Title: Genomic Recoding Broadly Obstructs the Propagation of Horizontally Transferred Genetic Elements

Authors:

Natalie J. Ma[1,2,3] & Farren J. Isaacs[1,2,4]
[1]Department of Molecular, Cellular & Developmental Biology, Yale University, New Haven, CT 06520, USA
[2]Systems Biology Institute, Yale University, West Haven, CT 06516, USA
[3]natalie.ma@yale.edu
[4]Correspondence should be addressed to F.J.I. (farren.isaacs@yale.edu, Phone: 203.737.3156, Fax: 203.737.3109)

SUPPLEMENTAL TABLES AND FIGURES

Table S1, HTGE-encoded UAG-ending genes the distance to the next stop codon, related to Figures 1, 2, and 3. Available as separate Excel file

Table S2 – Doubling times of strains carrying RK2 plasmid variants, related to Figure 3

Strain	RK2 variant	Doubling Time (min)
+UAG+RF1	No plasmid	63.80
+UAG+RF1	*pRK2*	73.42
+UAG+RF1	*pRK2 trfA*$_{TAA}$	70.60
+UAG+RF1	*pRK2rec*	71.81
ΔUAG+RF1	No plasmid	89.46
ΔUAG+RF1	*pRK2*	96.51
ΔUAG+RF1	*pRK2 trfA*$_{TAA}$	96.97
ΔUAG+RF1	*pRK2rec*	95.73
ΔUAGΔRF1	No plasmid	89.81
ΔUAGΔRF1	*pRK2*	114.57
ΔUAGΔRF1	*pRK2 trfA*$_{TAA}$	98.36
ΔUAGΔRF1	*pRK2rec*	96.59

Table S3, Metagenomic analysis of UAG-ending genes for *Enterobacteria* and *Escherichia* phage genomes archived on NCBI's Viral Genomes Resource, Related to Figure 1. Available as separate Excel file

Table S4 - *Escherichia coli* MG1655-derived strains used in this study, related to Experimental Procedures

Unique ID	Designation	Genotype	Resistance Markers
NJM 431	ECNR2GIB.prfA+.ΔλRed	*ΔmutS:zeo, ΔtolC, bioAB+*	Zeocin
NJM 432	C31GIB.ΔλRed	*0TAG, ΔmutS:zeo, ΔprfA, ΔtolC, bioAB+*	Zeocin
NJM 433	C31GIB.prfA+.ΔλRed	*0TAG, ΔmutS:zeo, ΔtolC, bioAB+*	Zeocin
NJM 795	ECNR2.ΔmutS:zeocin.ΔλRed	*ΔmutS:zeo, tolC in WT position*	SDS, Zeocin
NJM 796	C31.final.ΔmutS:zeocin.ΔprfA.ΔλRed	*0TAG, ΔmutS:zeo, ΔprfA, tolC in WT position, lacZ-*	SDS, