An Optimal Lysis Time Maximizes Bacteriophage Fitness in Quasi-continuous Culture

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ABSTRACT

Optimality models have a checkered history in evolutionary biology. While optimality models have been successful in providing valuable insight into the evolution of a wide variety of biological traits, a common objection is that optimality models are overly simplistic and ignore organismal genetics. We revisit evolutionary optimization in the context of a major bacteriophage life history trait, lysis time. Lysis time refers to the period spanning phage infection of a host cell and its lysis, whereupon phage progeny are released. Lysis time, therefore, directly determines phage fecundity assuming progeny assembly rate is maximized. Noting that previous tests of lysis time optimality rely on batch culture, we implemented a quasi-steady state system to observe productivity of a panel of isogenic phage λ mutants differing in lysis time. We report that λ phage productivity in our experiments is maximized around an optimal lysis time of 65 min, which is the lysis time of the λ "wildtype" strain. We discuss this finding in light of recent results that lysis time variation is also minimized in the λ "wildtype" strain.

INTRODUCTION

Evolutionary biologists have long been interested in the power of natural selection to refine biological adaptations (Smith 1978; Endler 1986; Lewontin 1989; Crow 1993). Generally, views on the power of natural selection fall into two camps: one emphasizing the ability of natural selection to optimize biological traits (Endler 1986; Parker and Smith 1990) and the other stressing the limitations of natural selection in face of evolutionary tradeoffs and genetic details (Lewontin 1989; Crow 1993) affecting phenotypic traits. Because of the relative simplicity of their life histories (for review see Abedon 2008), bacteriophages (phages) are an ideal arena to test predictions regarding optimality evolution, thus several studies have applied optimization

principles to theoretically predict how these traits will evolve in response to changing ecological conditions (Abedon 1989; Wang et al. 1996; Abedon et al. 2001; Bull et al. 2004; Bull 2006; Bonachela and Levin 2014).

However, despite clear predictions, experimental tests have generally failed to confirm theoretical predictions. T7, ST-1, and ΦX174 phages with theoretically predicted suboptimal lysis times failed to evolve predicted optimal lysis times (Heineman et al. 2007; Chantranupong and Heineman 2012). While the evolution of T7 phage with a deleted lysin gene qualitatively supported optimality predictions, it was noted that abolition of the lysis function did not extend the latent period, thus had no effect on the expected tradeoff between latent period and burst size (Heineman et al. 2007). ST-1 qualitatively, but not quantitatively, adapted as predicted by modeling, but ΦX174 failed to adapt at all (Chantranupong and Heineman 2012). In another study, experimental evolution of phage RB69 did result in selection for shorter lysis time mutants in high host density cultures, but it is unclear whether the lysis time of these mutants were optimal for the experimental conditions (Abedon et al. 2003). A study employing isogenic lambda phages with different lysis times found the model predictions for both the optimum and fitness to be different from the experimental estimations (Wang 2006).

However, we note that these studies used batch cultures to examine host-phage interactions, and phage fitnesses were estimated using absolute growth rates. These conditions may not effectively mimic the kinds of conditions experienced by phages in their natural habitats. To revisit the question of phage optimal lysis time, we employed a different approach, which was inspired by Bonachela *et. al* (2014), who modeled host-virus interactions in natural environments in terms of steady state conditions, such as those seen in continuous culture systems. A typical example of a continuous culture system is a two-stage chemostat. In the first stage, a chemostat

receives a flow of fresh media to maintain a continuous culture of bacteria. Fresh host bacteria from the first chemostat are fed into a second chemostat containing a population of phages. Both host cells and phages are washed out from the second chemostat at a specified rate. This system can mimic steady states that exist in natural environments, such as the gastrointestinal tract where peristaltic movements can maintain a continuous flow.

In this study, we maintained phage λ and its host, *Escherichia coli*, under quasi-steady state conditions. To initiate experiments, λ holin mutants differing in their lysis times (Dennehy and Wang 2011; Singh and Dennehy 2014; Kannoly et al. 2020) were added to exponentially replicating batch cultures of *E. coli*. After a short period of growth, λ phages were filtered from host cells, and a small fraction was transferred into a fresh exponential culture. At no time did these phages experience stationary phase host cells during the course of the experiment.

In principle, differences in lysis times should result in differences in phage productivity (Levin et al. 1977; Wang 2006). We predicted that there exists an optimum for lysis time that maximizes phage production under these quasi-steady state conditions. By contrast, strains with suboptimal lysis times should show declining phage populations in this experimental context. Our results show that phage production in these quasi-steady state conditions is maximized at lysis times approaching 65 min, which, perhaps not coincidentally, is the lysis time of the phage λ wildtype. These results suggest an optimum lysis time that maximizes phage progeny production.

RESULTS AND DISCUSSION

Lysis is the last step in the infectious cycle of lytic bacteriophage. It results in the release of virions by disrupting the host cell envelope. Lysis timing in many phage is controlled by a single protein, holin, which accumulates in the cell membrane up to a genetically determined time,

whence it nucleates generating membrane-permeabilizing holes (Wang et al. 2000, 2003; Dennehy and Wang 2011; Singh and Dennehy 2014; Kannoly et al. 2020).

To study the effect of lysis time on phage productivity, we employed a panel of λ phages harboring mutations in the holin gene *S*, which delay or hasten the lysis event following induction of the lytic cycle. These λ mutants were used to initiate batch cultures containing a fixed concentration of *E. coli* cells at a multiplicity of infection (MOI) of 0.001, then purified, diluted 1000-fold, and transferred to fresh cultures at 3 hr intervals, generating quasi-steady state conditions. A low MOI also minimizes multiple infections per cell and ensures multiple generations without depleting host cells to such an extent that the cell number becomes a limiting factor for phage growth. Furthermore, the short duration of growth prevents *E. coli* densities from being sufficiently high enough to enter stationary phase. A 3 h infection allows at least three generations of reproduction for wild type λ such that the MOI ≈ 1 at the end of phage growth.

Ideally, this study would be performed using actual chemostats, but we choose not to pursue this option because it would limit the number of holin mutants and degree of replication that we could easily manage. Moreover, it is well known that *E. coli* growth is consistent (i.e., in a steady state) as long as exponential growth conditions are maintained (Fishov et al. 1995) so, in physiological terms, *E. coli* growth is not inherently different between chemostats and our quasi-steady state transfers. Our study is also limited to a total of five transfers approximating fifteen phage generations, beyond which phage adaptation would begin to impact our results.

Five such serial transfers were completed, and the phage titers were obtained after every transfer (Fig. 1). Our data show that phage progeny production following five transfers was maximized with a peak close to 65 - 69 min (Fig. 2; Fig. 3). Interestingly the phage λ wildtype strain falls within this range (Fig. 2; Fig. 3). Outside this range, phage progeny production drops

118 precipitously up to six orders of magnitude (Fig. 2; Fig. 3). This outcome demonstrates the strong
119 influence of lysis timing on the reproductive success of the phage.

120 Conceptually, phage production per generation depends on the burst size, which is
121 positively correlated to the lysis time (Wang 2006; Baker et al. 2016). The exact shape of this
122 correlation is a matter of debate (Baker et al. 2016). A phage genotype with a short lysis time and
123 hence a low burst size would result in an overall reduction in phage productivity. Thus, fewer
124 phages are carried over to the next transfer. Conversely, one might intuitively assume that, for
125 delayed-lysing strains, the phage productivity will be significantly higher. However, a delay in
126 lysis traps a significant number of phages within cells, which fail to make it to the next transfer.
127 Although delaying lysis increases the number of offspring, it also delays the generation time. This
128 trade-off makes the optimum sensitive to environmental conditions such as dilution due to a
129 constant flow rate. Our experimental set up simulates such a dilution, and the strains with lysis
130 times that deviate from the optimum show reduced phage productivity over subsequent transfers
131 (Figs. 2 and 3).

132 In a previous study using phage λ holin mutants, we demonstrated that holin accumulation
133 thresholds generate precision in lysis timing, and that noise in lysis timing is reduced in mutants
134 with lysis times closer to the wild type value (Ghusinga et al. 2017; Kannoly et al. 2020). These
135 results showing that the variation in lysis timing is minimized support the idea of an optimal lysis
136 time. If there was no lysis time optimum, it would be difficult to explain why lysis time noise
137 minimization seems to be selectively favored. This conclusion contrasts previous studies that have
138 suggested that mutations increasing lysis time variance will be favored by selection (Bull et al.
139 2011; Baker et al. 2016). It was hypothesized that since phage burst size increases linearly
140 (Hutchison and Sinsheimer 1966; Wang 2006), early lysis events would contribute more to

141 population growth than late lysis events detracts (Bull et al. 2011; Baker et al. 2016). However,
142 this outcome only holds if the rate of phage production is linear. If phage assembly is non-linear,
143 it may be the case that noise minimization is favored. We hope to clarify this possibility with future
144 experiments. Interestingly, Storms *et. al* (2014) have reported that T4 phage productivity in a cell
145 about to undergo cell division was almost three times greater than the productivity in a young,
146 newly formed cell (Baker et al. 2016). We speculate that if the contribution of early lysis events to
147 population growth is balanced by larger bursts in the later stages of infection, mutations that
148 increase lysis time variance may not be favored after all (Baker et al. 2016).

149 Taken together, minimizing lysis time variance may factor in the evolution of holin. In this
150 study, we used holin mutants to further explore the effect of lysis time on phage productivity under
151 quasi-steady state conditions. Again, mutants with lysis times closer to that of the wildtype showed
152 a consistent increase in phage productivity compared to those with suboptimal lysis times.
153 Optimality models can reveal the underlying ecological contexts influencing evolutionary
154 processes. Our results suggest that the wildtype λ might already be selected for conditions where
155 a constant dilution results in a continuous wash out. Further experiments to test this hypothesis
156 might involve direct competition of wildtype against mutants under similar conditions. Also, long-
157 term experimental evolution under the same conditions should reveal if phage strains with
158 suboptimal lysis times could evolve towards optimality.

159 Our results add additional support for the idea that life history traits can evolve to optimal
160 values. Much work has been conducted experimentally assessing the validity of optimality models
161 with varying degrees of success (Kozlowski 1999; Dekel and Alon 2005; Miller et al. 2006; Camps
162 et al. 2007; Poelwijk et al. 2011*b*, 2011*a*; Nakabayashi 2012; Graves and Weinreich 2017). At this
163 point, it is clear that an unnuanced account of optimality evolution may fail because of genetic

164 details or constraints (Bull et al. 2004). Nonetheless, optimality models have provided valuable
165 insights into biological traits ranging from the genetic code to animal behavior (Charnov 1976;
166 Parker and Smith 1990; Jensen et al. 2006; Roff et al. 2006; Noblin et al. 2008; Baranov et al.
167 2009; Henshaw 2018; Ruess et al. 2019). Similarly, optimality can provide greater insight into the
168 evolution of bacteriophage life history traits.
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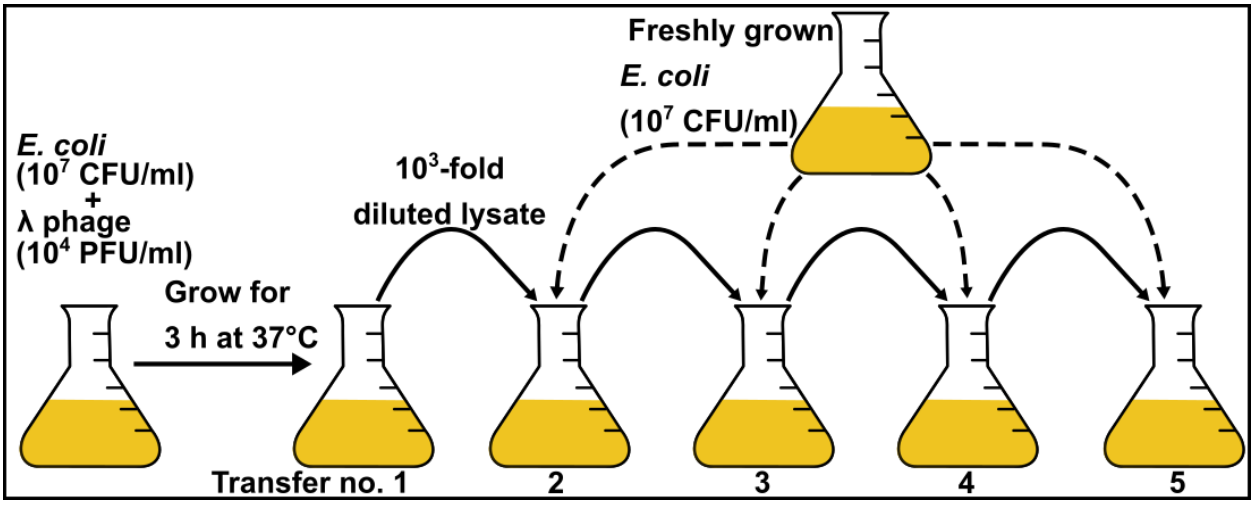

FIG. 1. Serial transfers. For serial transfers, λ phage strains were cocultured with exponentially growing host cells, filtered to separate phages, and diluted 1000-fold to start the next transfer with freshly growing cultures. After every transfer, the filtered phage lysates were titered using plaque assays.

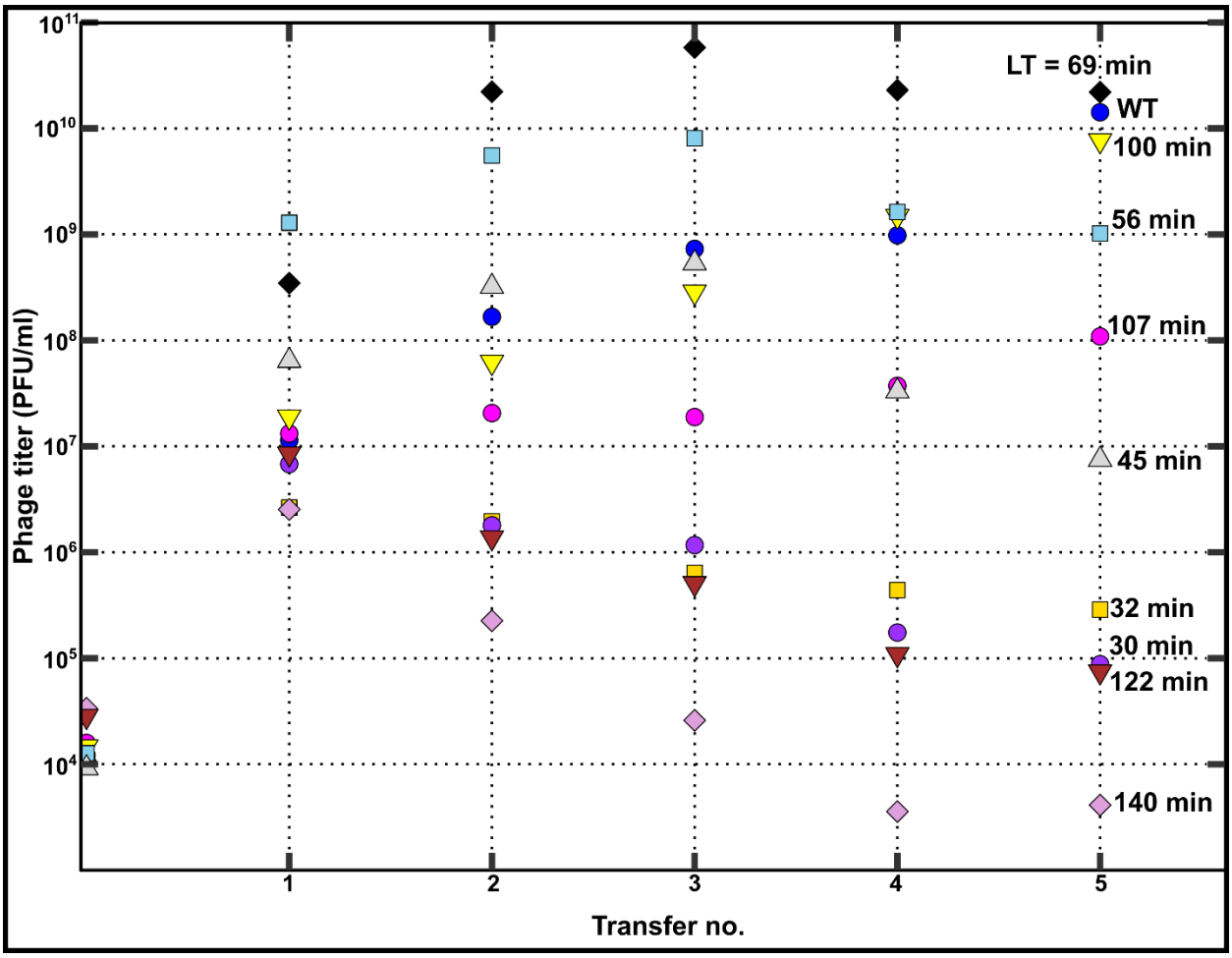

FIG. 2. Titers of λ strains with different lysis times (LT) after each transfer. Lysis times are from single-cell estimates.

