Title: Engineering Modular Viral Scaffolds for Targeted Bacterial Population Editing

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SUMMARY

Bacteria are central to human health and disease, but the tools available for modulating and editing bacterial communities are limited. New technologies for tuning microbial populations would facilitate the targeted manipulation of the human microbiome and treatment of bacterial infections. For example, antibiotics are often broad spectrum in nature and cannot be used to accurately manipulate bacterial communities. Bacteriophages can provide highly specific targeting of bacteria, but relying solely on natural phage isolation strategies to assemble well-defined and uniform phage cocktails that are amenable to engineering can be a time-consuming and labor-intensive process. Here, we present a synthetic-biology strategy to modulate phage

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

INTRODUCTION

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

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

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

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

RESULTS

Yeast platform for bacteriophage genome engineering.

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

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

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

Rebooting bacteriophages from PCR products via the yeast platform.

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

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

Swapping tail fibers enables modulation of phage host range.

To engineer phages with tunable host ranges, we first selected two model phages, T7 and T3, which are obligate lytic phages originally isolated as a member of the seven "Type" phages that grow on *E. coli* B (Demerec and Fano, 1945). T7 and T3 have linear genomes that share high homology with each other, in which the primary host determinant is the product of gene *17*

(gp17), the tail fiber (Dunn and Studier, 1983; Pajunen et al., 2002). Alterations in the gp17 sequence have been linked to the recognition of different host receptors and shifting host ranges (Molineux, 2006). Thus, we hypothesized that exchanging gene *17* or fragments of gene *17* between T7, T3, and their relatives could be used to tune their host specificities (Figure 2B). This is supported by previous data on naturally occurring hybrids between T7 and T3 whose host range was mostly dictated by which gene *17* they harbored (Lin et al., 2012).

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

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

T3 viral chassis, which carried engineered gene *17* alleles composed of fragments from the other phage. Specifically, we created six synthetic phages: T7 phage with the wild-type T7 tail fiber ($T7_{WT}$), T7 phage with 410 amino acids of the C-terminal region of the T3 tail fiber ($T7_{T3(C\text{-}gp17)}$), T7 phage with the entire T3 tail fiber ($T7_{T3(gp17)}$), T3 phage with the wild-type T3 tail fiber ($T3_{WT}$), T3 phage with 405 amino acids of the C-terminal region of the T7 tail fiber ($T3_{T7(C\text{-}gp17)}$), and T3 phage with the entire T7 tail fiber ($T3_{T7(gp17)}$). $T7_{WT}$ and T7 phages are the same at the genetic level; however, $T7_{WT}$ phage was created by capturing the T7 genome in yeast and then rebooting this phage genome in bacteria and served as a control for the faithfulness of the reconstruction process, whereas T7 was obtained from ATCC. The same applies to $T3_{WT}$ and T3. Each phage was assembled in yeast via six PCR fragments and was rebooted via transformation into *E. coli* 10G (example schematics in Figure 2A). No unexpected mutations were found in the heterologous gp17 regions of the rebooted phages.

To examine the host specificities of our six engineered phages, we performed plaque formation assays on a range of *E. coli, Klebsiella*, and *Yersinia pseudotuberculosis* (*Y. ptb*) strains (Figure 3). $T3_{T7(C\text{-}gp17)}$ and $T3_{T7(gp17)}$ plaqued on *E. coli* BW25113 and *E. coli* MG1655 at a similar EOP as T7 and $T7_{WT}$, while T3, $T3_{WT}$, $T7_{T3(C\text{-}gp17)}$, and $T7_{T3(gp17)}$ had $>10^5$-fold-reduced EOPs on these strains. In addition, T3, $T7_{T3(C\text{-}gp17)}$, and $T7_{T3(gp17)}$ plaqued on *E. coli* ECOR16 while T7, $T3_{T7(C\text{-}gp17)}$, and $T3_{T7(gp17)}$ did not. In addition, we also synthesized a codon-optimized version of the tail fiber of the T7-like enterobacteriophage 13a and created synthetic T7 phages containing the entire 13a tail fiber ($T7_{13a(gp17)}$) or the C-terminal region of the 13a tail fiber ($T7_{13a(C\text{-}gp17)}$) (Figure 2E, Table S2). Although T7 and $T7_{WT}$ did not plaque on *E. coli* ECOR16, both $T7_{13a(gp17)}$ and $T7_{13a(C\text{-}gp17)}$ were able to do so efficiently (Figure 3). These results demonstrate that the C-terminal region of gp17 is a major host range determinant and that new

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

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

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

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

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

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

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

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

Alternatively, the gene *17* product from K11 may require a function or factor that is absent from T7. Cuervo *et al.* reported that the tail of T7 phage, which assembles independently of the head, is assembled from a dodecamer of gp11 (the adaptor) and an hexamer of gp12 (the nozzle) (Figure 5A) onto which 6 trimers of gp17 attach (Cuervo et al., 2013). T7's six tail fibers attach at the interface between the adaptor and nozzle, thus making contacts with both proteins. The adaptor ring is responsible for the attachment of the preformed tail to the prohead via interactions with the portal composed of 12 subunits of gp8. The homology between the gp8 of T7 and K11 (80% identity at the amino acid level) is much higher than the homology between the gp11 and gp12 proteins of T7 and K11 (60 and 61% identity, respectively), which led us to suspect that replacing all three tail genes of T7 with their K11 equivalents (gp11, gp12, and gp17) could be necessary to create functional virions (Figure 5B). Indeed, both T7 with K11 tail components ($T7_{K11(gp11\text{-}12\text{-}17)}$) and K11 with T7 tail components ($K11_{T7(gp11\text{-}12\text{-}17)}$) were successfully engineered into functional phages that exhibited tail-dependent host ranges. Specifically, $T7_{K11(gp11\text{-}12\text{-}17)}$ infected *Klebsiella* sp. 390 and did not target *E. coli*, while $K11_{T7(gp11\text{-}12\text{-}17)}$ infected *E. coli*, but did not plaque on *Klebsiella* (Figure 5C and Figure 3). The yeast-based phage engineering platform enabled the facile construction of these phages via one-step genome construction even though gene *11* and *12* are physically separated from gene *17*, a feat that no other phage engineering method can currently achieve. To further validate the ability of synthetic $T7_{K11(gp11\text{-}12\text{-}17)}$ to target *Klebsiella*, we performed a time-course experiment that

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

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

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

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

DISCUSSION

In this study, we utilized an efficient yet simple yeast-based platform for phage engineering to modulate phage host ranges for several members of the T7 phage family. Traditional phage engineering strategies, such as *in vitro* manipulation, allele-exchange within bacterial hosts, and phage crossing via co-infection of bacteria (Beier et al., 1977; Garcia et al., 2003; Lin et al., 2011) have been used to modulate phage host range (Tetart et al., 1998; Trojet et al., 2011; Yoichi et al., 2005), but these strategies are inefficient and unable to achieve multiple genetic modifications in a single step. Screening for a desired mutation after classical crossing or recombination experiments can require PCR, restriction digestion, or plaque hybridization on hundreds of individual plaques, which are all costly and time-consuming methods. Conversely, our strategy rarely requires the screening of more than a few yeast clones. Specifically, we found that at >25% of our yeast clones contained properly assembled phage genomes (composed of up to 11 DNA fragments) that could be used to generate functional plaques after transformation into bacteria. Previously, a scheme for engineering phage T4 through electroporation of PCR products was devised (Pouillot et al., 2010), but it is based on a particular feature of the genetic regulation of T4 and cannot easily be applied to other phage families. Recently, the 5.4 kb filamentous coliphage ϕX174 was assembled in yeast in order to stably store the genome and aid in phage refactoring (Jaschke et al., 2012). In this approach, the majority of the genome assembly was performed *in vitro* and the YAC cloning was mostly used to store the resulting genome, whereas the majority of the genome engineering in our approach stems from the actual

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

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

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

EXPERIMENTAL PROCEDURES

Strains, vector, and primers. Phages T7 (ATCC BAA-1025-B2, NC_001604) and T3 (ATCC 110303-B3, AJ318471) were laboratory stocks. Phages K1E (NC_007637), K1F (NC_007456), K1-5 (NC_008152), SP6 (NC_004831), and K11 (EU734173) were kindly provided by Ian Molineux (The University of Texas at Austin). Phage LUZ19 (NC_010326) was kindly provided by Rob Lavigne (KU Leuven). Phage gh-1 (ATCC 12633-B1, NC_004665) was obtained from ATCC. Synthetic phages are listed in Table S4. *Saccharomyces cerevisiae* BY4741 (*MAT*a *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*) was obtained from Thermo Scientific. *Escherichia coli* strains BL21 [B, F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm*], DH5α [K–12, F^- λ^- Φ80d *lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA*1 *endA*1 *hsdR*17 (r_K^- m_K^+) *phoA supE*44 *thi*-1 *gyrA*96 *relA*1], 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 *recA*1 *endA*1
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

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

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

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

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

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

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

Extraction of captured phage genomes. Individual yeast transformants were picked into SD-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 μl of extracted DNA were electroporated
416 into 20 - 25 μl cells in a 2 mm gap electroporation cuvette (Molecular BioProducts) at 2,500 V,
417 25 μF, 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 μl 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 μl 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 μl 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 μl 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 μl of ~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 μl 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 μl 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-(PFU of unadsorbed phage / original PFU in the BL21 and phage
451 mixture)]×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 (≈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%

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

Microbiome editing assays. Overnight cultures of *E. coli* Nissle 1917, *Klebsiella* sp. 390, and *Y. pseudotuberculosis* IP2666 were diluted 1:200 into LB and grown to log-phase (~10^8 CFU/ml), 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 treated with phage lysates (MOI ~0.1) and incubated at 30°C. At each time point, bacteria were collected, washed twice with 0.95% saline, serially diluted, plated onto LB, LB containing 25 μg/ml carbenicillin (VWR), and LB containing 1 μg/ml triclosan (VWR), and incubated at 30°C. Colonies were enumerated to calculate CFU/ml.

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

AUTHOR CONTRIBUTIONS

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

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590
591

FIGURE LEGENDS

Figure 1. Yeast platform for phage engineering. (**A**) Schematic illustrating the workflow to capture and reboot phages using our yeast platform. An entire phage genome or PCR products spanning an entire phage genome are transformed into yeast cells along with a linearized yeast replicon fragment from the yeast artificial chromosome, pRS415. In yeast, the phage genome is assembled and captured in the YAC by gap-repair cloning. The resulting YAC-phage DNA is extracted and transformed into host bacteria. Active phages are produced from the YAC-phage DNA and generate plaques on a lawn of host bacteria. (**B**) To determine the phage sensitivity of the *E. coli* 10G strain, high-titer phage lysates (>10^9 PFU/ml) were spotted onto 10G lawns. The 10G strain was sensitive only to infection by T7 and T3 phages. (**C**) Rebooting T7 and T3 phages from purified phage genomes. We electroporated 10 ng of genomic DNA into 10G, mixed chloroform-treated lysates with *E. coli* BL21 and LB soft agar, poured the mixtures onto LB plates, and incubated at 37°C. (**D**) One-time phage propagation assays. Rebooting phages from purified phage genomes via 10G. We electroporated 10-200 ng of phage genomic DNA into 10G and incubated the cells at 37°C for 1 h. We then treated the bacteria with chloroform. After centrifugation, supernatants were mixed with host bacteria and LB soft agar, poured onto LB plates, and incubated at 30 or 37°C. All tested phage genomes, including non-*E. coli* phages, could be rebooted in *E. coli* 10G cells. Host bacteria: IJ1668 K-12 hybrid; K1 capsule for K1E, K1F, and K1-5 phages, IJ612 *Salmonella typhimurium* LT2 for SP6 phage, *Pseudomonas aeruginosa* PAO1 for LUZ19 phage, *Pseudomonas putida* C1S for gh-1 phage, and *Klebsiella* sp. 390 for K11 phage. (**E**) An example of capturing and rebooting a phage through the yeast platform. An excised YAC pRS415 amplicon and the T7 genome were co-transformed in yeast 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 ($T7_{T3(C\text{-}gp17)}$). 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 μL
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 ~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 *17*). We synthesized 13a's gene *17* and assembled it with the rest of

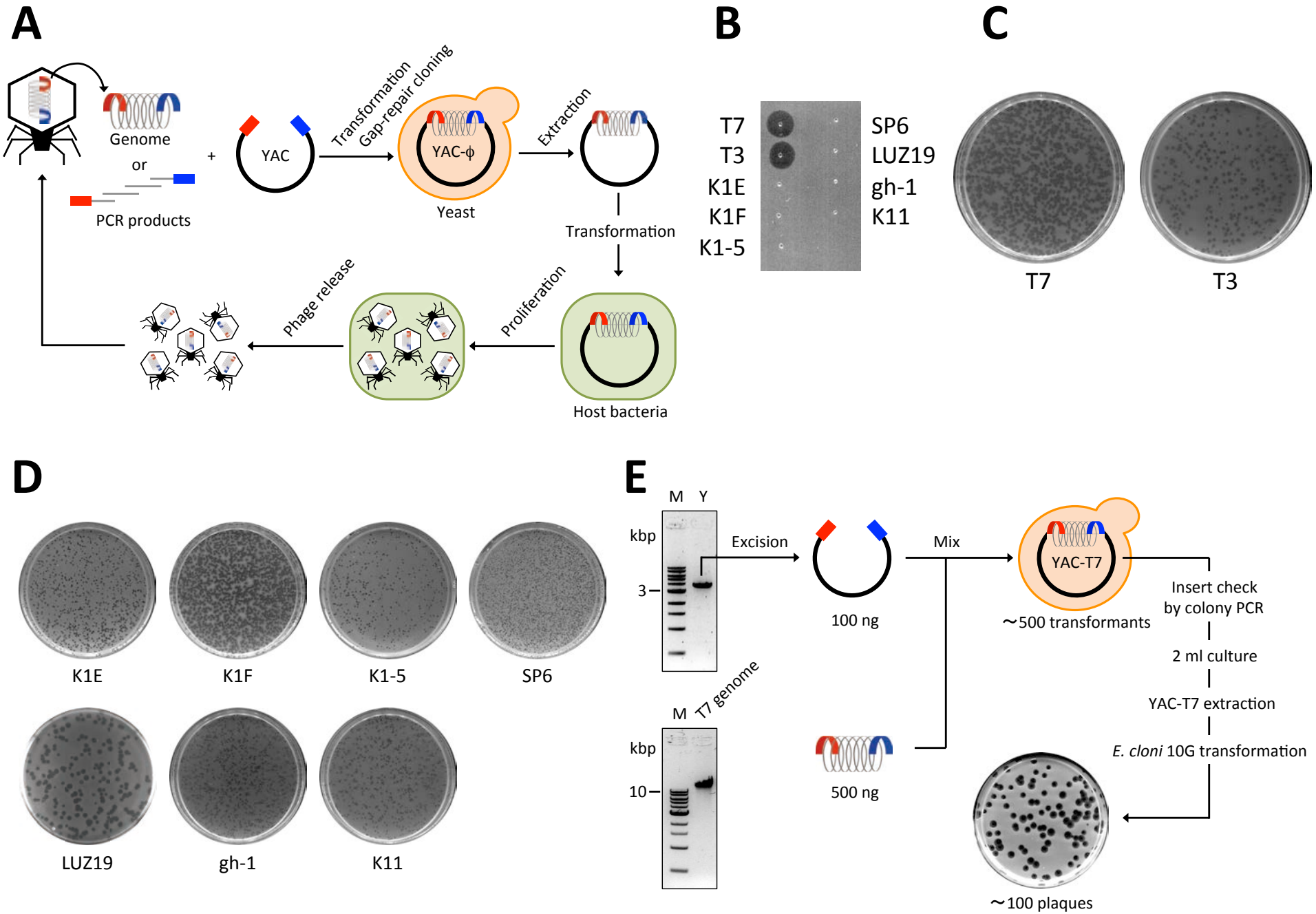
638 the T7 genome via overlapping PCR products in yeast. The YAC-phage DNA was extracted and
639 used for transformation. M, 1 kb DNA size marker (NEB). Y, YAC amplicon.
640
641 **Figure 3. Plaque formation assays with natural, reconstructed wild-type, and synthetic**
642 **phages.** Bacterial lawns were spotted with 2.5 μL 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 $T3_{R(gp17)}$. ~10^8 CFU/ml bacteria and ~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

components, a tubular structure and tail fibers. The tubular structure consists of an upper dodecameric ring made of adaptor protein gp11 and a pyramidal hexameric complex of the nozzle protein gp12. The tail fiber protein gp17 interacts with the interface between gp11 and gp12 (Cuervo et al., 2013). (**B**) Schematics illustrating the construction of synthetic hybrids between phages T7 and K11. Whole genomes were amplified as overlapping PCR amplicons as shown on the gel. Appropriate fragments were co-transformed and assembled in yeast. YAC-phage genomes were extracted and used for transformation. We swapped K11 genes *11*, *12* and *17* into T7 to create $T7_{K11(gp11\text{-}12\text{-}17)}$ and T7 genes *11, 12* and *17* into K11 to create $K11_{T7(gp11\text{-}12\text{-}17)}$. M, 1 kb DNA size marker (NEB). Y, YAC amplicon. (**C**) Plaquing of $T7_{K11(gp11\text{-}12\text{-}17)}$ and $K11_{T7(gp11\text{-}12\text{-}17)}$ on *E. coli* BL21 and *Klebsiella* sp. 390. Ten-fold serial dilutions of phage lysates were spotted on bacterial lawns and incubated for 4 h at 37°C. These pictures were cut from Figure 3. Bottom panels show images of individual plaques. NP, no plaque. (**D**) Killing curves of *Klebsiella* sp. 390 treated with $T7_{K11(gp11\text{-}12\text{-}17)}$. ~$10^8$ CFU/ml bacteria and ~10^7 PFU/ml phage were used (MOI ~0.1). The data are presented as the mean of three independent experiments, and the error bars represent the SEM. Small error bars are obscured by symbols. The detection limit was 2.0×10^3 CFU/ml.

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

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

Figure 1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T3
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K1F
K1-5
SP6
LUZ19
gh-1
K11
C
T7
T3
D
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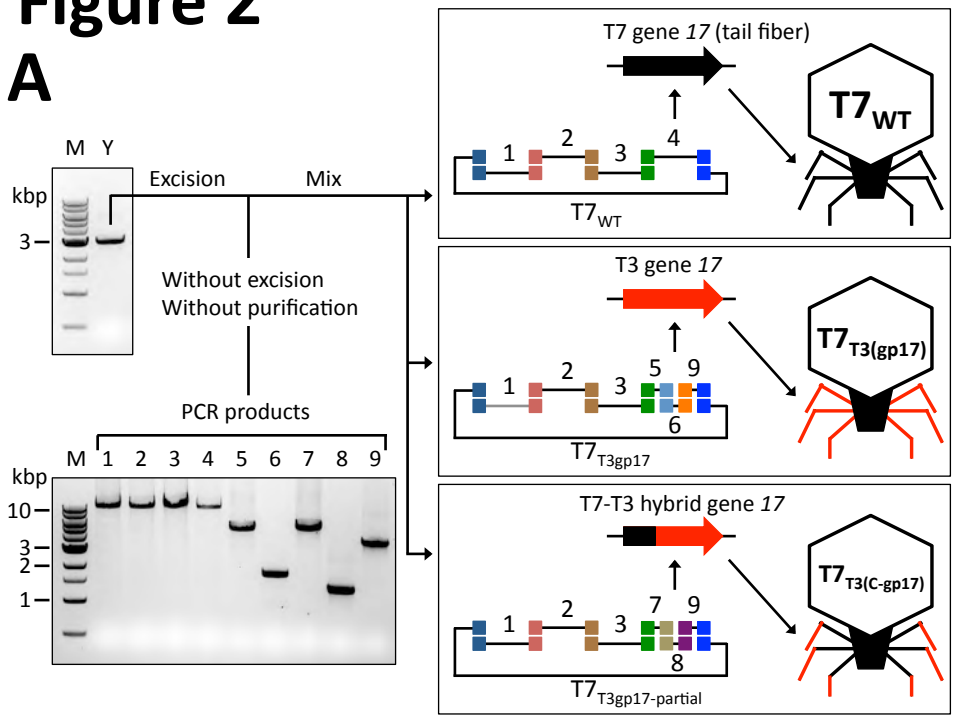
Figure 2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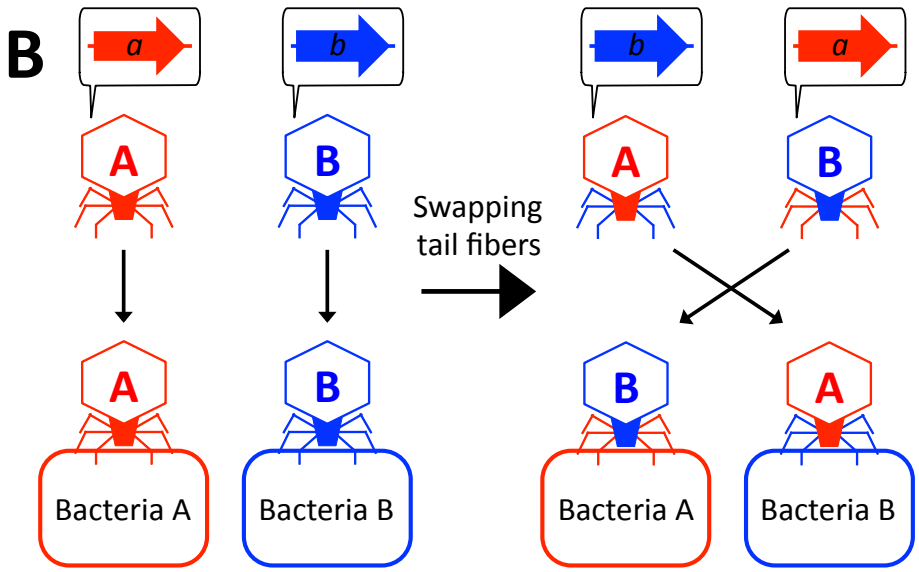
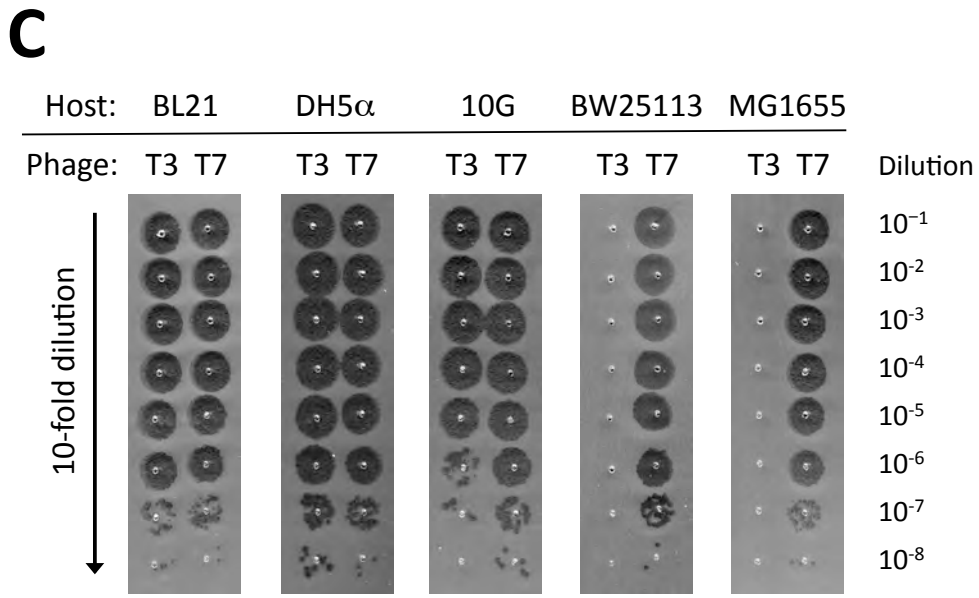
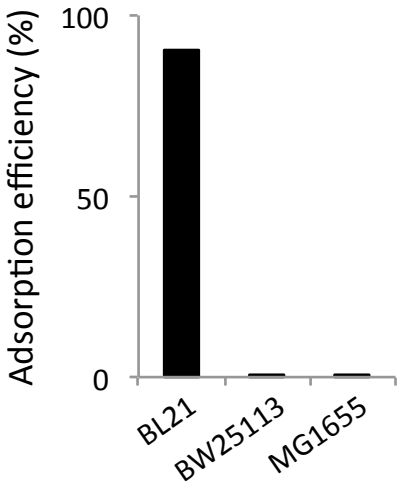
PCR products
M 1 2 3 4 5 6 7 8 9
kbp
10
3
2
1
T7 gene 17 (tail fiber)
T7WT
T7WT
T3 gene 17
T7T3(gp17)
T7T3gp17
T7-T3 hybrid gene 17
T7T3(C-gp17)
T7T3gp17-partial


B
a
b
A
B
Bacteria A
Bacteria B
Swapping
tail fibers


C
Host: BL21 DH5α 10G BW25113 MG1655
Phage: T3 T7 T3 T7 T3 T7 T3 T7 T3 T7
Dilution
10-fold dilution
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8


D
Adsorption efficiency (%)
100
50
0
BL21
BW25113
MG1655

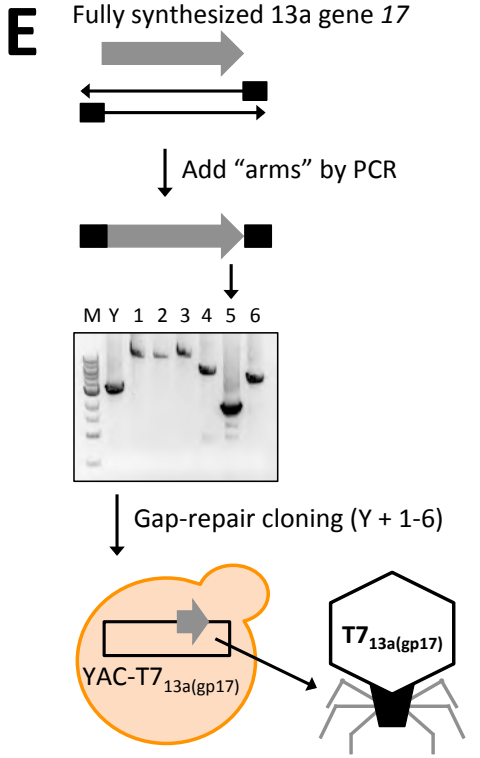

E
Fully synthesized 13a gene 17
Add "arms" by PCR
M Y 1 2 3 4 5 6
Gap-repair cloning (Y + 1-6)
YAC-T713a(gp17)
T713a(gp17)

Figure 3

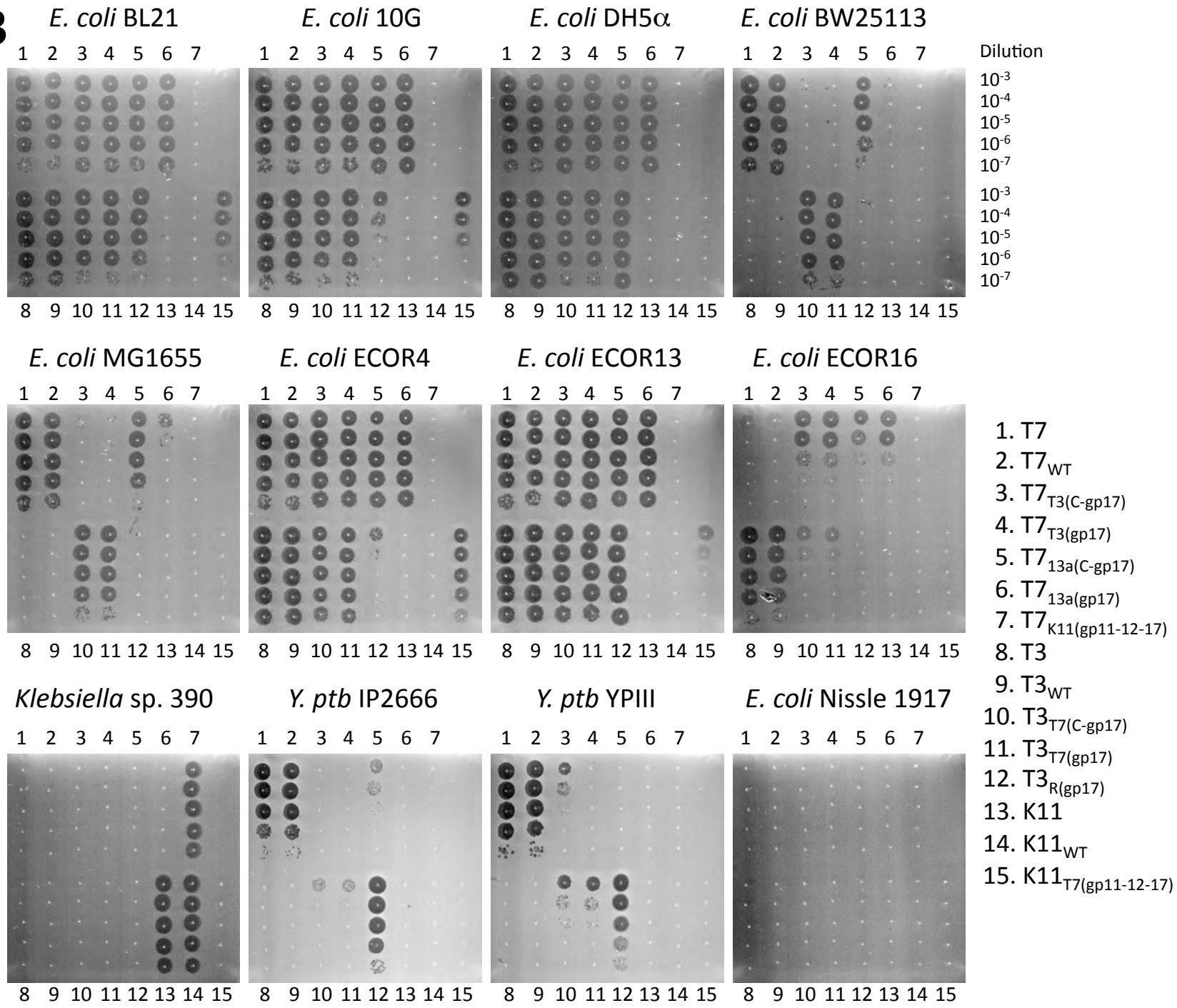
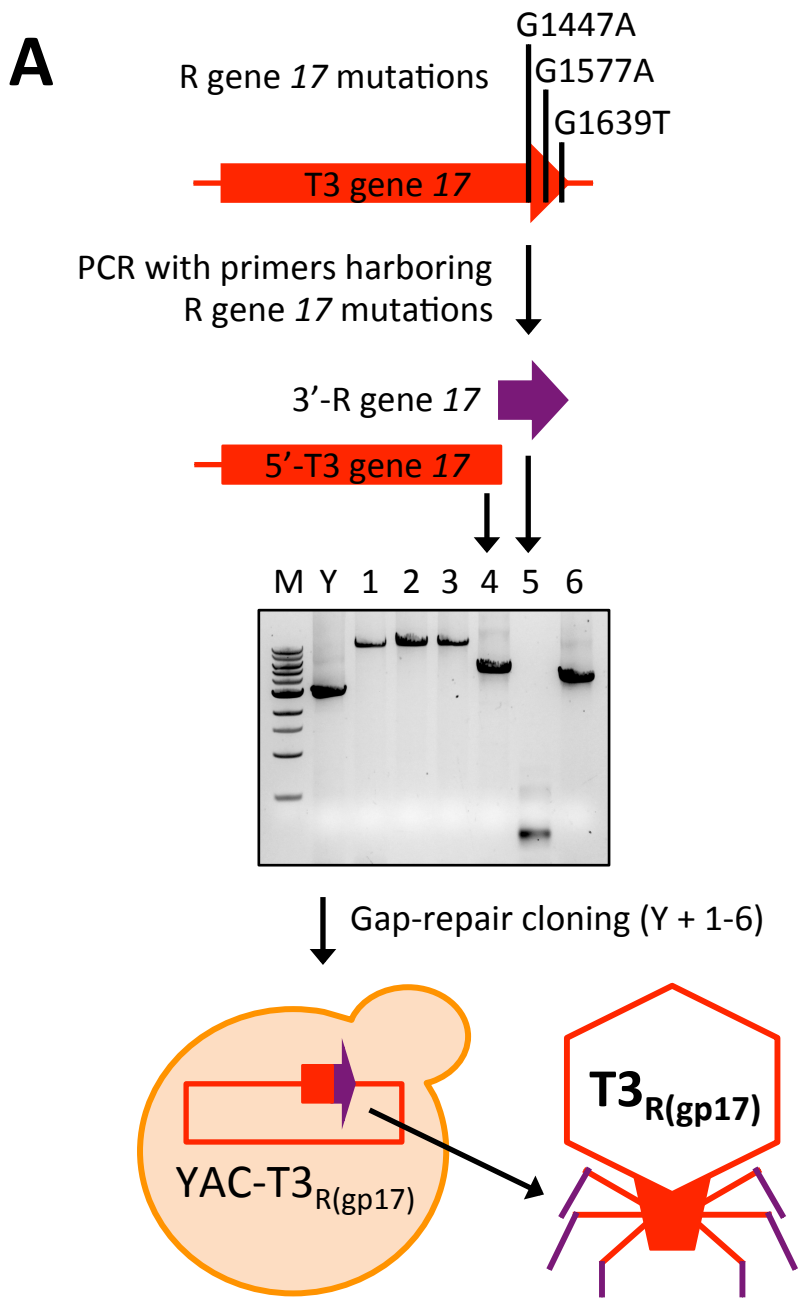
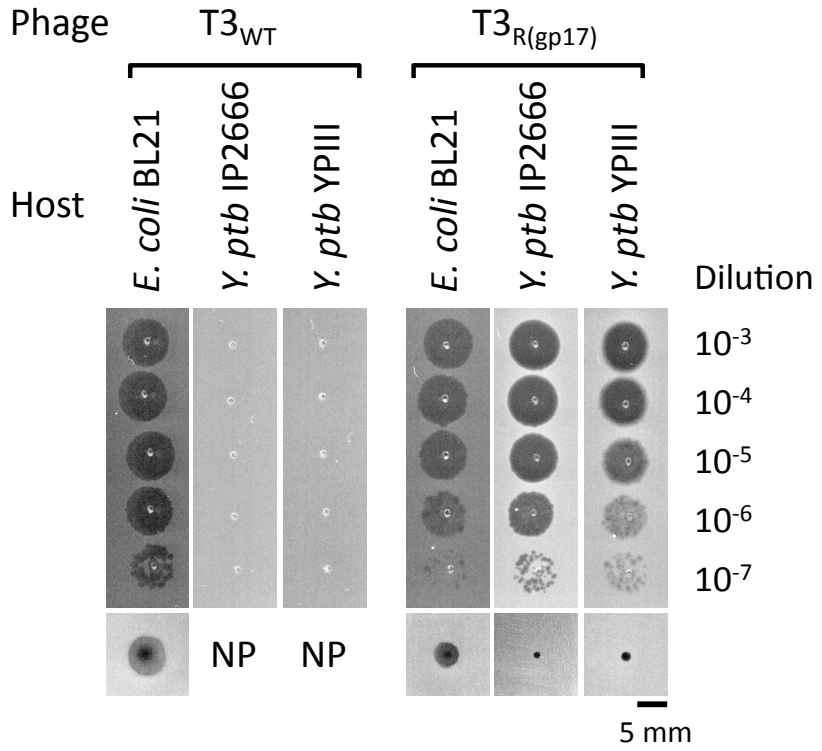

E. coli BL21
E. coli 10G
E. coli DH5α
E. coli BW25113
E. coli MG1655
E. coli ECOR4
E. coli ECOR13
E. coli ECOR16
Klebsiella sp. 390
Y. ptb IP2666
Y. ptb YPIII
E. coli Nissle 1917
1 2 3 4 5 6 7
8 9 10 11 12 13 14 15
Dilution
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
1. T7
2. $T7_{WT}$
3. $T7_{T3(C\text{-}gp17)}$
4. $T7_{T3(gp17)}$
5. $T7_{13a(C\text{-}gp17)}$
6. $T7_{13a(gp17)}$
7. $T7_{K11(gp11\text{-}12\text{-}17)}$
8. T3
9. $T3_{WT}$
10. $T3_{T7(C\text{-}gp17)}$
11. $T3_{T7(gp17)}$
12. $T3_{R(gp17)}$
13. K11
14. $K11_{WT}$
15. $K11_{T7(gp11\text{-}12\text{-}17)}$

Figure 4


A
R gene 17 mutations
G1447A
G1577A
G1639T
T3 gene 17
PCR with primers harboring
R gene 17 mutations
3'-R gene 17
5'-T3 gene 17
M Y 1 2 3 4 5 6
Gap-repair cloning (Y + 1-6)
YAC-T3R(gp17)
T3R(gp17)


B
Phage
T3WT
T3R(gp17)
Host
E. coli BL21
Y. ptb IP2666
Y. ptb YPIII
E. coli BL21
Y. ptb IP2666
Y. ptb YPIII
Dilution
10-3
10-4
10-5
10-6
10-7
NP
NP
5 mm

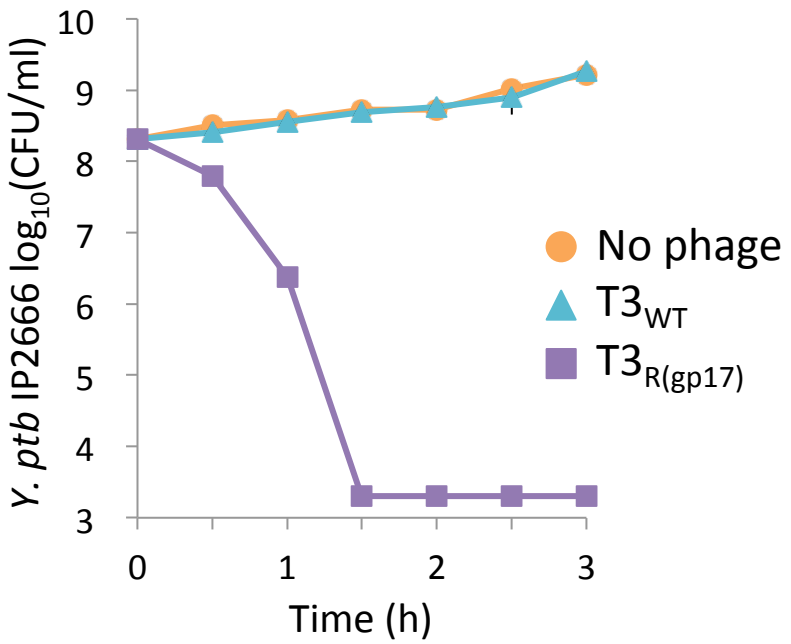
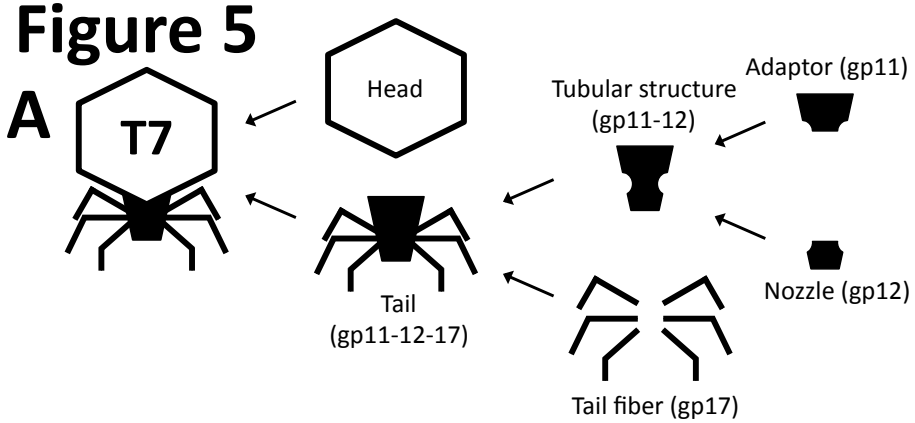
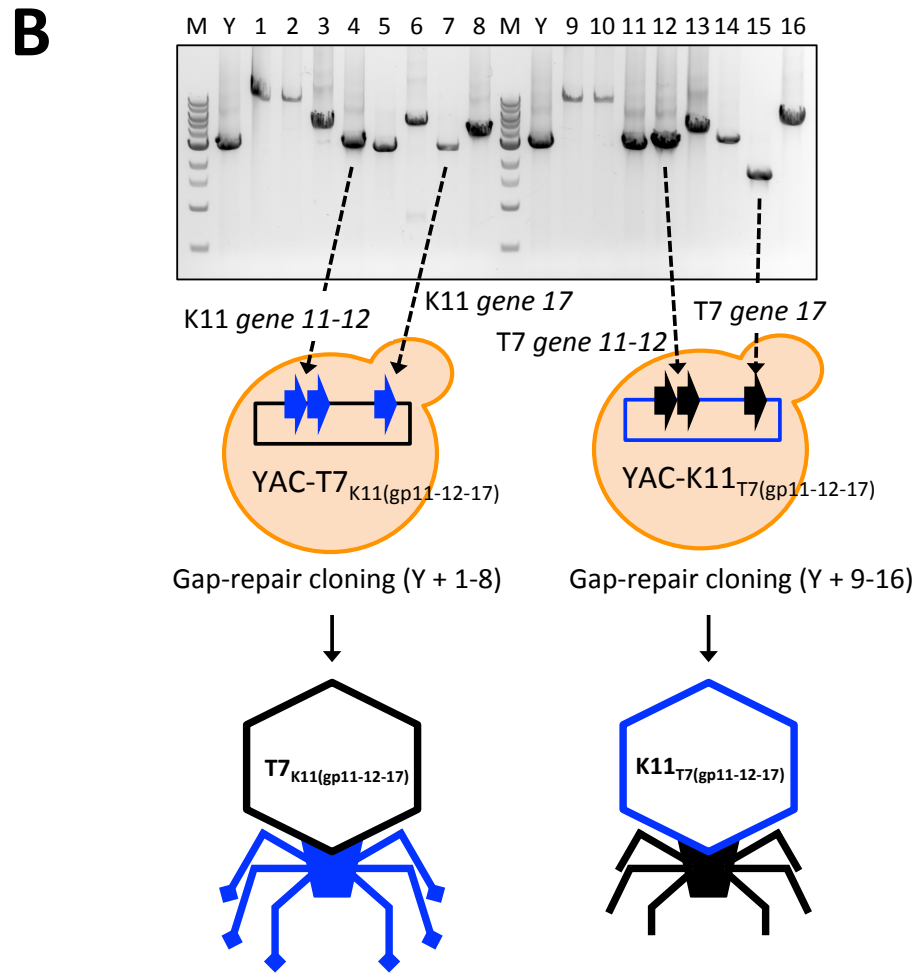
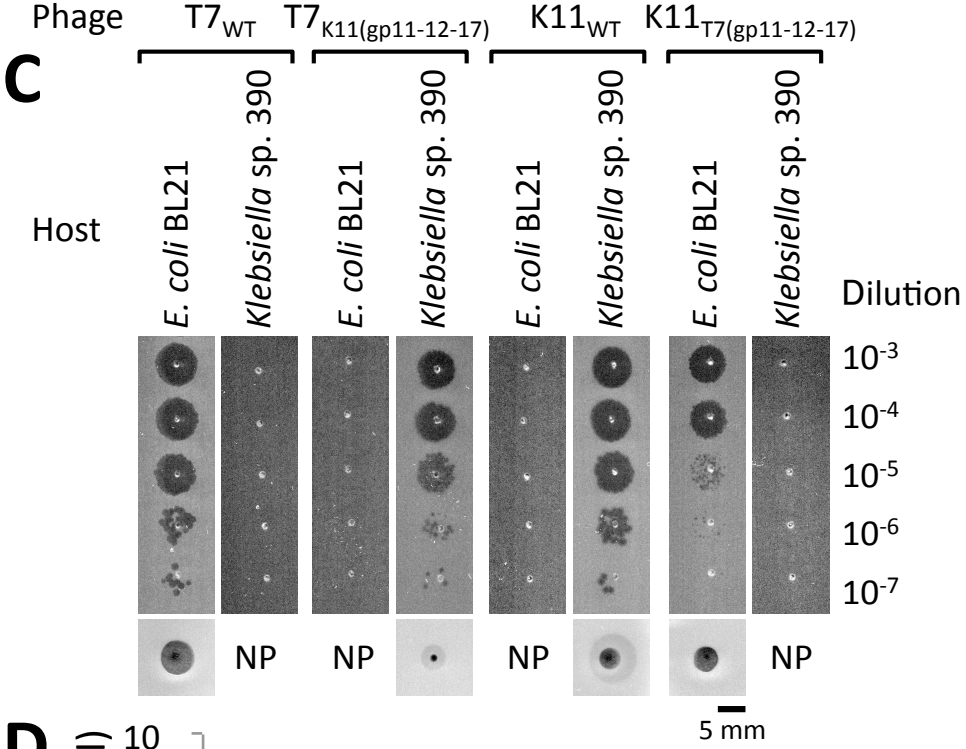

C
Y. ptb IP2666 log10(CFU/ml)
10
9
8
7
6
5
4
3
0
1
2
3
Time (h)
No phage
T3WT
T3R(gp17)

Figure 5


A
T7
Head
Tubular structure
(gp11-12)
Adaptor (gp11)
Tail
(gp11-12-17)
Nozzle (gp12)
Tail fiber (gp17)


B
M Y 1 2 3 4 5 6 7 8 M Y 9 10 11 12 13 14 15 16
K11 gene 11-12
K11 gene 17
T7 gene 11-12
T7 gene 17
YAC-T7K11(gp11-12-17)
YAC-K11T7(gp11-12-17)
Gap-repair cloning (Y + 1-8)
Gap-repair cloning (Y + 9-16)
T7K11(gp11-12-17)
K11T7(gp11-12-17)


C
Phage
T7WT
T7K11(gp11-12-17)
K11WT
K11T7(gp11-12-17)
Host
E. coli BL21
Klebsiella sp. 390
Dilution
10-3
10-4
10-5
10-6
10-7
NP
5 mm

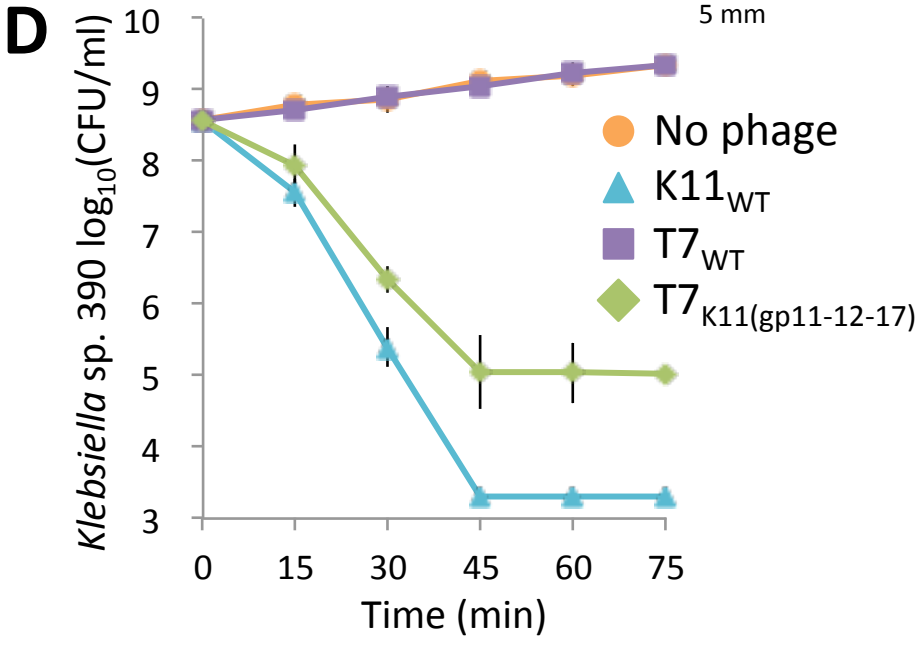
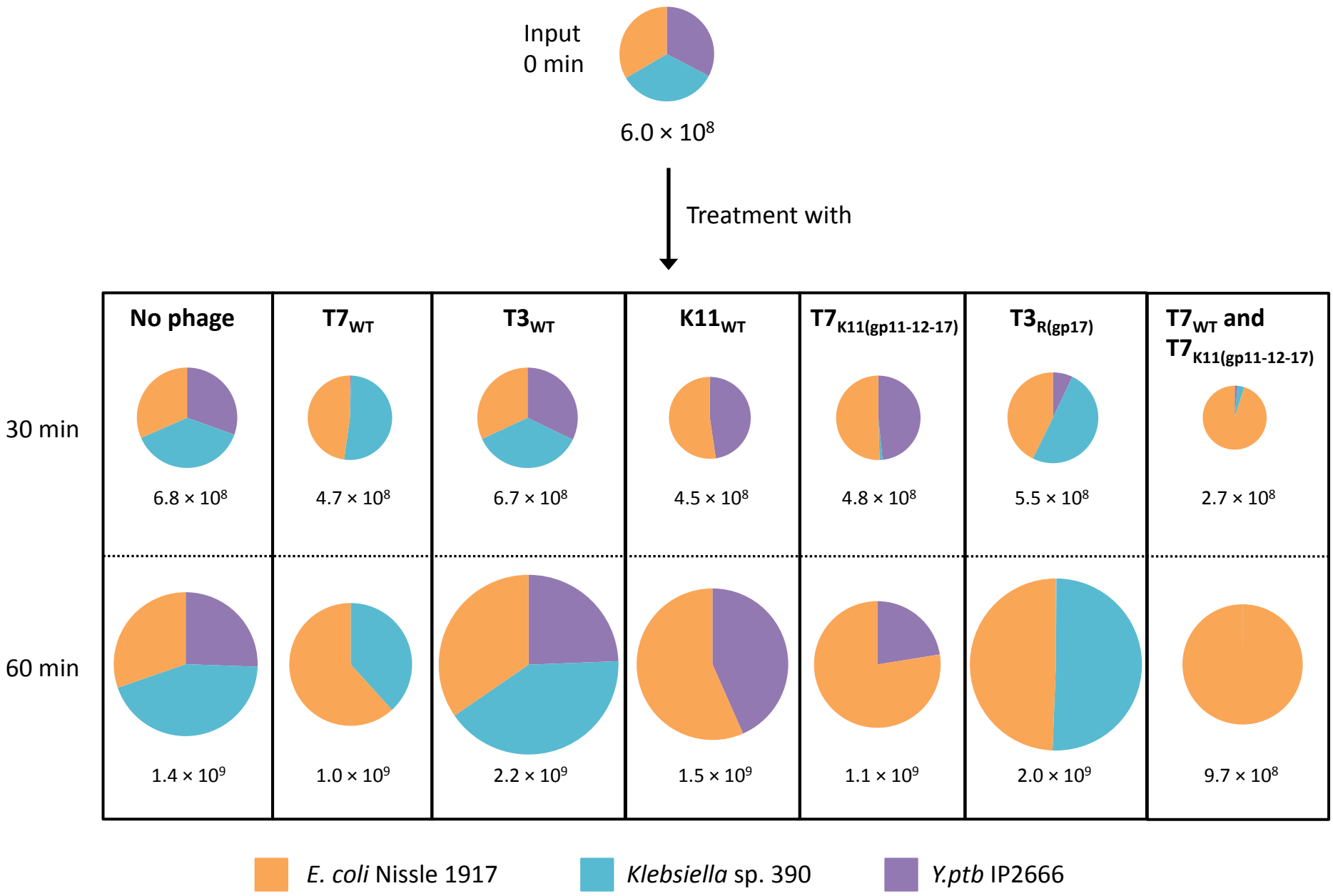

D
Klebsiella sp. 390 log10(CFU/ml)
Time (min)
No phage
K11WT
T7WT
T7K11(gp11-12-17)

Figure 6


Input
0 min
6.0 × 10^8
Treatment with
No phage
T7WT
T3WT
K11WT
T7K11(gp11-12-17)
T3R(gp17)
T7WT and T7K11(gp11-12-17)
30 min
6.8 × 10^8
4.7 × 10^8
6.7 × 10^8
4.5 × 10^8
4.8 × 10^8
5.5 × 10^8
2.7 × 10^8
60 min
1.4 × 10^9
1.0 × 10^9
2.2 × 10^9
1.5 × 10^9
1.1 × 10^9
2.0 × 10^9
9.7 × 10^8
E. coli Nissle 1917
Klebsiella sp. 390
Y.ptb IP2666

Supplemental Information

Figure S1. Plaque formation assays with $T7_{K11(gp11\text{-}12\text{-}17)}$ and $K11_{T7(gp11\text{-}12\text{-}17)}$, related to Figure 5. To confirm correct EOPs of $T7_{K11(gp11\text{-}12\text{-}17)}$ and $K11_{T7(gp11\text{-}12\text{-}17)}$ phages, 2.5 μL of 10-fold serially diluted phages were spotted onto bacterial lawns and incubated at 37°C. $K11_{T7(gp11\text{-}12\text{-}17)}$ adopted the host range of $T7_{WT}$ while $T7_{K11(gp11\text{-}12\text{-}17)}$ adopted the host range of K11, thus demonstrating that tail component swapping can lead to acquisition of novel host ranges.

Figure S2. Antimicrobial susceptibilities of *E. coli* Nissle 1917, *Klebsiella* sp. 390, and *Y. pseudotuberculosis* IP2666, related to Figure 6. (**A**) Five microliters of each overnight cultures (>10^9 CFU/ml) for each bacteria were streaked on LB plates with or without antibiotics. Plates were incubated at 30°C for 24 h. *Klebsiella* sp. 390 was naturally resistant to 25 mg/ml carbenicillin and *Y. ptb* IP2666 was naturally resistant to 1 mg/ml triclosan, while *E. coli* Nissle 1917 was sensitive to both. (**B**) Diluted log-phase cultures were plated onto LB with or without antibiotics. After incubation at 30°C for 18-24 h, colonies were enumerated.

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

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

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

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

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

Figure S1

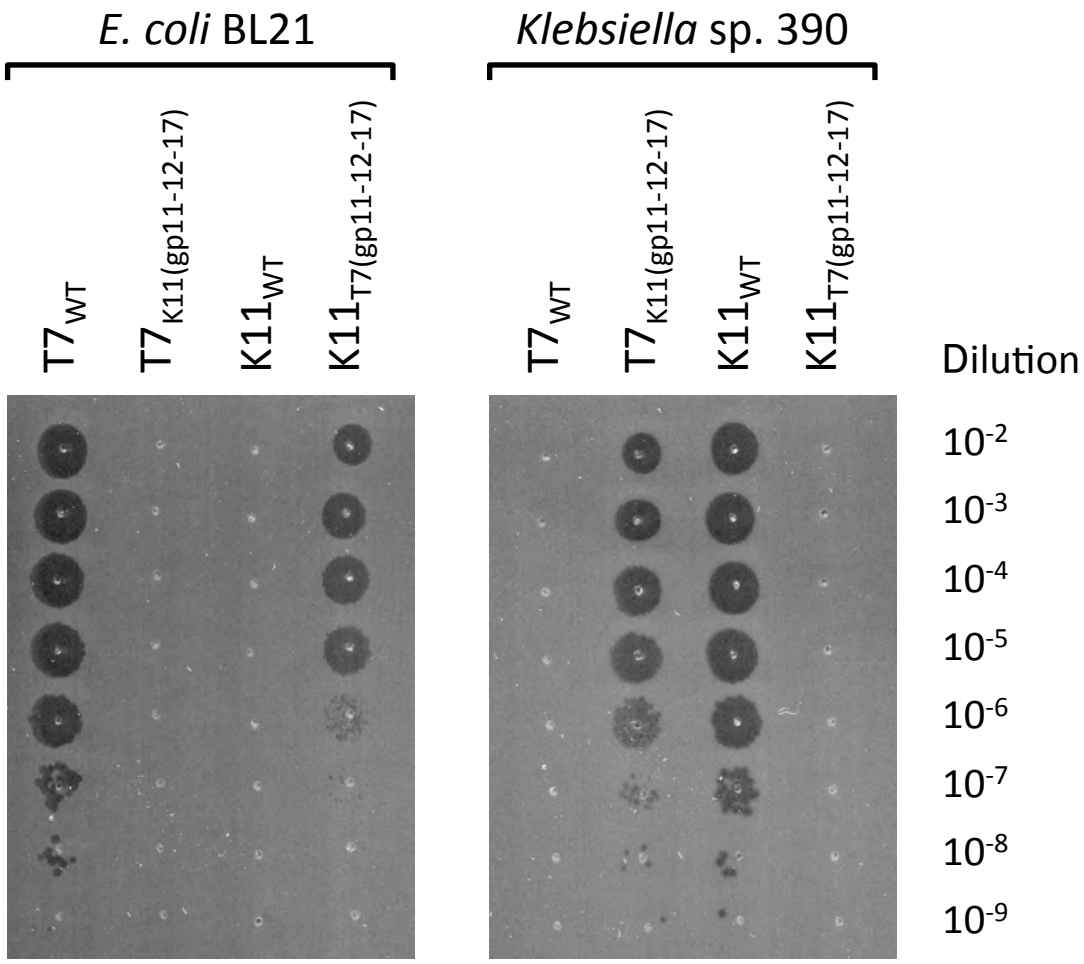
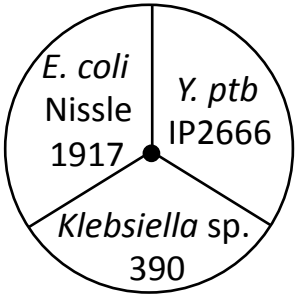

E. coli BL21
Klebsiella sp. 390
$T7_{WT}$
$T7_{K11(gp11-12-17)}$
$K11_{WT}$
$K11_{T7(gp11-12-17)}$
$T7_{WT}$
$T7_{K11(gp11-12-17)}$
$K11_{WT}$
$K11_{T7(gp11-12-17)}$
Dilution
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}

Figure S2

A


E. coli
Nissle
1917
Y. ptb
IP2666
Klebsiella sp.
390

B

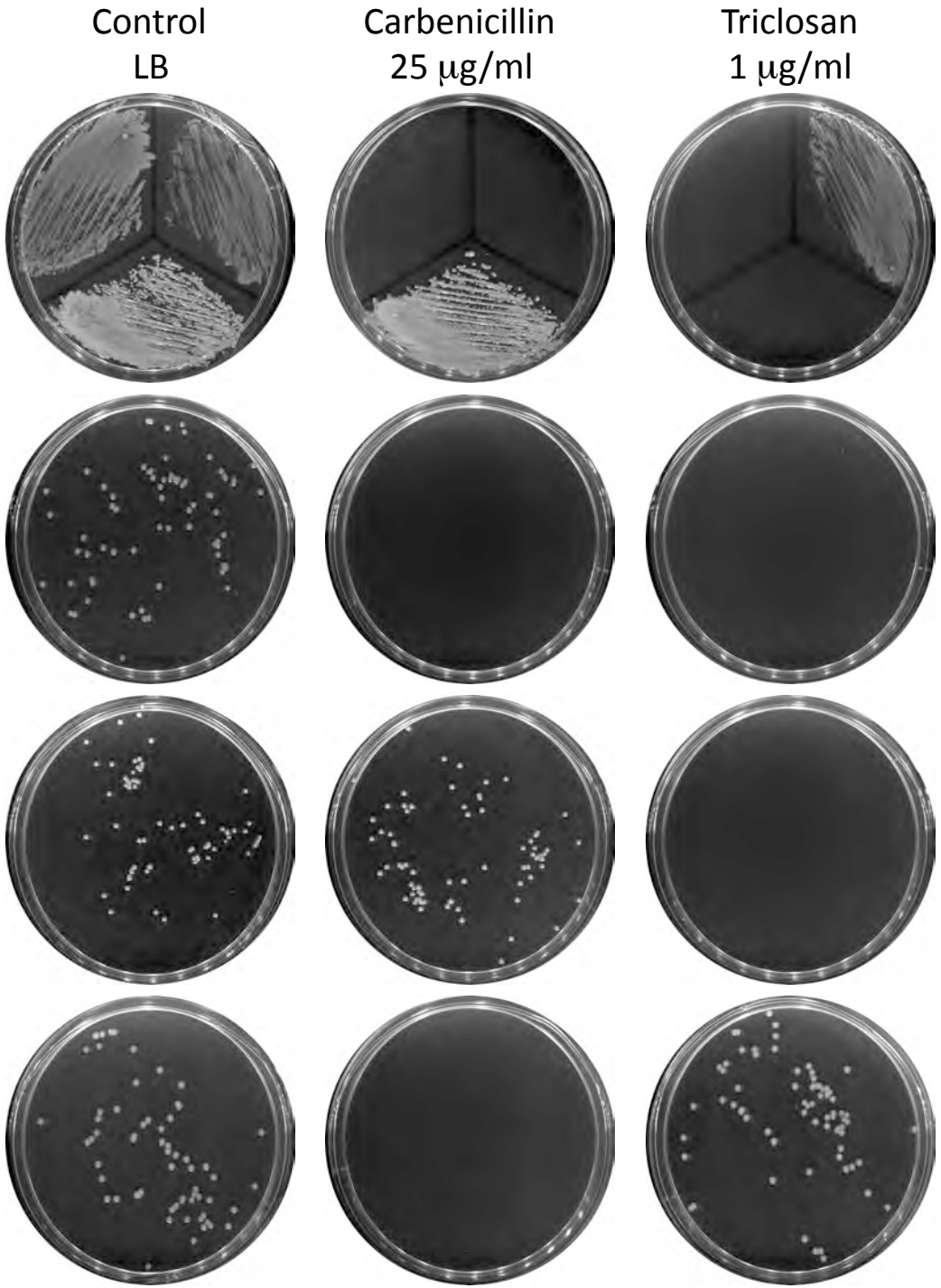

Control
LB
Carbenicillin
25 μg/ml
Triclosan
1 μg/ml
E. coli Nissle 1917
Klebsiella sp. 390
Y. ptb IP2666

Table S1

One-time phage propagation assay.

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

Table S2

Sequence of codon-optimized 13a gene *17*.

ATGGCGAATGTGATTAAGACCGTTCTGACGTATCAGTTAGATGGATCCAATAGCGATTTTAATATTCCATTTGAAT
ACCTGGCGCGCAAATTTGTCGCCGTGACGCTGATTGGGGTTGATCGCAAGGTATTAACCATTAACACCGACTAT
CGCTTTGCTACGCGCACGACCATCTCTCTCACTAAGGCGTGGGGACCGGCGGATGGTTATACTACCATCGAGTT
GCGCCGCGTTACGTCGACAACTGACCGCCTCGTGGACTTTACCGACGGTAGCATTCTGCGTGCGTACGATCTTA
ACGTGGCCCAGATTCAGACAATCCACGTCGCAGAAGAAGCACGTGACCTGACTGCCGACACCATTGGCGTAAA
CAATGACGGCCATCTGGATGCGCGCGGTCGCCGTATTGTCAATTTGGCGAACGCCGTTGATGATCGTGATGCGG
TGCCGCTCGGCCAACTCAAAACTATGAATCAGAACAGTTGGCAGGCTCGCAACGAGGCACTGCAATTCCGCAA
TGAAGCCGAAACTTTTCGCAATCAGGCGGAAGGTTTTAAAAACGAGAGCGGTACTAACGCCACTAACACGAA
ACAGTGGCGCGACGAGACAAAAGGCTTCCGCGACGAAGCGGAACAATTTAAGAACACCGCGGGTCAGTATG
ATACATCCGCGGGTAACAGCGCGAGCGCTGCCCATCAGAGCGAAGTAAACGCAGAAAACAGTGCGACCGCGT
CCGCTAATAGCGCCCACCTCGCCGAGCAACAGGCCGATCGCGCGGAGCGTGAAGCTGATAAACTCGGCAACTT
TAATGGTCTTGCTGGTGCGATCGACAAGGTCGACGGCACAAACGTGTATTGGAAAGGTAACATCCATGCAAAC
GGTCGCCTCTACATCACGACCAATGGCTTCGACTGCGGTCAATATCAGCAGTTCTTTGGTGGCGATACGAATCG
CTACAGCGTGATGGAATGGGGTGACGATAACGGGTGGCTGATGTATGTGCAGCGCCGTGAATGGACCACCGCA
ATCGGCGGCAACATTCAGTTGGTTGTCAACGGTCAGATCATCACCCAGGGCGGTGCCATGACGGGGCAACTGA
AACTTCAGAATGGTCACGTTCTGCAATTGGAATCCGCCAGTGACAAAGCGCATTATATTTTATCAAAGGATGGC
AACCGTAATAATTGGTATATTGGTCGTGGATCGGATAACAATAACGACTGCACGTTTCACTCCTACGTTCACGGT
ACCACGTTAACCTTGAAACAGGATTATGCAGTGGTCAACAAACACTTTCATGTGGGACAGGCGGTCGTGTCAA
CTGATGGCAATATCCAGGGCACCAAATGGGGTGGTAAATGGCTGGATGTTTATTTAAATGATACATACGTTAAGA
AAACGATGGCTTGGACACAAGTGTGGGCTGCGGACTCGGGTAAATACCTCCCGGGTGGGAGTCAAACTGATA
CTCTGCCGCAGGATCTGCGTTTCCGCAACATCTGGATTCGTACGCGTAACAACTATTGGAACTTTTTTCGCACGG
GCCCGGATGGGATCTATTTCCTGTCGGCTGAAGGAGGTTGGCTGAAATTTCAGATCCACTCAAACGGTCGCGT
CTTCAAAAACATCTCTGATCGCGACGCACCCCCGACCGCAATCGCTGTGGAAGACGTTTAA

Table S3

Microbiome editing assay.

