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Extending the Host Range of Bacteriophage Particles for DNA Transduction

Highlights

- Hybrid phage particles extend DNA transduction into new bacteria
- Directed evolution optimizes particles' DNA transduction into new bacteria
- Optimized hybrid particles specifically and efficiently transduce desired DNA

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In Brief

Yosef et al. develop a generalizable technology for efficient DNA delivery using phage particles. They demonstrate efficient DNA delivery across several bacterial genera and further develop a directed-evolution tool that optimizes this delivery.

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Extending the Host Range of Bacteriophage Particles for DNA Transduction

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SUMMARY

A major limitation in using bacteriophage-based applications is their narrow host range. Approaches for extending the host range have focused primarily on lytic phages in hosts supporting their propagation rather than approaches for extending the ability of DNA transduction into phage-restrictive hosts. To extend the host range of T7 phage for DNA transduction, we have designed hybrid particles displaying various phage tail/tail fiber proteins. These modular particles were programmed to package and transduce DNA into hosts that restrict T7 phage propagation. We have also developed an innovative generalizable platform that considerably enhances DNA transfer into new hosts by artificially selecting tails that efficiently transduce DNA. In addition, we have demonstrated that the hybrid particles transduce desired DNA into desired hosts. This study thus critically extends and improves the ability of the particles to transduce DNA into novel phage-restrictive hosts, providing a platform for myriad applications that require this ability.

INTRODUCTION

Pathogen resistance to antibiotics is an increasing threat to human health, requiring the development of novel antimicrobial agents and strategies (see, for example, the World Health Organization's 2017 report: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1). Bacterial viruses, also known as bacteriophages or phages, are re-emerging as tools used in traditional and novel approaches for overcoming this threat (Bikard et al., 2014; Citorik et al., 2014; Harper and Enright, 2011; Merril et al., 2003; Yosef et al., 2015). Temperate and transducing phages have been recently shown to effectively transfer DNA into bacteria for therapeutic or prophylactic treatments against antibiotic-resistant bacteria. These novel technologies demonstrate that injecting manipulated DNA by phages (DNA transduction) may occasionally be superior to direct bacterial killing (e.g., by lytic phages). Importantly, DNA transduction can selectively target resistant pathogens and provide "good" bacteria with tools to effectively compete against the "bad" ones. Two independent studies from the groups of Lu and Marraffini have shown that phages transferring a tailored CRISPR-Cas system may be used to specifically target resistant bacteria both in vivo and ex vivo (Bikard et al., 2014; Citorik et al., 2014). Our group has shown that using phages to transfer DNA, encoding dominant sensitive genes or CRISPR-Cas, results in bacterial sensitization to antibiotics (Edgar et al., 2012; Goren et al., 2017; Yosef et al., 2014, 2015). These technologies rely on the phages' ability to transduce DNA into the desired bacterial host strains; therefore, overcoming the major limitation of phages' narrow host range is the key to further developing and applying these novel technologies.

DESIGN

For the last century, efforts have been made to extend the phages' host range. Various studies have shown that the phage host range can be extended by infecting a desired host with numerous phages and consequently selecting those mutant phages whose mutation enables them to propagate in desired hosts (see, for example, Garcia et al., 2003; Lazarus and Gunnison, 1947; Molineux, 2005). More recently, Lu and colleagues have shown that phages of the T7 group can be programmed to recognize desired hosts. Because the tail/tail fiber proteins of T7 phage determine the recognition of different hosts (Ando et al., 2015), Lu and colleagues hypothesized that swapping tail/tail fibers from different sources would enable the phage to recognize different hosts. To this end, they constructed different phage genomes by a unique synthetic biology platform, based on the Gibson assembly of different PCR fragments (Gibson et al., 2008). The assembled genomes, having swapped tail/tail fiber genes with those from different phages, were replicated in yeast cells (Ando et al., 2015; Goren et al., 2015). The resulting phage genomes were then transformed into *Escherichia coli*, which produced infective hybrid particles that were tested for their ability to infect different hosts. These experiments paved the way for producing hybrid particles that extend the host range of T7 phage.

Despite this progress, these host-range-extending techniques all focus on phages that propagate in the desired host rather than those that transduce DNA. Phage propagation relies on multiple steps: adsorption; DNA injection; DNA replication; dependence on factors in the host that are essential for its propagation;

overcoming host defense mechanisms; and lysis of the host cell. Owing to the various key steps required, T7 phage cannot propagate in many hosts, even if its DNA is transduced into them. Unlike phage propagation, DNA transduction by phages requires only adsorption, overcoming defense mechanisms, and DNA-injection capabilities. This explains why more hosts could be prone to DNA transduction without supporting phage propagation. Nevertheless, identifying such hosts is complex, because linkage between the phage host's range and the ability to transduce DNA should first be established. A platform that enables phages to transduce DNA into new hosts will significantly extend their host range and pave the way for advanced genetic manipulations and analyses.

Transducing particles also manifest several critical technological advantages over phages. Unlike the production of phages, the integrity of the packaged phage genome is not essential for producing transducing particles. In fact, the only requirement is that all the components of the phage particles should be encoded in the producing host (either in *cis* or in *trans*). Expression of some components in *trans* dramatically simplifies the generation of the hybrid particles, because these components can be expressed in plasmids that are constructed using conventional molecular biology techniques rather than the laborious and costly synthetic biology techniques used to generate swapped phage genomes (Ando et al., 2015; Figure S1). The phage genomes used to produce the transducing particles lack self-propagation capacity (due to the absence of critical genes that are expressed in *trans*). Thus, the production of transducing particles is safer and more controllable, and it consequently may require a more relaxed approval procedure by regulatory agencies. Therefore, using transducing particles is superior in several technological aspects over using infectious phages.

To isolate phages that transduce DNA into hosts that do not support their propagation, we first determined the transduction ability of hybrid capsids with tail/tail fibers derived from different phages on various pathogenic hosts (for simplicity, tail/tail fibers are henceforth referred to as "tails"). We also substantially improved the initial transduction efficiency of some of these hybrid particles. To this end, we developed GOTraP (general optimization of transducing particles), a platform to link the phenotype (i.e., transduction of DNA) with the desired genotype that allows this transduction (i.e., mutations in the tail-encoding genes). Importantly, GOTraP allows us to select phages with tails that are compatible with desired hosts. Lastly, we showed that the desired DNA, encoding a packaging signal, can be transduced into the pathogenic strain, thus demonstrating that DNA can specifically be transduced using the programmed transducing particles.

RESULTS

Hybrid T7 Phages Efficiently Transduce DNA into Novel Hosts

Because T7 phage can package and transduce plasmids, we hypothesized that different tails, determining different host specificities, will allow DNA transduction into desired hosts regardless of the phage's ability to successfully propagate in these hosts. The T7 phage used to package the plasmids lacked its tail genes (genes 11, 12, and 17). Consequently, its maturation into a transducing particle depends on the tail genes that are produced by the transduced plasmid, in *trans*. Thus, plasmid transduction can occur only if the tail gene products are assembled into a hybrid particle and only if this tail recognizes the receptor of the transduced host (Figure 1A). Desired hybrids allow plasmid transduction that confers antibiotic resistance to the target host. To test these hypotheses, we designed plasmids encoding the tail genes of 15 different phages as well as an antibiotic resistance marker and a T7-packaging sequence (Chung and Hinkle, 1990; Table S1). We produced 15 different hybrid particle lysates in *E. coli* harboring different plasmids. These lysates contain hybrid particles encapsulating the T7 genome or the plasmid at ~1:1 ratio (Chung and Hinkle, 1990). We then tested the transduction efficiency of each of the produced hybrid particle lysates in 12 different target hosts. We determined the transduction efficiency by counting the number of colony-forming units acquiring the antibiotic resistance marker encoded by the transduced plasmid. As expected, hybrid particles produced in hosts encoding T7 tail proteins efficiently transduced DNA into BW25113Δ*trxA*, a derivative of *E. coli* K-12 that does not support T7 phage propagation, owing to lack of *trxA* (a host gene encoding the T7 DNA polymerase subunit; Modrich and Richardson, 1975; Figure 1B; Methods S1). Hybrid particles displaying the *Klebsiella* phage K11 transduced DNA efficiently into *Klebsiella* sp. 390 (Figure 1B). This result is expected, because this hybrid was shown to successfully infect this host (Ando et al., 2015). Other *Klebsiella* strains that we tested exhibited DNA transduction by at least one of the hybrid particles (Figure 1B). *Enterobacter cloacae* and *Enterobacter aerogenes* were also transduced at low-to-intermediate efficiency by some of the hybrid particles (Figure 1B). The deviation in the number of compatible hybrids within the same genus of bacteria can vary dramatically (e.g., Sen4513 versus Sen4510). Some hosts could only be transduced by a single hybrid particle, demonstrating that testing multiple hybrids is essential for finding compatible combinations of at least one hybrid particle and a new host. Some hosts, on the other hand, were transduced efficiently by multiple hybrid particles (e.g., Sso4727 was transduced by 12 out of the 15 hybrid particles tested), demonstrating that finding compatible hybrid particles is probably feasible for many bacterial hosts. Significantly, the extension of the phage range to most of the strains presented in Figure 1B, which do not support T7 propagation (except K390), could not be accomplished via traditional methods that require phage propagation. Taken together, these experiments show that the approach may significantly extend the utilization of phages for transducing DNA into new hosts; this could not be tested in previous studies because phage propagation was required.

GOTraP Generates Phages with Increased Transduction Efficiency

We hypothesized that we could improve the transduction efficiency by selecting plasmids that were transduced more efficiently due to modified tail genes that they encode. We assumed that, in a DNA transduction experiment, plasmids encoding such modified tails would be transduced more efficiently than plasmids encoding the parental tails simply because the particles

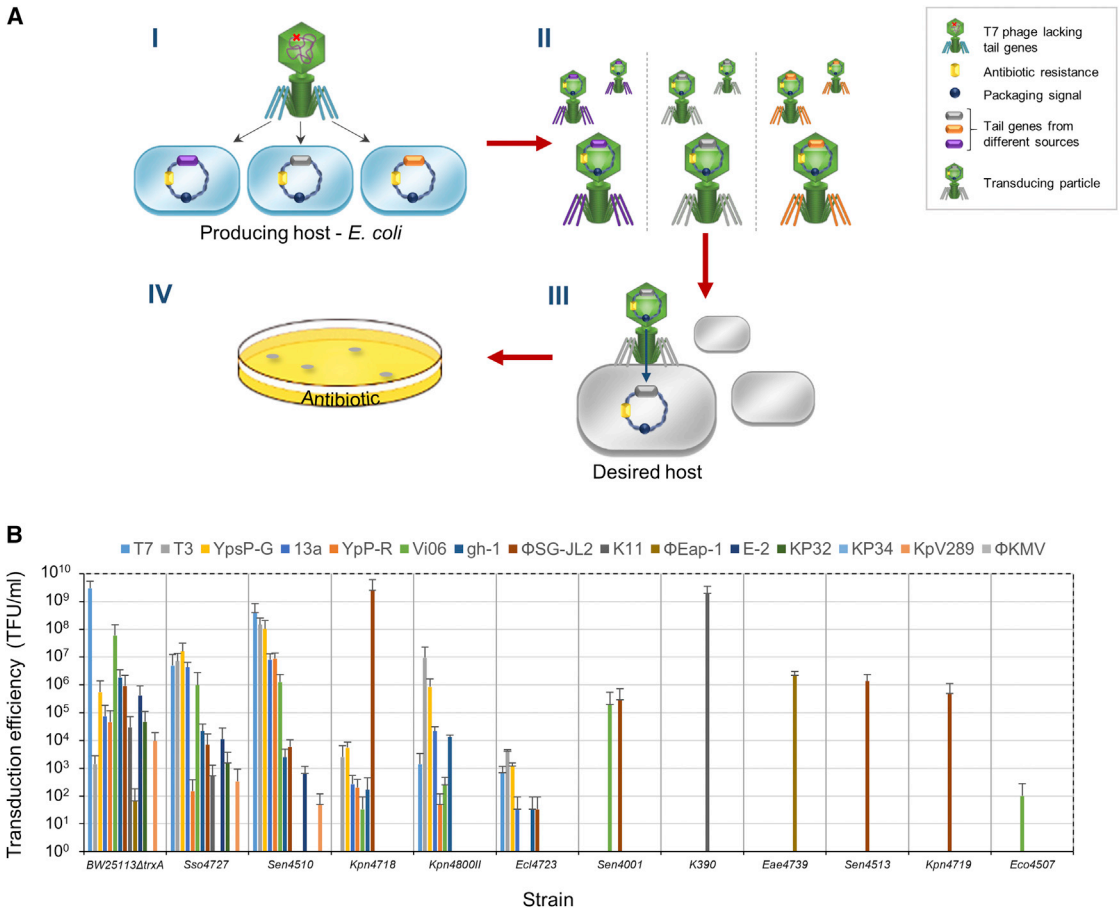

Figure 1. Identifying Hybrid T7 Particles Able to Transduce DNA to Novel Hosts

(A) Schematic depiction of the procedure. (I) T7 phages lacking their tail genes are produced in *E. coli* hosts encoding tails from various sources in a packable plasmid. (II) The resulting particle-lysate contains hybrid T7 particles with novel tail genes (as well as hybrid particles packaging the T7 genome; not shown). (III) These particles are incubated with a host strain that does not necessarily support T7 phage propagation. Particles having compatible tail proteins that recognize this host inject the plasmid. (IV) Hosts acquiring the plasmid are selected on antibiotic-containing plates. The efficiency of transduction is then determined.

(B) DNA transduction of different hosts by different hybrid particles. Bars represent the average ± SD of the number of transduced colonies per mL (TFU/mL) by the indicated hybrid particles (T7 core capsid hybridized with tails derived from phages T7, T3, YpsP-G, 13a, YpP-R, Vi06, gh-1, ΦSG-JL2, K11, ΦEap-1, E-2, KP32, KP34, KpV289, and ΦKMV) into the indicated host. Experiments were repeated independently at least three times for each set of hosts. BW25113Δ*trx*, *E. coli* Δ*trxA*; Eae4739, *Enterobacter aerogenes* ATCC 51697; Ecl4723, *Enterobacter cloacae* subsp. cloacae ATCC 13047; Eco4507, *Escherichia coli* ATCC 25922; K390, *Klebsiella* sp. 390; Kpn4718, *Klebsiella pneumoniae* subsp. pneumoniae ATCC 10031; Kpn4719, *Klebsiella pneumoniae* subsp. pneumoniae ATCC 13882; Kpn4800II, *Klebsiella pneumoniae* subsp. pneumoniae ATCC 9997; Sen4001, *Salmonella enterica* subsp. enterica serovar Typhimurium str. LT2; Sen4510, *Salmonella enterica* serovar Arizonae str. SARC 5; Sen4513, *Salmonella enterica* serovar Enteritidis PT4; Sso4727, *Shigella Sonnei* 4727.
See also Table S2.

displaying them transduce DNA better than particles displaying parental tails. Following several transduction cycles in which transduced plasmids are collected and used to produce more hybrid particles, we would be able to isolate mutant plasmids encoding tail proteins with enhanced transduction capability (Figure 2A). This rationale led us to evolve the procedure we termed GOTraP.

As a feasibility test for GOTraP, we first examined whether it enriches tail proteins in *E. coli* lacking *waaC* and *trxA* (BW25113Δ*trxA*Δ*waaC*). This host does not support T7 phage propagation due to lack of *trxA* and does not encode the T7 host receptor, lipopolysaccharide (LPS), because it lacks *waaC*, a gene required for LPS biosynthesis (Qimron et al., 2006). Therefore, this host does not support either T7 phage growth or DNA transduction. We speculated that we would be able to isolate a T7 particle with an altered compatible tail protein that would be able to transduce it. Ethyl methanesulfonate (EMS) mutagenesis was used to generate random mutations in packable plasmids encoding the T7 tail proteins (Methods S1). *E. coli* hosts harboring these plasmids were then used to produce particles displaying the plasmid-encoded mutant tails. The resultant particles were then used to transduce the restrictive *E. coli* BW25113Δ*trxA*Δ*waaC*. Transductants were selected using an antibiotic and counted (Figure 2B; cycle 0). These transductants were pooled, and their plasmids were extracted and transformed to fresh *E. coli* hosts that were used for producing

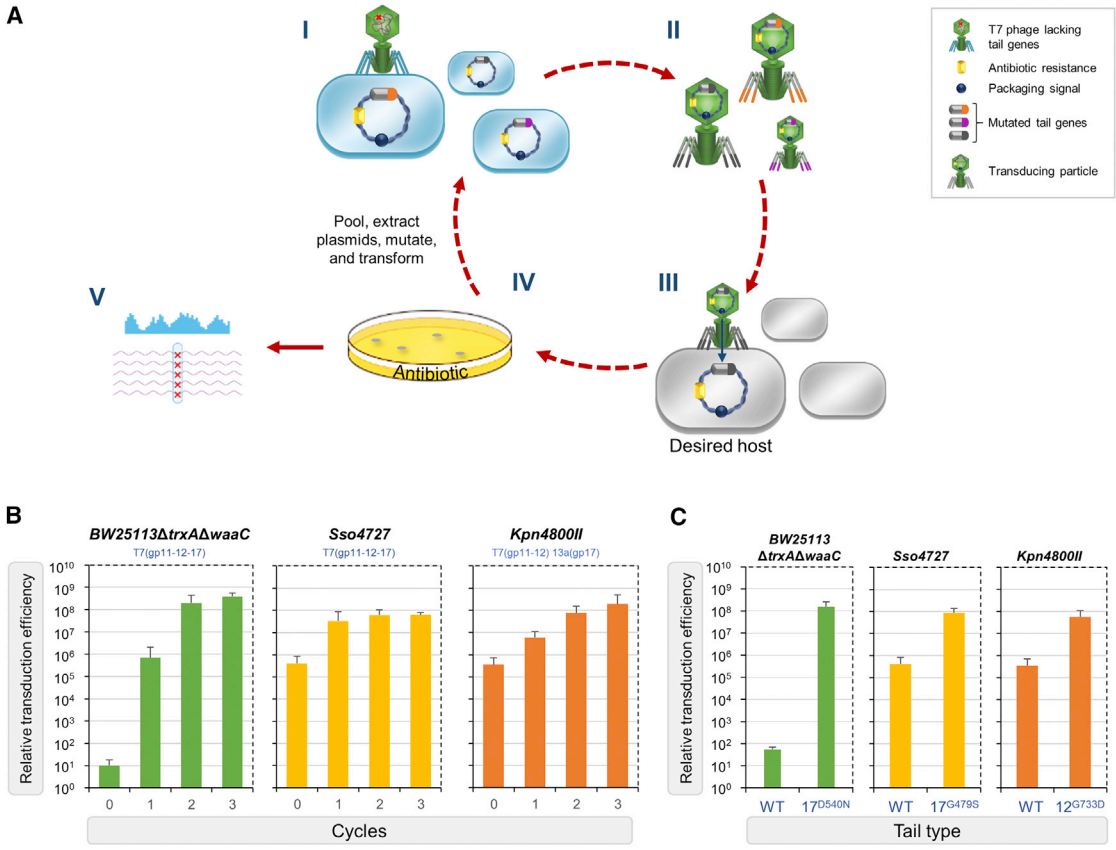

Figure 2. Enhancing the Ability of Hybrid T7 Particles to Transduce DNA to Novel Hosts

(A) Schematic depiction of GOTraP. (I) T7 phages lacking their tail genes are used to infect *E. coli* hosts encoding randomly mutated tails in a packable plasmid. (II) The resulting phage-lysate contains numerous variants of particles that have packaged the plasmid encoding their mutated tail genes. (III) These particles are incubated with a host strain that does not necessarily support phage propagation. Particles having compatible tail proteins that recognize this host with improved efficiency will inject the plasmid better than the parental or other mutant tails. (IV) Hosts that have acquired the plasmid are selected on antibiotic-containing plates due to the antibiotic marker encoded by the plasmid. Plasmids are extracted, transformed into *E. coli* hosts, and mutated; this procedure is repeated several times to select optimized tail gene products infecting the new host. (V) Plasmids are sequenced, and mutations that improve transduction are identified.

(B) Enhancing the transduction efficiency of different hybrid particles using GOTraP. Particle lysates produced in wild-type-*E.-coli*-harboring plasmids encoding the indicated tails (blue font) were mutated using EMS. The lysates were used to transduce the indicated hosts (bold font). Transductants were plated on antibiotic-containing agar plates and counted. Plasmids from these transductants were extracted, mutated, and transformed into fresh wild-type *E. coli*. These hosts were used to produce new particle lysates that served to transduce the indicated host. The number of transduced bacteria was counted in untreated lysates (cycle 0) and in lysates undergoing the indicated number of cycles (cycles 1, 2, and 3). Bars represent the average number of transduced bacteria ± SD normalized to the transduction efficiency of the same hybrid particle on the reference strains (*E. coli*Δ*trxA* for *E. coli*Δ*trxA*Δ*waaC* and for *Sso*4727; *Sso*4727 for *Kpn*4800II) in three independent experiments.

(C) Validating the effect of the obtained mutation on DNA transduction. *E. coli* harboring a plasmid encoding either the parental tail genes (wild-type [WT]) or the indicated mutant gene was used to prepare hybrid particles. Bars represent the average number of transduced bacteria ± SD normalized to the transduction efficiency of the hybrid particles on the above reference strains in three independent experiments.

an additional lysate of transducing particles. Transduction using this lysate produced over 10^4-fold more colonies than did the first lysate (Figure 2B; cycle 1), and two more repeated cycles produced ~10^7-fold more colonies than did the first lysate (Figure 2B). These results indicate that the transduced plasmid, which is re-packaged by T7 phage and re-transduced, encodes a mutated-compatible tail to the restrictive host. To validate this, we sequenced plasmids purified from cycle 3. We identified a mutation in gene 17, D540N, a position next to a residue known to allow T7 phage adsorption to *E. coli* LPS mutants (Qimron et al., 2006). A plasmid introduced with this specific mutation increased the transduction efficiency by ~10^6-fold over the parental plasmid (Figure 2C). This result confirmed that the identified mutation is responsible for the observed phenotype. Four independent experiments resulted in an identical independent mutation, demonstrating the reproducibility of GOTraP and its efficiency in evolving a novel tail compatible with the new host.

To further show that GOTraP also works on strains other than *E. coli*, we used it to improve the transduction efficiency of a particle that transduced with intermediate efficiency a clinical isolate of *Shigella sonnei* (a strain not supporting T7 phage propagation; Table S2). To this end, mutated plasmids, encoding T7 tail

proteins, were transduced into *S. sonnei*, extracted, and then transformed back into *E. coli* hosts. These hosts were consequently used to produce new lysates. The new lysates were used to re-transduce *S. sonnei*, and these cycles were repeated three times. Following the first, second, and third cycle, we continuously monitored an increased titer of transducing particles of ~50-, 120-, and 150-fold over the initial efficiency, respectively, probably due to the enrichment of mutated plasmids with higher transduction efficiency (Figure 2B). This efficiency stabilized at the third cycle, where most likely all plasmids were mutants with increased transduction efficiency. DNA sequencing of extracted plasmids revealed a mutation in gene 17, resulting in a glycine substitution for serine at position 479. To validate that indeed this mutation is the sole mutation that is responsible for the increased transduction efficiency, we mutated the parental plasmid at this position and monitored its transduction efficiency compared with the unmutated plasmid. As expected, the mutated plasmid increased its transduction efficiency by ~150-fold over the parental plasmid (Figure 2C). This result confirmed that the identified mutation is responsible for the observed phenotype. We further showed that hybrid particles containing non-T7 tails can also be optimized by GOTraP. A hybrid constituted of T7 capsid and tails, but with a tail fiber derived from phage 13a, was optimized to transduce DNA into *Klebsiella pneumonia* 4800II. Also in this case, three cycles sufficed to significantly improve the transduction efficiency of the hybrid particle (Figure 2B). A mutation was identified in gene 12, resulting in a glycine to aspartate substitution at position 733. In this case too, a mutated plasmid constructed with this specific mutation resulted in increased transduction efficiency compared to the control plasmid (Figure 2C). Overall, these results show that GOTraP can significantly increase the efficiency of DNA transduction of particles across multiple bacterial genera.

Efficient Transduction of a Plasmid Using the Optimized Particles

Finally, we wished to demonstrate the ability to re-program phage capsids to enable transduction of desired plasmids, not encoding the tail genes, into new hosts. To this end, we introduced two plasmids into the *E. coli* host that was used to produce the particles: a non-packable plasmid encoding the parental or the mutated tail genes obtained in the GOTraP and a packable plasmid carrying an antibiotic marker (Figure 3A). We speculated that the produced particles harbored the packable plasmid and that the particles produced in hosts encoding mutated tails will transduce this plasmid more efficiently than those produced with the parental tails. The lysates were used to transduce *E. coli*Δ*trxA*Δ*waaC*, *Shigella sonnei* 4727, and *Klebsiella pneumonia* 4800II. The strains were consequently inoculated on plates supplemented with the antibiotic to which the transduced plasmid confers resistance and counted the next day. Indeed, as expected, we observed the transduction of the packable plasmid, corresponding with the measured transduction efficiency of the parental and mutant tails (Figure 3B). Transfer of a plasmid encoding the packaging signal (but not the tail genes) was also observed using other hybrid particles that were produced in hosts encoding compatible tails identified in Figure 1 (Figure S2). As a control for specificity, we plated the transduced cells on plates containing the antibiotic resistance conferred by the tail-encoding plasmid. The non-packable plasmid was transduced at least 100-fold less efficiently than the plasmid carrying the packaging signal, indicating that the packable plasmid is specifically delivered by at least a factor of 100-fold over the non-packable plasmid (Figure S2). These experiments showed that the hybrid particle capsids may indeed be programmed to efficiently and specifically promote transduction of desired DNA plasmids into new hosts.

DISCUSSION

We have designed T7-hybrid particles that transduce DNA into novel hosts. Importantly, this approach does not require phage propagation in these hosts; thus, in contrast to previous methods, it can also be used to extend the spectrum of hosts to those that do not support phage propagation. These hosts constitute a considerable proportion of the host repertoire (Table S2). To date, the dependence on the phage propagation ability limited the detection of novel phage-host interactions. For example, a recent study by Lu's group succeeded in assembling hybrid T7 phage capsids having tails from different phages (Ando et al., 2015). This study managed to extend the host range of these hybrid particles to a few strains of hosts other than *E. coli* (one strain of *Klebsiella* and two strains of *Yersinia*). Significantly, we extended the spectrum of hosts for several species of *Klebsiella*, *Salmonella*, *Escherichia*, *Shigella*, and *Enterobacter*, which could not be identified by Lu's approach because none of them supports T7 propagation (Table S2). Our study also demonstrated DNA transduction by a hybrid particle into the same *Klebsiella* strain identified in Lu's study (Figure 1B; K390). We have also optimized the efficiency of transduction into new hosts by developing GOTraP, a platform that selects for optimized DNA-transducing hybrid particles. Lastly, we demonstrated that the hybrid particles can be programmed to specifically transfer DNA into desired hosts.

The hybrid particles have several key advantages over natural temperate phages, which can also deliver DNA. First, customizing such modular phage particles is more efficacious than isolating and characterizing natural temperate phages for each host. Second, the hybrid particles deliver only the desired plasmid DNA, whereas a replication-competent phage DNA is not transduced. This is in contrast to DNA delivery by temperate phages in which phage genes with potentially undesirable side effects are also delivered into the targets. Third, using a single type of modular hybrid particle overcomes many challenges associated with using cocktails of several different phages, such as ease of production, regulation, and biological monitoring.

We demonstrated the optimization of DNA transduction by altering the tail's compatibility with the host receptor. Nevertheless, other factors, such as restriction enzymes in the host, may also pose an obstacle for DNA transduction. These obstacles can also be overcome by GOTraP, because it selects mutants that enhance their DNA transduction efficiency regardless of the underlying mechanism. The selection of mutants that overcome these obstacles is enabled due to the linkage between

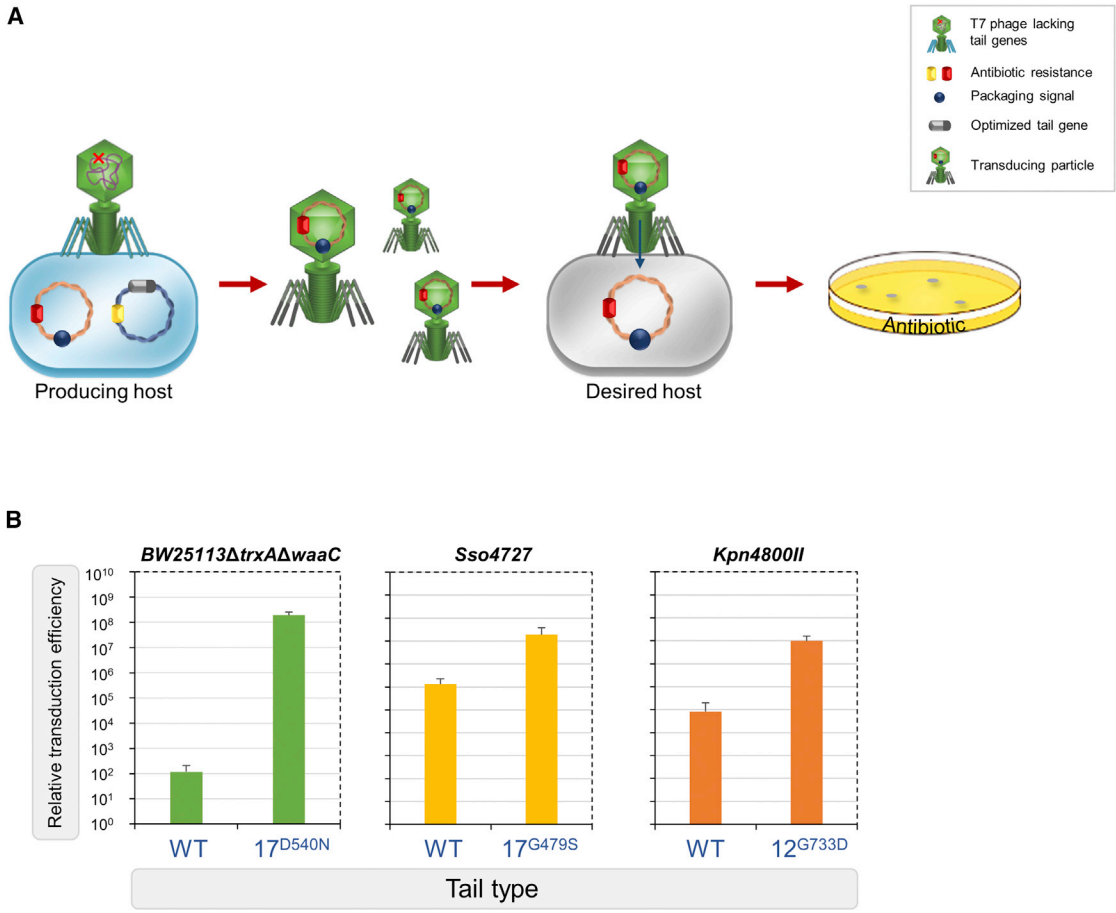

Figure 3. Production of Hybrid Particles for Plasmid Transduction into Specific Hosts

(A) Schematic depiction of the procedure. *E. coli* cells used to produce hybrid particles harbored two plasmids: one encoding the tail proteins (without packaging signal) and the other encoding the T7 packaging signal. The produced hybrid particle lysates were used to transduce DNA into the indicated hosts. The hosts were then inoculated on Luria-Bertani (LB) agar plates supplemented with the antibiotic to which the plasmid with the packaging signal confers resistance.

(B) Comparison between the transduction efficiencies supported by the parental (WT) or the improved mutated tails. Bars represent the average number of transduced bacteria ± SD normalized to the transduction efficiency of the same hybrid particle on a reference strain (*E. coli*Δ*trxA* for *E. coli*Δ*trxA*Δ*waaC* and for *Sso*4727; *Sso*4727 for *Kpn*4800II) in three independent experiments.

See also Figure S2.

the transduced DNA and the phenotype that it confers, i.e., increased transduction efficiency. Thus, if a restriction recognition site blocks efficient transfer of DNA, plasmids acquiring mutations at this site will transduce better and become enriched. Furthermore, this method can be used to isolate improved inhibitors of restriction enzymes by substituting the plasmid-encoding tail genes for genes encoding restriction enzyme inhibitors (e.g., gene 0.3 of T7 phage; Studier, 1975). This linkage enables one to enrich the optimal transduced DNA, encoding the optimal genes whose products allow efficient transduction. Thus, GOTraP is a broad-scope technology that could potentially resolve numerous aspects of DNA transduction efficiency.