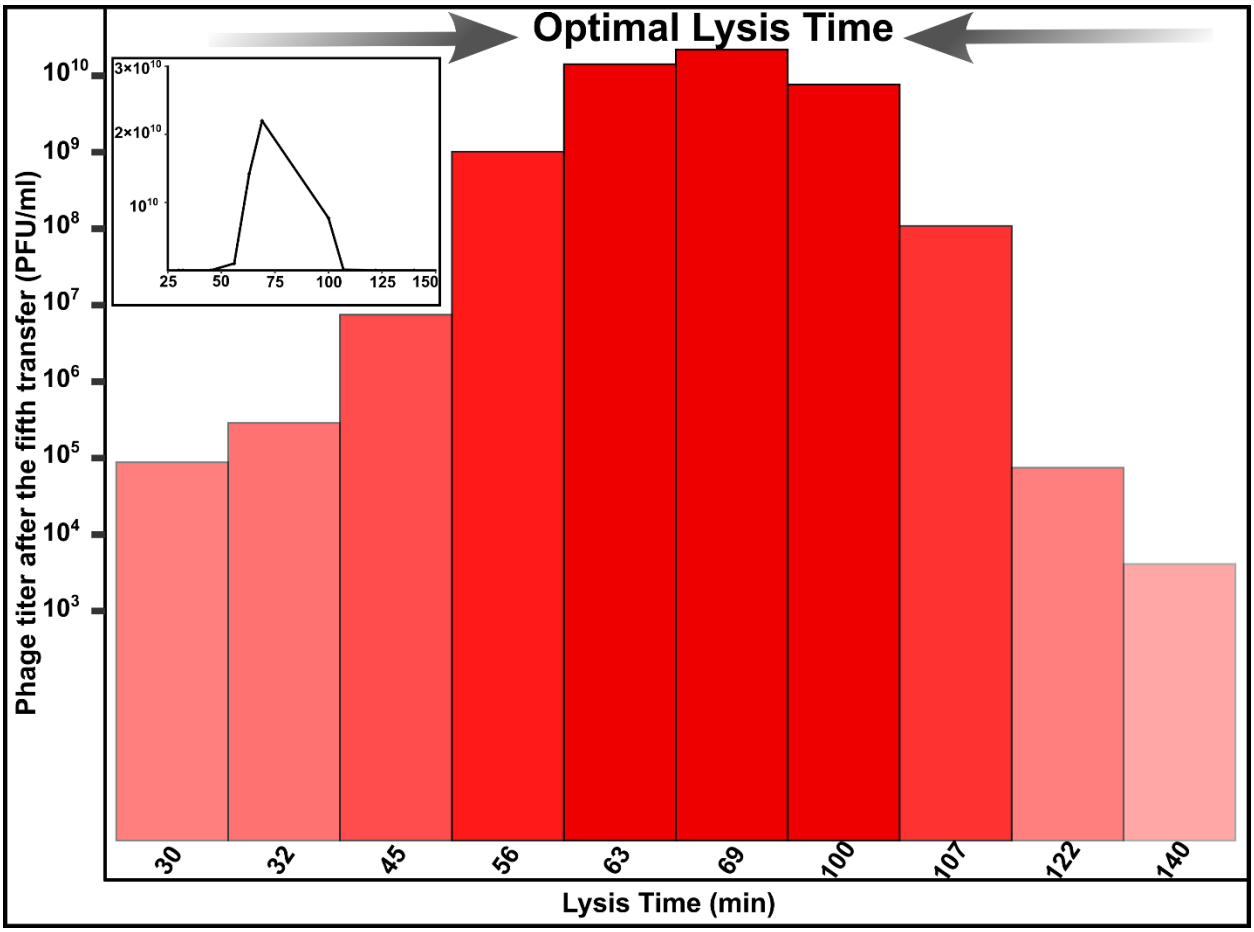

FIG. 3. Titers of λ strains with different lysis times after the fifth transfer (Log scale). Titers increase as the lysis time approaches optima. Inset shows the same data on a linear scale.

