Supplementary Material For:

The case for trypsin release of affinity-selected phages

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Phage constructs

Trypsin-release vectors

The cloning-site sequences of the trypsin-release vectors and their corresponding biotinylated testers are diagrammed in Figure S1. In Type 88 (1) vector f88TR1 (GenBank Accession no. HM355480), as in vector f88–4 (Accession no. AF218363), the cloning site lies in an IPTG-inducible synthetic gene *VIII* that supplements the wild-type gene *VIII* in the same genome. In Type 3 (1) vector f3TR1 (Accession no. HM355479), as in vector fUSE5 (Accession no. AF218364), the cloning site lies in the genome's sole gene *III*, which is constitutively expressed. Like all vectors derived from fd-tet (Accession no. AF217317.1), including f88–4 and fUSE5, both f88TR1 and f3TR1 confer tetracycline resistance on the host cell, and have disrupted, non-functional minus-strand origins (2). In addition, in f3TR1 (but not f88TR1), a 263-bp deletion corresponding to fd-tet positions 8266 to 8528 eliminates 108 nucleotides of the disrupted minus strand origin, leaving only a 25-nucleotide remnant. In both vectors, the two *Bgl*I sites shown are the only ones in the genome, an engineered silent mutation in the *tetA* tetracycline resistance gene having removed a third *Bgl*I site.

Biotinylated tester virions

The f88TR1-AviTag and f3TR1-AviTag testers (Figure S1) display multiple copies of the AviTag peptide, which is a substrate for the biotinylating enzyme BirA (3). The f88TR1-AviTag tester was biotinylated on 10 of its ~100 displayed AviTag peptides by propagation in the BirA-overexpressing host AVB100 (Avidity, Inc., Aurora, CO, USA). The f3TR1-AviTag tester was biotinylated on all five of its displayed AviTag peptides by the BirA enzyme in vitro according to the supplier's recommendations (Avidity). Biotinylated virions were purified and freed of unbound biotin by multiple precipitations with polyethylene glycol (PEG) as described below. Biotinylation levels were determined as described (4). Digestion in solution with 2.5 µM trypsin in phosphate buffer (0.1 M NaH_2PO_4, pH 7.0 with NaOH) for 1–5 h at 37°C sufficed to remove all biotins without decreasing infectivity (data not shown).

Materials and methods

Propagation of phages in liquid culture

NZY medium (5) supplemented with 0.2 µg/mL tetracycline was inoculated with cells infected or transfected with fd-tet–derived phages, which bear an inducible tetracycline resistance determinant. The culture was shaken vigorously at 37°C for a few hours to allow induction of tetracycline resistance by the sub-inhibitory concentration of tetracycline, after which additional tetracycline was added to a full inhibitory concentration of 15–20 µg/mL and shaking continued overnight (≥12 h) at 37°C. The culture was cleared of cells by two successive 20-min centrifugations at ~3500 and 9500× *g* (the exact speed are not important), yielding a doubly-cleared culture supernatant with ~5 × 10^{11} virions/mL.

Partial purification of virions by PEG precipitation

To 1 volume of cleared virion solution (e.g., doubly cleared culture supernatant as described in the previous subsection) was added 0.15 volumes of PEG/NaCl (5). The solution was mixed thoroughly, and if the virion concentration was less than ~10^{13} virions/mL (roughly 20× the concentration in culture supernatant) allowed to stand overnight in the refrigerator. The precipitated virions were pelleted by centrifuging at 4°C for 30 min at ~5000× *g* or higher; the supernatant was decanted, the centrifuge bottle or tube was centrifuged again for 5 min with the pellet oriented centrifugally, and residual supernatant was removed by aspiration. The pellet was dissolved in the desired buffer, and the solution was cleared of insoluble material by centrifugation at 4°C for 10 min at 10,000× *g*. Two successive PEG precipitations from doubly cleared culture supernatant (the second one from 1/25 of the volume of the original supernatant) yielded virions that were sufficiently pure for almost all purposes. The virions can be stored at 4°C for years.