Generating phage particles for DNA transduction may be used in molecular biology, e.g., to establish transduction systems for hosts for which we currently lack such genetic manipulation systems. Importantly, the principles described here could be used to generate other platforms for DNA delivery into other groups of bacterial hosts. For example, a phage infecting Gram-positive hosts could potentially be developed to transduce an entire group of Gram-positive bacteria using the presented technology. Indeed, phages have been shown to transduce DNA across different species (Chen and Novick, 2009; Kenzaka et al., 2010). We even envision, in the far future, the use of certain manipulated phage capsids to transduce eukaryotes, such as yeasts and even higher organisms, creating an exciting and novel platform for introducing DNA into desired animal cells (Clark and March, 2004).

The ability to transduce a variety of hosts with several optimized T7 particles will enable easy editing of certain bacterial populations both specifically and efficiently. We and others have demonstrated the potential editing of microbial populations using the CRISPR-Cas system (Bikard et al., 2014; Citorik et al., 2014; Yosef et al., 2015). In these strategies, transducing particles may transfer a tailor-made CRISPR-Cas system to eliminate

antibiotic resistance determinants in pathogens found in patients or on hospital surfaces or that stem from natural flora, such as skin and intestines. Thus, particles obtained through the platforms described in here may be applied in these settings, providing a significant weapon to the dwindling arsenal against antibiotic-resistant pathogens.

Limitations

As indicated above, the GOTraP platform may be used to optimize transfer of a DNA molecule that carries a recognition site of a restriction system found in the host cell. In such cases, mutations that either delete or modify the recognition site will enhance the transfer of the molecule. Nevertheless, in cases where several recognition sites are encoded in different regions on the DNA molecule, a simple deletion will delete extensive parts of the desired molecule, and simultaneous alterations of all sites are less likely to evolve. Therefore, GOTraP will be less effective in optimizing such a DNA molecule. If multiple restriction-recognition sites are suspected, it would be beneficial to test several plasmid backbones for DNA transfer. Some of these plasmids may, by chance, have one or no recognition site. The restriction-recognition site could be identified by analyzing the mutations obtained in successfully transduced plasmids, and consequently, appropriate modifications of these sites (with silent mutations where required) could be introduced into other desired constructs.

Another limitation of the technology is that the selection of transduced bacteria is based on an antibiotic marker suitable for *E. coli*. In general, no barriers are posed for related species in terms of codon usage, promoter use, and other factors affecting acquisition of resistance. Nevertheless, for distant bacterial genera, the antibiotic resistance marker used for selection may not properly be expressed. To overcome this limitation, bioinformatics should be used to determine the codon usage, promoter type, and other factors required for optimal expression of the resistance marker in the target strain.

A significant limitation of the technology is to evolve optimized receptor-tail interactions in cases requiring multiple simultaneous mutations in the tail genes. Assuming that the mutagenesis procedure yields a mutation in 10^{-3} bp/generation, then based on the formula 10^{-3n}, where n = number of simultaneous mutations required, a double simultaneous mutation will occur in 10^{-6} of the plasmids, a triple simultaneous mutation will occur in 10^{-9} of the plasmids, and so on. Thus, in order to optimize an interaction that requires four simultaneous mutations, one would need to have a library of $\sim 10^{12}$ plasmids, and for five simultaneous mutations, a library of 10^{15} would be required. Such a large library may in some cases be impossible to generate. However, it should be emphasized that, if an incremental benefit is derived from each mutation, then theoretically, several cycles of GOTraP would eventually result in the desired optimization. To partially overcome this limitation, one could induce a much greater mutagenesis rate, in a limited genetic region. In addition, significant increase in the volume of the culture would enlarge the size of the library, which in turn reduces this limitation.

Lastly, the molecular size of the transferred DNA is limited to the maximal size that the phage capsid can encapsulate. For T7 phage, this limit is ~43 kbp (Molineux, 2005). Other phages, packaging larger size of DNA molecules, such as T4, could be used to overcome this limitation. Developing other phages for DNA transduction and GOTraP could be feasible in light of the proof of principle presented here for T7 phage.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Construction of strains
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  - Preparing lysates of T7 TFU
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- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES
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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, four tables, and Methods and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2017.04.025.

AUTHOR CONTRIBUTIONS

I.Y., M.G.G., R.G., S.M.-M., and U.Q. conceived and designed the experiments as well as analyzed the data. I.Y., M.G.G., R.G., and S.M.-M. performed the experiments. U.Q. wrote the manuscript with input from all authors.

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REFERENCES

Ando, H., Lemire, S., Pires, D.P., and Lu, T.K. (2015). Engineering modular viral scaffolds for targeted bacterial population editing. Cell Syst. *1*, 187–196.

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L., and Mori, H. (2006). Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. *2*, 2006.0008.

Bessler, W., Freund-Mölbert, E., Knüfermann, H., Rduolph, C., Thurow, H., and Stirm, S. (1973). A bacteriophage-induced depolymerase active on Klebsiella K11 capsular polysaccharide. Virology *56*, 134–151.

Bikard, D., Euler, C.W., Jiang, W., Nussenzweig, P.M., Goldberg, G.W., Duportet, X., Fischetti, V.A., and Marraffini, L.A. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat. Biotechnol. *32*, 1146–1150.

Chen, J., and Novick, R.P. (2009). Phage-mediated intergeneric transfer of toxin genes. Science *323*, 139–141.

Chung, Y.B., and Hinkle, D.C. (1990). Bacteriophage T7 DNA packaging. I. Plasmids containing a T7 replication origin and the T7 concatemer junction are packaged into transducing particles during phage infection. J. Mol. Biol. *216*, 911–926.

Citorik, R.J., Mimee, M., and Lu, T.K. (2014). Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat. Biotechnol. *32*, 1141–1145.

Clark, J.R., and March, J.B. (2004). Bacteriophage-mediated nucleic acid immunisation. FEMS Immunol. Med. Microbiol. *40*, 21–26.

Datsenko, K.A., and Wanner, B.L. (2000). One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. USA *97*, 6640–6645.

Edgar, R., Friedman, N., Molshanski-Mor, S., and Qimron, U. (2012). Reversing bacterial resistance to antibiotics by phage-mediated delivery of dominant sensitive genes. Appl. Environ. Microbiol. *78*, 744–751.

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

Gibson, D.G., Benders, G.A., Andrews-Pfannkoch, C., Denisova, E.A., Baden-Tillson, H., Zaveri, J., Stockwell, T.B., Brownley, A., Thomas, D.W., Algire, M.A., et al. (2008). Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. Science *319*, 1215–1220.

Goren, M.G., Yosef, I., and Qimron, U. (2015). Programming bacteriophages by swapping their specificity determinants. Trends Microbiol. *23*, 744–746.

Goren, M., Yosef, I., and Qimron, U. (2017). Sensitizing pathogens to antibiotics using the CRISPR-Cas system. Drug Resist. Updat. *30*, 1–6.