184 # MATERIALS AND METHODS

185 ## Bacterial and phage strains

186 All bacterial and phage strains used in this study are listed in Table 1.

Strain	Genotype	Source
CGSC#: 6152[a]	*E. coli* MC4100 (λ-)	(Casadaban 1976)
JJD3	MC4100 (*λ cI857 S*)	(Wang 2006)
JJD246	MC4100 (*λ cI857 S105*$_{H7D}$)	INW
JJD248	MC4100 (*λ cI857 S105*$_{F94C}$)	INW
JJD391	MC4100 (*λ cI857 S105*$_{A16G/K92Q}$)	INW
JJD404	MC4100 (*λ cI857 S105*$_{I21V}$)	INW
JJD405	MC4100 (*λ cI857 S105*$_{V45G}$)	INW
JJD414	MC4100 (*λ cI857 S105*$_{L90I}$)	INW
JJD423	MC4100 (*λ cI857 S105*$_{F27Y}$)	INW
JJD432	MC4100 (*λ cI857 S105*$_{S89W}$)	INW
JJD436	MC4100 (*λ cI857 S105*$_{K92N}$)	INW

187 [a]Coli Genetic Stock Center; INW, Ing-Nang Wang (unpublished data)

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Plaque assays

The plaque assays were designed for minimizing variation in plaque size and enabling plaque counting. The *E. coli* strain MC4100 was grown overnight in TB broth (5 g NaCl and 10 g tryptone in 1 L water) plus 0.2% maltose at 37°C. The overnight culture was diluted with equal volume of TB + maltose and grown for another 1.5 h. 100 ul of these cells were mixed with appropriately diluted phage lysates and incubated at room temperature for 20 min to allow pre-adsorption. This mixture was then added to 2.5 ml of molten H-top agar (Miller 1992), gently vortexed, and overlaid onto freshly prepared plates containing 35 ml LB agar. The plates were then incubated at 37°C and plaques were counted after 18–22 h.

Thermal induction of lysogens to obtain phage lysates

Lysogens were grown overnight in LB media at the permissive temperature of 30°C. Overnight cultures were diluted 100-fold and grown in a 30°C shaking incubator till an OD_{600} of 0.3–0.4 was reached. For heat induction, the cultures were transferred to a 42°C shaking water bath for 20 min. Following induction, the cultures were transferred to a 37°C shaking incubator until lysis. The phage lysates were filtered and titered using plaque assay.

Single-cell lysis time determination

The protocol for determining single-cell lysis times has been described previously (Dennehy and Wang 2011). Briefly, a 200-µl aliquot of exponentially growing culture (OD_{600} = 0.3–0.4) of lysogens was chemically fixed to a 22 mm square glass coverslip, which was pretreated with 0.01% poly-L-lysine (mol. wt. 150 K–300 K; Millipore Sigma, St. Louis, MO) at room temperature for 10 min. Using this coverslip, a perfusion chamber (RC-21B, Warner Instruments, New Haven,

CT) was assembled and immediately placed on a heated platform (PM2; Warner Instruments, New Haven, CT). The heated platform was mounted on an inverted microscope stage (TS100, Nikon, Melville, NY), and the perfusion chamber was infused with heated LB at 30°C (Inline heater: SH-27B, dual channel heating controller: TC-344B; Warner Instruments, New Haven, CT). The chamber temperature was elevated to 42°C for 20 min and thereafter maintained at 37°C until ~95% cells were lysed. Videos of cell lysis were recorded using an eyepiece camera (10X MiniVID™; LW Scientific, Norcross, GA). The lysis times of individual cells were visually determined using VLC™ media player. Lysis time was defined as the time required for a cell to disappear after the temperature was increased to 42°C. For each lysogen, an average of approximately 100 cells was used to calculate the lysis time.

Serial Transfers

To initiate the transfers, an *E. coli* (~10^7 CFU/ml) culture growing exponentially at 37°C in a shaking incubator (200 rpm) was infected with a phage lysate (~10^4 PFU/ml). The infection could proceed for 3 h, after which phages were separated by filtration using a 0.2 µ syringe filter (Pall Corp.). The filtered lysate was diluted 1000-fold to start the next transfer using freshly growing cells (~10^7 CFU/ml). These steps were repeated four more times for a total of five serial transfers (Fig. 1). Plaque assays were performed at the end of each transfer to determine the titers of phage lysates.

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238 Wang, and Daniel Weinreich.

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