Spectrophotometric quantification of virions

Virion samples that had been purified by a suitable method (e.g., by two successive PEG precipitations as described in the previous subsection) were diluted to ~2.5 × 10^{12} virions/mL in a non-UV–absorbing buffer between pH 5 and pH 9, and scanned spectrophotometrically from 220 to 320 nm using the same buffer as reference. The absorption spectrum has a characteristic broad plateau at ~260–280 nm, with a shallow maximum at ~269 nm (6). Virion concentration was calculated as

$$\text{virions/mL} = \frac{(OD_{269} - OD_{320}) \times 6.083 \times 10^{16}}{\text{number of nucleotides/virion}} \quad \text{[Eq. S1]}$$

Subtraction of the optical density (OD) at 320 nm was meant to correct crudely for light scattering, since there was little absorption by virion chromophores at that wavelength; the conversion factor 6.083 × 10^{16} is the number of viral DNA nucleotides per mL in a phage solution that gives an absorption of 1 at 269 nm (6).

Preparation of high-density log-phase culture

NZY medium was inoculated with *Escherichia coli* K91BlueKan (7) and shaken overnight at 37°C; a 20-µL aliquot of the overnight culture was used to inoculate 20 mL Terrific Broth [Tartof and Hobbs (8) but without kanamycin] in a 250-mL baffled culture flask shaken vigorously (at least 230 revolutions/min) at 37°C until the OD_{600} of a 1/10 dilution reached 0.125 (~3 h); the culture was poured into a sterile 50-mL tube, incubated at 37°C without shaking for 5 min to allow regeneration of sheared F pili, and used within ~5 min for infection.

Titering tetracycline-resistant colony-forming units (cfu) and calculating infectivity

Phage samples were diluted in TBS/gelatin (5) to concentrations of ~10^6 virions/mL. Ten-microliter droplets of phage dilutions were deposited on the inner walls of sterile 17 × 100-mm polypropylene snap-cap

Type 88 vector f88TR1

signal peptidase / trypsin-sensitive tether / trypsin / mature wild-type pVIII

L S F A▼A G A G A R▼R▼A P A E G D
CTAAGCTTTGCCGCAGGGCTGGTGCCCGTCGGGCGCCGGCAGAAGGTGAT
GATTCGAAACGGCGTCCCGACCACGGGCAGCCCGCGGCCGTCTTCCACTA
*Bgl*I stuffer *Bgl*I

Type 88 tester f88TR1-AviTag

signal peptidase / displayed AviTag peptide / biotin / trypsin

A▼A **G L N D I F E A Q K I E W H E** G R▼R▼A
GCCGCAGGTCTGAACGACATCTTCGAAGCGCAGAAAATCGAATGGCACGAAGGTCGTCGGGCG
CGGCGTCCAGACTTGCTGTAGAAGCTTCGCGTCTTTTAGCTTACCGTGCTTCCAGCAGCCCGC

Type 3 vector f3TR1

signal peptidase / trypsin-sensitive tether / trypsin / mature wild-type pIII

Y S H S▼A D *---frameshift---* G A R▼R▼A G A E T V
TATTCTCACTCGGCCGACGGGGCCCGGCCGCTGGGGCCCGCCGTGCTGGGGCCGAAACTGTT
ATAAGAGTGAGCCGGCTGCCCCGGGCCGGCGACCCCGGGCGGCACGACCCCGGCTTTGACAA
*Bgl*I stuffer *Bgl*I

Type 3 tester f3TR1-AviTag

signal peptidase / displayed AviTag peptide / biotin / trypsin

S▼A D **G L N D I F E A Q K I E W H E** A G A R▼R▼A
TCGGCCGACGGTCTGAACGACATCTTCGAAGCGCAGAAAATCGAATGGCACGAAGCTGGGGCCCGCCGTGCT
AGCCGGCTGCCAGACTTGCTGTAGAAGCTTCGCGTCTTTTAGCTTACCGTGCTTCGACCCCGGGCGGCACGA