Harper, D.R., and Enright, M.C. (2011). Bacteriophages for the treatment of Pseudomonas aeruginosa infections. J. Appl. Microbiol. *111*, 1–7.

Kenzaka, T., Tani, K., and Nasu, M. (2010). High-frequency phage-mediated gene transfer in freshwater environments determined at single-cell level. ISME J. *4*, 648–659.

Kiro, R., Molshanski-Mor, S., Yosef, I., Milam, S.L., Erickson, H.P., and Qimron, U. (2013). Gene product 0.4 increases bacteriophage T7 competitiveness by inhibiting host cell division. Proc. Natl. Acad. Sci. USA *110*, 19549–19554.

Lazarus, A.S., and Gunnison, J.B. (1947). The action of Pasteurella pestis bacteriophage on strains of Pasteurella, Salmonella, and Shigella. J. Bacteriol. *53*, 705–714.

Merril, C.R., Scholl, D., and Adhya, S.L. (2003). The prospect for bacteriophage therapy in Western medicine. Nat. Rev. Drug Discov. *2*, 489–497.

Modrich, P., and Richardson, C.C. (1975). Bacteriophage T7 deoxyribonucleic acid replication invitro. Bacteriophage T7 DNA polymerase: an an emzyme composed of phage- and host-specific subunits. J. Biol. Chem. *250*, 5515–5522.

Molineux, I.J. (2005). The T7 group. In The Bacteriophages, S.T. Abedon and R.L. Calendar, eds. (Oxford: Oxford University Press), pp. 275–299.

Qimron, U., Marintcheva, B., Tabor, S., and Richardson, C.C. (2006). Genomewide screens for Escherichia coli genes affecting growth of T7 bacteriophage. Proc. Natl. Acad. Sci. USA *103*, 19039–19044.

Studier, F.W. (1975). Gene 0.3 of bacteriophage T7 acts to overcome the DNA restriction system of the host. J. Mol. Biol. *94*, 283–295.

Yosef, I., Goren, M.G., Kiro, R., Edgar, R., and Qimron, U. (2011). High-temperature protein G is essential for activity of the Escherichia coli clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system. Proc. Natl. Acad. Sci. USA *108*, 20136–20141.

Yosef, I., Kiro, R., Molshanski-Mor, S., Edgar, R., and Qimron, U. (2014). Different approaches for using bacteriophages against antibiotic-resistant bacteria. Bacteriophage *4*, e28491.

Yosef, I., Manor, M., Kiro, R., and Qimron, U. (2015). Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria. Proc. Natl. Acad. Sci. USA *112*, 7267–7272.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Escherichia coli: NEB 5-alpha	New England Biolabs Inc.	Cat#C2987H
Chemicals, Peptides, and Recombinant Proteins		
Taq DNA Polymerase	Lamda Biotech	Cat#D118
Phusion High-Fidelity DNA Polymerase	New England Biolabs	Cat#M0530L
Quick Ligation Kit	New England Biolabs	Cat#M2200L
T4 Polynucleotide Kinase	New England Biolabs	Cat#M0201L
Antarctic Phosphatase	New England Biolabs	Cat#M0289L
BamHI	New England Biolabs	Cat#R3136S
*Bsp*HI	New England Biolabs	Cat#R0517S
*Nae*I	New England Biolabs	Cat#R0190S
*Nde*I	New England Biolabs	Cat#R0111S
*Spe*I	New England Biolabs	Cat#R0133S
*Xba*I	New England Biolabs	Cat#R0145S
*Xho*I	New England Biolabs	Cat#R0146S
Ethyl Methanesulfonate	Sigma-Aldrich	Cat#M0880
Experimental Models: Organisms/Strains		
E. coli: BW25113: F$^-$, Δ*(araD-araB)567, ΔlacZ4787(::rrnB-3),* λ$^-$, *rph-1,* Δ*(rhaD-rhaB)568, hsdR514*	Baba et al., 2006	N/A
E. coli: BW25113Δ*trxA*::kan: F$^-$,Δ*(araD-araB)567, ΔtrxA::kan, ΔlacZ4787 (::rrnB-3),* λ$^-$, *rph-1,* Δ*(rhaD-rhaB)568, hsdR514*	Baba et al., 2006	Keio collection: JW5856
E. coli: BW25113Δ*trxA*: F$^-$,Δ*(araD-araB)567,* Δ*trxA, ΔlacZ4787(::rrnB-3),* λ$^-$, *rph-1,* Δ*(rhaD-rhaB)568, hsdR514*	This paper	N/A
E. coli: BW25113Δ*trxA*Δ*waaC:* F$^-$,Δ*(araD-araB)567,* Δ*trxA*, Δ*waaC::kan,ΔlacZ4787 (::rrnB-3),* λ$^-$, *rph-1,* Δ*(rhaD-rhaB)568, hsdR514*	This paper	N/A
Shigella sonnei: Sso4727	ATCC	ATCC:9290
Salmonella enterica: Sen4510: *Salmonella enterica* serovar arizonae str. SARC5	Ohad Gal-Mor, Sheba Medical Center, Tel Aviv	N/A
Klebsiella pneumoniae: Kpn4718	ATCC	ATCC:10031
K. pneumoniae: Kpn4800II	ATCC	ATCC:9997
Enterobacter cloacae: Ecl4723	ATCC	ATCC:13047
S. enterica: Sen4001: *Salmonella enterica* subsp. enterica serovar Typhimurium str. LT2	Sima Yaron, Technion, Israel	N/A
Klebsiella sp. 390: K390	Bessler et al., 1973	N/A
E. aerogenes: Eae4739	ATCC	ATCC:51697
S. enterica: Sen4513: *Salmonella enterica* subsp. enterica serovar Enteritidis PT4	Ohad Gal-Mor, Sheba Medical Center, Israel	N/A
K. pneumoniae: Kpn4719	ATCC	ATCC:13882
E. coli: Eco4507	ATCC	ATCC:25922
T7 phage:T7$_{\Delta 17:trxA\text{-}FRT}$	This paper	N/A
T7 phage:T7$_{\Delta 17}$	This paper	N/A
T7 phage:T7$_{\Delta 17\Delta(11\text{-}12):trxA\text{-}FRT}$	This paper	N/A
T7 phage:T7$_{\Delta(11\text{-}12\text{-}17)}$	This paper	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
See Table S3 for a list of primers used in this study	N/A	N/A
Recombinant DNA		
pGEM-T Vector Systems	Promega	Cat#A1360
See Table S3 for a list of plasmids used in this study	N/A	N/A
Other		
Phage tail proteins used in this study	This paper	Table S1
Detailed protocols for preparing lysates of T7, transduction assays, and GoTraP	This paper	Methods S1

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Udi Qimron (ehudq@post.tau.ac.il).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Bacterial strains were grown in LB medium supplemented with the indicated antibiotics at 37°C with constant shaking of 250 RPM.