Treatment	Time after treatment	log_{10} (mean ± SEM CFU/ml) *E. coli* Nissle 1917	*Klebsiella* sp. 390	*Y. ptb* IP2666
	0 min	8.300 ± 0.036	8.307 ± 0.086	8.290 ± 0.155
No phage	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
$K1\text{-}5_{WT}$	K1-5 wild-type	Synthesized from PCR products
$SP6_{WT}$	SP6 wild-type	Synthesized from PCR products
$gh\text{-}1_{WT}$	gh-1 wild-type	Synthesized from PCR products
$K11_{WT}$	K11 wild-type	Synthesized from PCR products
$T7_{T3(C\text{-}gp17)}$	$T7_{WT}$ gene *17* (1-447)-T3 gene *17* (448-1677)	T7 with T7-T3 hybrid tail fiber
$T7_{T3(gp17)}$	$T7_{WT}$ Δgene *17*, T3 gene *17*	T7 with T3 tail fiber
$T7_{13a(C\text{-}gp17)}$	$T7_{WT}$ gene *17* (1-450)-13a gene *17* (451-1677)	T7 with T7-13a hybrid tail fiber
$T7_{13a(gp17)}$	$T7_{WT}$ Δgene *17*, 13a gene *17*	T7 with 13a tail fiber
$T7_{K11(gp11\text{-}12\text{-}17)}$	$T7_{WT}$ Δgene (*11 12 17*), K11 gene (*11 12 17*)	T7 with K11 tail
$T3_{T7(C\text{-}gp17)}$	$T3_{WT}$ gene *17*(1-447)-T7 gene *17*(448-1662)	T3 with T3-T7 hybrid tail fiber
$T3_{T7(gp17)}$	$T3_{WT}$ Δgene *17*, T7 gene *17*	T3 with T7 tail fiber
$T3_{R(gp17)}$	$T3_{WT}$ Δgene *17*, R gene *17*	T3 with R tail fiber
$K11_{T7(gp11\text{-}12\text{-}17)}$	$K11_{WT}$ Δgene (*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	GTGTTACCTTGAGTGTCTCTCTGTGTCCCT**TGTCTCATGAGCGGATACATATTTGAATGT**	To amplify YAC for capturing T7 genomic DNA
5'T7-pRS415-R-2	GGGGGAACTTTAGGTCCGTACACTGTGAGA**CCTTGTTCATGTGTGTTCAAAAACGTTATA**	
3'T3-pRS415-F-4	CAGTATGATAGTACATCTCTATGTGTCCCT**TGTCTCATGAGCGGATACATATTTGAATGT**	To amplify YAC for capturing T3 genomic DNA
5'T3-pRS415-R-2	GGGGGTACTTTGGGTTCTTGAACTATGAGA**CCTTGTTCATGTGTGTTCAAAAACGTTATA**	
LUZ19_ASB_Y2_Fw	TCCTGTCGGGTGGTGGTGCGGGAGTGGCTA**TGTCTCATGAGCGGATACATATTTGAATGT**	To amplify YAC for capturing LUZ19 genomic DNA
LUZ19_ASB_Y2_Rev	GGAAGGGTGGGCTGATCAGAGTCGGGAGGG**CCTTGTTCATGTGTGTTCAAAAACGTTATA**	
pRS415-F-4	**TGTCTCATGAGCGGATACATATTTGAATGT**	To amplify YAC for capturing PCR products of T7, T3, gh-1, K11, and synthetic phages
pRS415-R-2	**CCTTGTTCATGTGTGTTCAAAAACGTTATA**	
PST255	**CCTGTACTTCCTTGTTCATGTGTGTTCAAA**	To amplify YAC for capturing PCR products of K1E, K1F, K1-5, and SP6
PST256	**ATAAACAAATAGGGGTTCCGCGCACATTTC**	
pRS415-R-2-1-30-F	**TATAACGTTTTTGAACACACATGAACAAGG**TCTCACAGTGTACGGACCTAAAGTTCCCCC	
9971-10000-R	ATTACGCGATGACAGTAGACAACCTTTCCG	For $T7_{WT}$
9960-9989-F	TGCAGCAATACCGGAAAGGTTGTCTACTGT	Primer pairs:
19930-19959-R	ATATGTCTCCTCATAGATGTGCCTATGTGG	pRS415-R-2-1-30-F and 9971-10000-R
19920-19949-F	ACTTGTGACTCCACATAGGCACATCTATGA	9960-9989-F and 19930-19959-R
29890-29919-R	GAATAACCTGAGGGTCAATACCCTGCTTGT	19920-19949-F and 29890-29919-R
29880-29909-F	GACATGATGGACAAGCAGGGTATTGACCCT	29880-29909-F and pRS415-F-4-39908-39937-R
pRS415-F-4-39908-39937-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGAGACACTCAAGGTAACAC	
pRS415-R-2-T3-1-30-F	**TATAACGTTTTTGAACACACATGAACAAGG**TCTCATAGTTCAAGAACCCAAAGTACCCCC	
T3-9971-10000-R	ACGGAACCTCCTTCTTGGGTTCTTTGACGC	For $T3_{WT}$
T3-9961-9990-F	CCAGTGGCTGGCGTCAAAGAACCCAAGAAG	Primer pairs:
T3-19931-19960-R	GGAAGTCGGTTCATCGCTAAGCACGATTGC	pRS415-R-2-T3-1-30-F and T3-9971-10000-R
T3-19921-19950-F	TGGCGATGATGCAATCGTGCTTAGCGATGA	T3-9961-9990-F and T3-19931-19960-R
T3-29891-29920-R	GATGCAACGTTCAGCGCAGCACTTTCGGCA	T3-19921-19950-F and T3-29891-29920-R
T3-29881-29910-F	TTGTAGTTGGTGCCGAAAGTGCTGCGCTGA	T3-29881-29910-F and pRS415-F-4-T3-38179-38208-R
pRS415-F-4-T3-38179-38208-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACATAGAGATGTACTATCATACTG	
K1E-1	**TTTGAACACACATGAACAAGGAAGTACAGG**TCTCGCCCTCGCCCTCGCCGGATTTTCCCC	
K1E-2	TAGTTATATGCTAATTCATTGTGGTACTTAT	For $K1E_{WT}$
K1E-3	CAGGTCTAAGAAAGAGGCTGCTTTGTTTGA	Primer pairs:
K1E-4	AGTGGAGAACTTCTCCCATAGCTTAGGTAT	K1E-1 and K1E-2
K1E-5	ATTTGGAGTATGGAGGTAAGCGGTCTAAGA	K1E-3 and K1E-4
K1E-6	GCTTATTGCGCTCAAGGTCTTCATCAGCAT	K1E-5 and K1E-6
K1E-7	CTGAGGATGAATTGAACTTCCGTATTCAGA	K1E-7 and K1E-8
K1E-8	**GAAATGTGCGCGGAACCCCTATTTGTTTAT**TAGCCCACACCCTCACACCCTGTCAAACAC	
K1F-1	**TTTGAACACACATGAACAAGGAAGTACAGG**TCTCACAGTTCAAGAACCTCAAGTCTCCCC	
K1F-2	ACGATGAACGTCTGGCCCACACCAATCTTG	For $K1F_{WT}$
K1F-3	GACTGTGAAGCGTGAAGTCCTGACAATCGA	Primer pairs:
K1F-4	AAGTTGTGTTGTTCCTATAGTGATAGTTTA	K1F-1 and K1F-2
K1F-5	CAGGGAGCTGGTGAGGACATGGCTCAAGCC	K1F-3 and K1F-4
K1F-6	GTCAGCGAACTTAACGCCTTGGTGACTAAA	K1F-5 and K1F-6
K1F-7	CGTATGCCAATCCGTGAAGGAGAGGAGACC	K1F-7 and K1F-8
K1F-8	**GAAATGTGCGCGGAACCCCTATTTGTTTAT**GAGACTCAGAGACAGACCAATAGTCACCAC	
K1-5*1	**GTTTTTGAACACACATGAACAAGGAAGTACAGG**TCGCCCTCGCCCTCGCCGGGTTGT	
K1-5*2	GGAGAGTCAGAGGGCTTAAGGTTTACTGCT	
K1-5*3	TGCTATGCTACGCGATGCAGTAGGTGCGAA	
K1-5*4	CAGGGTCACGCATCTCATATGGGTCGAAGA	
K1-5*5	TGGACTTGCTCACCACTGAGGAGTTCCTCT	For $K1\text{-}5_{WT}$
K1-5*6	GCTTTGTCAGCCTGCTCAGGGAAGCAAGCA	Primer pairs:
K1-5*7	TAACTTCGCTGCTGGTCTGGAGTTCGCTCG	K1-5*1 and K1-5*2
K1-5*8	TGTGCACTTTGTTCTGCATTCCATGAGGCT	K1-5*3 and K1-5*4
K1-5*9	TGTGCATCTCTTAATAGAGACCCACCACTC	K1-5*5 and K1-5*6
K1-5*10	AAGAAGCTGAGTGGCTATCTGCTGCGCAGT	K1-5*7 and K1-5*8
K1-5*11	TCTAAGGATGCAGATCAGACTAAGCTAGCC	K1-5*9 and K1-5*10
K1-5*12	GCCTTAGCTCGTAACTCTTCTTCCGCAATA	K1-5*11 and K1-5*12
K1-5*13	TAAAACCGAAGTGTCAGACTTAGGTAAAGC	K1-5*13 and K1-5*14
K1-5*14	TATTGCCGCCCCAGCTTACATTCTGTTTAA	K1-5*15 and K1-5*16
K1-5*15	TTGACGGGTTTTATCCAGAAGGATACTTCA	K1-5*17 and K1-5*18
K1-5*16	GCTATCTCCTATTACTTTCCAACCCTCCCT	
K1-5*17	TTGAGCGGCCTATTACTAGCCAATCTTCAT	
K1-5*18	**GAAATGTGCGCGGAACCCCTATTTGTTTAT**TAGCCCACGCCCTCACACCCTGTCAATCCC	
SP6-1	**TTTGAACACACATGAACAAGGAAGTACAGG**TCTCTCGGCCTCGGCCTCGCCGGGATGTCC	
SP6-2	CGTCCTGATGTACTGGTAGGTGAGTGCGGA	
SP6-3	ATTTGGTGGATGAAGGAAGGGCCGACGAAT	
SP6-4	TTCTCCGTGTAGTTATAGCCTTTCCATATA	
SP6-5	CGGCTTGCTTTTTGAGAAGGCATTCCCCGA	For $SP6_{WT}$
SP6-6	AAGATAATAACTTTGAGGTAATCTTTCATC	Primer pairs:
SP6-7	AGATTATGTGTATGGTCGTGATGTCAAAAT	SP6-1 and SP6-2
SP6-8	CTGGAACCTTAGCTGCCTCAATGCGAGGTG	SP6-3 and SP6-4
SP6-9	CATTTCAAGCAGTAGGTCTGGCACAAAAGG	SP6-5 and SP6-6
SP6-10	CTTGTTTGTCAAAGATTTCAGGTACTTGAC	SP6-7 and SP6-8
SP6-11	AGGAGGAGTATTTCTTCATAATGAAGAAGG	SP6-9 and SP6-10
SP6-12	CCACATACGCATCTGATTAGCTTCAAAGTT	SP6-11 and SP6-12
SP6-13	GCAGTTAAAGAGCGCGATGAAGCGAAGAAG	SP6-13 and SP6-14
SP6-14	TCAATCCTCCAATAAGTCTACGCTGGCCTT	SP6-15 and SP6-16
SP6-15	GCAAATACGATTGGTGTAGGTCAGATGACC	SP6-17 and SP6-18
SP6-16	TAAACCTCCTATTACTATCCAGCCCTCCCC	
SP6-17	TTGAGCGGCCTATTACTCACCAGTCTTCAC	
SP6-18	**GAAATGTGCGCGGAACCCCTATTTGTTTAT**TAGCCCACGCCCACACACGCTGTCAAGCGG	
pRS415-R-2-gh-1_1-30-F	**TATAACGTTTTTGAACACACATGAACAAGG**CCTCTCAAGGAAAACAGCCGAGAGTTCCCC	
gh-1_9971-10000-R	CTCGCTCACCAGATGACTCGCCATCACCCT	For $gh\text{-}1_{WT}$
gh-1_9960-9989-F	CGAGTATGTAAAGGGTGATGGCGAGTCATC	Primer pairs:
gh-1_19930-19959-R	GGAGTCCTTGGGGTCATCCCAAAGAACTCT	pRS415-R-2-gh-1_1-30-F and gh-1_9971-10000-R
gh-1_19920-19949-F	TGGCTGCATGAGAGTTCTTTGGGATGACCC	gh-1_9960-9989-F and gh-1_19930-19959-R
gh-1_29890-29919-R	CGAACGGTTGAAGTCCTGAGTGTGCGAGAT	gh-1_19920-19949-F and gh-1_29890-29919-R
gh-1_29880-29909-F	GGCATACGGCATCTCGCACACTCAGGACTT	gh-1_29880-29909-F and pRS415-F-4-gh-1_37330-37359-R
pRS415-F-4-gh-1_37330-37359-R	**ACATTCAAATATGTATCCGCTCATGAGACA**GACAGATAGGGGCCCATTGAGTTTCCCTAT	
pRS415-R-2-K11-1-30-F	**TATAACGTTTTTGAACACACATGAACAAGG**TCTCACAGTTTACACTTTTGGTTATCCCCC	
K11-9971-10000-R	ATTAGAAGTCATCGTCTTCTTCGGCTTCGC	For $K11_{WT}$