Figure S1. Sequence at the cloning sites of Type 88 and Type 3 trypsin-release vectors and their corresponding biotinylated testers. Details are in the "Phage constructs" section of the main text. The f88TR1-AviTag tester was biotinylated on 10 of its displayed AviTag peptides, scattered randomly along the length of the filamentous virion. The f3TR1-AviTag tester was biotinylated on all five of its displayed AviTag peptides, which are tightly clustered at one tip of the virion.

tubes (e.g., Cat. no. 14–956–1J; Fisher Scientific, Pittsburg, PA, USA), slanted so that the droplet doesn't roll down to the bottom of the tube. A 10-µL droplet of high-density log-phase culture (previous subsection) was deposited on each phage droplet; after 15 min at room temperature, the mixed droplets were washed down to the bottoms of the tubes with 1-mL portions of NZY supplemented with 0.2 µg/mL tetracycline, a sub-inhibitory concentration that induces expression of the tetracycline resistance gene in newly infected cells (see "Propagation of phage in liquid culture" section, above). The caps were replaced in the loose position, and the tubes were shaken upright at 37°C for 45 min, after which a 200-µL portion of each tube was spread on a Petri dish containing NZY nutrient agar supplemented with tetracycline at 40 µg/mL and kanamycin at 100 µg/mL (the K91BlueKan host cells are resistant to the latter antibiotic). The dishes were incubated at 37°C and colonies were counted after 16–24 h. The cfu/mL titer in the original 10-µL phage sample was calculated by multiplying the colony count by 510. The corresponding infectivity is the cfu/mL titer divided by the calculated number of virions/mL in the 10-µL sample. Infectivity determined in this way averages ~5%—roughly 10× lower than the plaque-forming infectivity of wild-type filamentous phage—and is quite variable from experiment to experiment, though less so between duplicates within the same experiment. The reason for low and variable infectivity lies in the fd-tet family's replication defect (2); this somewhat inconvenient feature of the family is offset by key advantages (9).

Trypsin release of biotinylated testers from a streptavidin-coated ELISA dish

Wells of a streptavidin-coated ELISA dish (Cat. no. 15500; Pierce Chemical Co., Rockford, IL, USA) were blocked overnight at 4°C with SuperBlock (Cat. no. 37515; Pierce) and washed with TBS/Tween (5). Serial dilutions of biotinylated tester virions (Figure S1) in SuperBlock supplemented with 0.5 percent v/v Tween 20 were applied to the wells, and the dish was incubated overnight at 4°C to allow binding. After blocking unused biotin binding sites (10 µM biotin in TBS/Tween for 1 h at room temperature) and washing with TBS/Tween to remove residual unbound virions (if any), some wells were treated with 125 µL 2.5-µM trypsin in phosphate/Tween (0.1 M NaH_2PO_4, pH 7.0 with NaOH, 0.5% v/v Tween 20), while others were treated with 125 µL phosphate/Tween alone. After incubation at 37°C (1.75–3.25 h), 175-µL aliquots of a high-density log-phase culture of infectible cells (see "Preparation of high-density log-phase culture" section, above) were added to some of the wells, after which the dish was incubated at room temperature for 1 h to allow released virions to infect cells. Aliquots (50 µL) of the infected wells were diluted into 1 mL NZY (5) supplemented with tetracycline at a sub-inhibitory concentration (0.2 µg/mL); the dilutions were shaken for 45 min at 37°C to allow expression of the phage-borne tetracycline resistance gene, after which a 200-µL aliquot of each culture was spread on a selective plate (NZY supplemented with tetracycline at 40 µg/mL and kanamycin at 100 µg/mL). Plates were incubated overnight at 37° and counted, yielding the colony counts reported in main-text Figure 1A. Meanwhile, after the 1-h room temperature infection period, the ELISA dish was washed with TBS/Tween to remove unbound phages, and unreleased virions were quantified by phage capture ELISA essentially as described (10), yielding the data reported in main-text Figure 1B.

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