3-T7-gp17-R T7-T3-gp17-F and pRS415-F-4-T3-38179-38208-R
T3-29881-29910-F R-gp17_1433-1462-R R-gp17_1433-1462-F R-gp17_1562-1654-R R-gp17_1625-1654-F pRS415-F-4-T3-38179-38208-R	TTGTAGTTGGTCCGAAAGTCTGCGCTGA TCTGAGAACCTCTCATGTAACCTACCAC GTGGTAGTTACATGAGAGAGGTTCTCAGA CTGTTGGAGGGCAGATATCTATCCGCTATGTTAAATACCTGCCATAGAGTATCTGGAATTTAGCCAACCGTCTCGGCTGAAAGGA ACATAGCGGATAGATATGCGCTCCAACAG ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACATAGAGATGACTACTCATACTG	For T3 _(H) (gp17) Primer pairs: T3-29881-29910-F and R-gp17_1433-1462-R R-gp17_1433-1462-F and R-gp17_1562-1654-R R-gp17_1625-1654-F and pRS415-F-4-T3-38179-38208-R
K11-19950-19979-F K11-22861-22890-R K11-22861-22890+T7gp11-F K11-25868-25897+T7gp12-R K11-25868-25897-F K11-29950-29979-R K11-29880-29909-F Up-K11-gp17-R T3/7-gp17-F K11-T7-gp17-R T7-K11-gp17-F pRS415-F-4-K11-41152-41181-R	ATTGCCGATGGTCAACCCCTAAGGCCCTC AATGTAAGCTCTCAAGCAAAAACCCCTCA TGAGGGTTTTTTGCTTAAAGGAGTACATTAATGCGCTCATACGATATGAACGTTGAGACT TAATTGAGCACACCATAAGGATTCCTCAGTTAAATACCGAACTCTCCGTAAGTAGTT CTGAAGGAATCCTTATGGTGTCTCAATTA CATCGTGTCTTGAACACATCGTACCCATC CGGGGACGCTGCTGAGGCTCAGATTCAAGAA TGTGACCTCCTTAGTTGAATGAGAAGGGG CCCTTCTCACTCAACTAAAGGAGGTCAATGGCTAACGTAATTAACACCGTTTTGACT TTGGTTAAATCACTCAGCATGTTGCTCTTACTGCTTCCACCATGATTGATTAGG CCTAATGCAATCATGGTGGAGAACGAGTAATAAGCATCAAAGAACTACTTTAAGTCCAC ACATTCAAATATGTATCCGCTCATGAGACA AGGGACACAGAGACACTCAACATATAGTGTG	For K11 _(T7gp11-12-17) Primer pairs: K11-19950-19979-F and K11-22861-22890-R K11-22861-22890+T7gp11-F and K11-25868-25897+T7gp12-R K11-25868-25897-F and K11-29950-29979-R K11-29880-29909-F and Up-K11-gp17-R T3/7-gp17-F and K11-T7-gp17-R T7-K11-gp17-F and pRS415-F-4-K11-41152-41181-R

Boldface means YAC pRS415 sequence.