METHOD DETAILS

Reagents, strains, and plasmids

Luria-Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl) and agar were from Acumedia. 2YT medium contained 1.6% (w/v) Bacto-tryptone (Acumedia), 1% (w/v) Bacto-yeast extract (Acumedia), and 0.5% (w/v) NaCl (Acumedia) in distilled water. Antibiotics were from Calbiochem. Ethyl methanesulfonate was from Sigma-Aldrich. Restriction enzymes, ligation enzymes, DNA modification enzymes, and Phusion® High-Fidelity DNA Polymerase were from New England Biolabs. The plasmids and oligonucleotides used in this study are listed in Table S3.

Plasmid construction

Plasmids were constructed using standard molecular biology techniques. DNA segments were amplified by PCR. Standard DNA digestions and ligations were carried out according to the manufacturer's instructions. Phage tail genes were codon optimized (Table S1) and synthesized by GenScript. The plasmids that were used in this study are listed in Table S3. Specific DNA templates and oligos used for plasmids construction are listed in Table S4.

Construction of strains

BW25113Δ*trxA*::kan was obtained from the Keio collection (Baba et al., 2006). BW25113Δ*trxA*Δ*waaC* was constructed by excising the kanamycin resistance marker using pCP20 (Datsenko and Wanner, 2000). The resulting strain (BW25113Δ*trxA*) was used as the acceptor strain in P1 transduction using BW25113Δ*waaC*::kan from the Keio collection as the donor strain. P1 transduction was carried out as described in Yosef et al. (2011).

Preparing phage T7 lacking tail genes

T7 phage, having *trxA-FLP* instead of gene 17, was constructed by homologous recombination as previously described (Kiro et al., 2013). Briefly, BW25113 cells carrying pGEMIY398F (encoding gp17) and pCAIY396 (encoding *trxA-FLP* with flanking homologous sequences to gene 17's ends) were grown overnight. Phages encoding *trxA* were then selected in BW25113Δ*trxA*/pGEMIY398F. FLP recombinase was used to excise the *trxA* gene from T7Δ17*trxA-FLP* phage by continuous propagation of the phage in BW25113/pGEMIY398F/pAC-FRT. T7Δ17 phages were finally isolated as described in Kiro et al. (2013).

To construct T7Δ(11-12-17), genes 11-12 were deleted from phage T7Δ17 using similar procedures. Briefly, BW25113 cells carrying pGEM3RCF (encoding genes 11, 12, and 17) and plasmid pCAIY405 (encoding *trxA-FLP* with flanking homologous sequences to ends of genes 11-12) were used for replacing genes 11-12 with *trxA-FLP*. Phages encoding *trxA* were then selected in BW25113Δ*trxA*/pGEM3RCF. FLP recombinase was used to excise the *trxA* gene from T717Δ(11-12):*trxA*-FRT phage by continuous propagation of the phage in BW25113/pGEM3RCF/pAC-FRT. T7Δ(11-12-17) phages were finally isolated as described in Kiro et al. (2013).

Preparing lysates of T7 TFU

A detailed protocol can be found in the Supplemental Information. Briefly, an overnight culture of the donor strain *E. coli* BW25113, harboring a plasmid encoding a T7 packaging signal and tail proteins, was diluted 1:5 in LB and aerated at 37°C for an additional hour. Cells were washed by centrifugation and re-suspended with fresh LB. The culture was then infected by ~$5*10^8$ PFU of T7Δ(11-12-17) at a multiplicity of infection (MOI) of ~2. The infected culture was aerated for 1.5h–2h at 37°C until lysis occurred. Chloroform was consequently added and the lysate was centrifuged for 2 min at maximum speed. The resulting lysates contained approximately half particles containing the phage DNA and half particles containing plasmid-encoding genes 11, 12, and 17. See also Figure S1. In experiments for determining the specificity of the transduction of plasmids, lysates were prepared as described above except that the overnight culture of *E. coli* BW25113 harbored two plasmids: a plasmid with a T7 packaging signal and an additional plasmid with tail proteins lacking a packaging signal.

Transduction assays

A detailed protocol can be found in the Supplemental Information. Briefly, recipient cells in their exponential growth phase were mixed in a 1:1 ratio (v/v) with serial dilutions of T7 TFU lysates prepared as described above. Cultures were incubated for 60 min at 37°C with shaking. Next, the cultures were plated on a LB-agar plate containing an appropriate antibiotic and incubated overnight at 37°C. TFU/ml counts were normalized to 10^8 transductants obtained in the indicated reference strain.

Preparing a plasmid library using EMS

Overnight cultures of *E. coli* BW25113 harboring a T7 packaging plasmid were diluted 1:50 in 5 mL of LB medium containing appropriate antibiotics. The culture was aerated at 37°C for several hours. Upon reaching mid log-phase, cells were washed twice and re-suspended in phosphate-buffered saline (PBS). EMS (Sigma-Aldrich) was then added to the culture at 1%, and cells were aerated at 37°C for 1 hr. Cells were then washed three time with PBS and then re-suspended in 50 mL LB supplemented with an appropriate antibiotic, and aerated at 37°C for 16 hr. The overnight cultures were used to prepare a library of T7 phages as described above.

GOTraP

A detailed protocol can be found in the Supplemental Information. Briefly, transduction assays with recipient cells and with T7 TFU lysate in total volume of 10 mL were carried out as described above. The cultures were inoculated on three 140 mm Petri dishes containing LB-agar plate supplemented with an appropriate antibiotic. These plates were incubated overnight at 37°C. Transductants were pooled from the selective plates using 7 mL LB and plasmids were extracted. These plasmids (100 ng) were re-transformed into BW25113 and inoculated on 140 mm LB plates supplemented with 50 μg/ml kanamycin. Following overnight incubation, ~1 X 10^6 transformants were collected in 7 mL LB, and mutagenized with EMS as described above. T7 lysates were prepared from the EMS-treated cells, and were used to transduce the recipient cells as described above. The T7 lysate was also used to transduce a reference strain for normalization of the TFU on the recipient cells.

QUANTIFICATION AND STATISTICAL ANALYSIS

The number of repeats for each experiment is indicated in the legend of the respective figure. Averages and SDs were calculated using Microsoft Excel 2013.

ADDITIONAL RESOURCES

Detailed Protocols

Methods S1 in the Supplemental Information details three protocols. Protocol 1 describes how to prepare T7 TFU lysates from a donor strain. Protocol 2 describes how to transduce a plasmid from a donor strain to a recipient host. Protocol 3 describes each step in the GOTraP procedure, designed to optimize DNA transduction into a recipient host.