K11-9900-9929-F	AGCGGACGAATCTCGCAGCCGTAAACCTCA	Primer pairs:
K11-19961-19990-R	TCATCACCTTCGAGGGCCTTAAGGGCTGAC	pRS415-R-2-K11-1-30-F and K11-9971-10000-R
K11-19950-19979-F	ATTGCCGCATGGTCAGCCCTTAAGGCCCTC	K11-9900-9929-F and K11-19961-19990-R
K11-29950-29979-R	CATCGTGTCCTTGAACACATCGTACCCATC	K11-19950-19979-F and K11-29950-29979-R
K11-29880-29909-F	CGGGGACGCTGCTGAGGCTCAGATTCAGAA	K11-29880-29909-F and pRS415-F-4-K11-41152-41181-R
pRS415-F-4-K11-41152-41181-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGACATCAACATATAGTGTC	
29880-29909-F	GACATGATGGACAAGCAGGGTATTGACCCT	
35042-35071-R	AACAGCATCGCGGTCATCCACGGCGTTCGC	For $T7_{T3(C\text{-}gp17)}$
T7(447)-T3(448)-gp17-F	GCGAACGCCGTGGATGACCGCGATGCTGTTCCGTTTGGTCAACTTAAGACCATGAACCAG	Primer pairs:
T7-T3-gp17-R	GACTACACGTCTTTCCTTGTGATTTACCAATTACACGTCCTCTACGGCTATTGCTGTTGG	29880-29909-F and 35042-35071-R
T3-T7-gp17-F	CCAACAGCAATAGCCGTAGAGGACGTGTAATTGGTAAATCACAAGGAAAGACGTGTAGTC	T7(447)-T3(448)-gp17-F and T7-T3-gp17-R
pRS415-F-4-39908-39937-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGAGACACTCAAGGTAACAC	T3-T7-gp17-F and pRS415-F-4-39908-39937-R
29880-29909-F	GACATGATGGACAAGCAGGGTATTGACCCT	
Up-gp17-R	TTGACCTCCTTAAAGTAAATCTAAGAGACT	For $T7_{T3(gp17)}$
gp17-F-1	AGTCTCTTAGATTTACTTTAAGGAGGTCAAATGGCTAACGTAATTAAAACCGTTTTGACT	Primer pairs:
T7-T3-gp17-R	GACTACACGTCTTTCCTTGTGATTTACCAATTACACGTCCTCTACGGCTATTGCTGTTGG	29880-29909-F and Up-gp17-R
T3-T7-gp17-F	CCAACAGCAATAGCCGTAGAGGACGTGTAATTGGTAAATCACAAGGAAAGACGTGTAGTC	gp17-F-1 and T7-T3-gp17-R
pRS415-F-4-39908-39937-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGAGACACTCAAGGTAACAC	T3-T7-gp17-F and pRS415-F-4-39908-39937-R
29880-29909-F	GACATGATGGACAAGCAGGGTATTGACCCT	
35045-35074-R	CGGAACAGCATCGCGGTCATCCACGGCGTT	For $T7_{T13a(C\text{-}gp17)}$
T7(450)-13a(451)-gp17-F	AACGCCGTGGATGACCGCGATGCTGTTCCGCTCGGCCAACTCAAAACTATGAATCAGAAC	Primer pairs:
T7-13a-gp17-R	GACTACACGTCTTTCCTTGTGATTTACCAATTAAACGTCTTCCACAGCGATTGCGGTCGG	29880-29909-F and 35045-35074-R
13a-T7-gp17-F	CCGACCGCAATCGCTGTGGAAGACGTTTAATTGGTAAATCACAAGGAAAGACGTGTAGTC	T7(450)-13a(451)-gp17-F and T7-13a-gp17-R
pRS415-F-4-39908-39937-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGAGACACTCAAGGTAACAC	13a-T7-gp17-F and pRS415-F-4-39908-39937-R
29880-29909-F	GACATGATGGACAAGCAGGGTATTGACCCT	
Up-gp17-R	TTGACCTCCTTAAAGTAAATCTAAGAGACT	For $T7_{T13a(gp17)}$
13a-gp17-F-1	AGTCTCTTAGATTTACTTTAAGGAGGTCAAATGGCGAATGTGATTAAGACCGTTCTGACG	Primer pairs:
T7-13a-gp17-R	GACTACACGTCTTTCCTTGTGATTTACCAATTAAACGTCTTCCACAGCGATTGCGGTCGG	29880-29909-F and Up-gp17-R
13a-T7-gp17-F	CCGACCGCAATCGCTGTGGAAGACGTTTAATTGGTAAATCACAAGGAAAGACGTGTAGTC	13a-gp17-F-1 and T7-13a-gp17-R
pRS415-F-4-39908-39937-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGAGACACTCAAGGTAACAC	13a-T7-gp17-F and pRS415-F-4-39908-39937-R
19920-19949-F	ACTTGTGACTCCACATAGGCACATCTATGA	
T7-24198-24227-R	ATAGTTCCTCCTTTCAGCAAAAAACCCCTC	
T7-24198-24227+K11gp11-F	GAGGGGTTTTTTGCTGAAAGGAGGAACTATATGAACATGCAAGATGCTTACTTTGGGTCT	For $T7_{K11(gp11\text{-}12\text{-}17)}$
T7-27227-27256+K11gp12-R	ATTTCGAGCCACCACAGGGAGAATATTTAATTAAATGCCGTTGGCTCGTCTGCTGTAGTT	Primer pairs:
T7-27227-27256-F	TTAAATATTCTCCCTGTGGTGGCTCGAAAT	19920-19949-F and T7-24198-24227-R
29890-29919-R	GAATAACCTGAGGGTCAATACCCTGCTTGT	T7-24198-24227+K11gp11-F and T7-27227-27256+K11gp12-R
29880-29909-F	GACATGATGGACAAGCAGGGTATTGACCCT	T7-27227-27256-F and 29890-29919-R
Up-gp17-R	TTGACCTCCTTAAAGTAAATCTAAGAGACT	29880-29909-F and Up-gp17-R
K11-gp17-F-1	AGTCTCTTAGATTTACTTTAAGGAGGTCAAATGGACCAAGACATTAAAACAGTCATTCAG	K11-gp17-F-1 and T7-K11-gp17-R
T7-K11-gp17-R	GACTACACGTCTTTCCTTGTGATTTACCAATTATAAAACAAATGATGCTAATCTCGAGAT	K11-T7-gp17-F and pRS415-F-4-39908-39937-R
K11-T7-gp17-F	ATCTCGAGATTAGCATCATTTGTTTTATAATTGGTAAATCACAAGGAAAGACGTGTAGTC	
pRS415-F-4-39908-39937-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGAGACACTCAAGGTAACAC	
T3-29881-29910-F	TTGTAGTTGGTGCCGAAAGTGCTGCGCTGA	
T3-33249-33278-R	AACAGCGTCGCGGTCATCCACAGCGTTCGC	For $T3_{T7(C\text{-}gp17)}$
T3(447)-T7(448)-gp17-F	GCGAACGCTGTGGATGACCGCGACGCTGTTCCGTTTGGTCAACTAAAGACCATGAACCAG	Primer pairs:
T3-T7-gp17-R	GTGGACTTAAAGTAGTTCCTTTGATGCTTATTACTCGTTCTCCACCATGATTGCATTAGG	T3-29881-29910-F and T3-33249-33278-R
T7-T3-gp17-F	CCTAATGCAATCATGGTGGAGAACGAGTAATAAGCATCAAAGGAACTACTTTAAGTCCAC	T3(447)-T7(448)-gp17-F and T3-T7-gp17-R
pRS415-F-4-T3-38179-38208-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACATAGAGATGTACTATCATACTG	T7-T3-gp17-F and pRS415-F-4-T3-38179-38208-R
T3-29881-29910-F	TTGTAGTTGGTGCCGAAAGTGCTGCGCTGA	
Up-gp17-R	TTGACCTCCTTAAAGTAAATCTAAGAGACT	For $T3_{T7(gp17)}$
gp17-F-1	AGTCTCTTAGATTTACTTTAAGGAGGTCAAATGGCTAACGTAATTAAAACCGTTTTGACT	Primer pairs:
T3-T7-gp17-R	GTGGACTTAAAGTAGTTCCTTTGATGCTTATTACTCGTTCTCCACCATGATTGCATTAGG	T3-29881-29910-F and Up-gp17-R
T7-T3-gp17-F	CCTAATGCAATCATGGTGGAGAACGAGTAATAAGCATCAAAGGAACTACTTTAAGTCCAC	gp17-F-1 and T3-T7-gp17-R
pRS415-F-4-T3-38179-38208-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACATAGAGATGTACTATCATACTG	T7-T3-gp17-F and pRS415-F-4-T3-38179-38208-R
T3-29881-29910-F	TTGTAGTTGGTGCCGAAAGTGCTGCGCTGA	
R-gp17_1433-1462-R	TCTGAGAACCTCCTCTCATGTAACTACCAC	For $T3_{R(gp17)}$
R-gp17_1433-1462-F	GTGGTAGTTACATGAGAGGAGGTTCTCAGA	Primer pairs:
R-gp17_1562-1654-R	CTGTTGGAGGCGCATATCTATCCGCTATGTTCTTAAATACCCTGCCATTAGAGTGTATCTGGAATTTTAGCCAACCGTCCTCGGCTGAAAGGA	T3-29881-29910-F and R-gp17_1433-1462-R
R-gp17_1625-1654-F	ACATAGCGGATAGATATGCGCCTCCAACAG	R-gp17_1433-1462-F and R-gp17_1562-1654-R
pRS415-F-4-T3-38179-38208-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACATAGAGATGTACTATCATACTG	R-gp17_1625-1654-F and pRS415-F-4-T3-38179-38208-R
K11-19950-19979-F	ATTGCCGCATGGTCAGCCCTTAAGGCCCTC	
K11-22861-22890-R	AATGTAAGCTCCTAACGAAAAAACCCCTCA	
K11-22861-22890+T7gp11-F	TGAGGGGTTTTTTCGTTAGGAGCTTACATTATGCGCTCATACGATATGAACGTTGAGACT	For $K11_{T7(gp11\text{-}12\text{-}17)}$
K11-25868-25897+T7gp12-R	TAATTGAGCACACCATAAGGATTCCTTCAGTTAAATACCGGAACTTCTCCGTAAGTAGTT	Primer pairs:
K11-25868-25897-F	CTGAAGGAATCCTTATGGTGTGCTCAATTA	K11-19950-19979-F and K11-22861-22890-R
K11-29950-29979-R	CATCGTGTCCTTGAACACATCGTACCCATC	K11-22861-22890+T7gp11-F and K11-25868-25897+T7gp12-R
K11-29880-29909-F	CGGGGACGCTGCTGAGGCTCAGATTCAGAA	K11-25868-25897-F and K11-29950-29979-R
Up-K11-gp17-R	TGTGACCTCCTTTAGTTGAATGAGAAGGGG	K11-29880-29909-F and Up-K11-gp17-R
T3/7-gp17-F	CCCCTTCTCATTCAACTAAAGGAGGTCACAATGGCTAACGTAATTAAAACCGTTTTGACT	T3/7-gp17-F and K11-T7-gp17-R
K11-T7-gp17-R	TTGGTTTAAATCATTCAGCATGTTGTCTCCTTACTCGTTCTCCACCATGATTGCATTAGG	T7-K11-gp17-F and pRS415-F-4-K11-41152-41181-R
T7-K11-gp17-F	CCTAATGCAATCATGGTGGAGAACGAGTAAGGAGACAACATGCTGAATGATTTAAACCAA	
pRS415-F-4-K11-41152-41181-R	**ACATTCAAATATGTATCCGCTCATGAGACA**AGGGACACAGAGACATCAACATATAGTGTC	

Boldface means YAC pRS415 sequence.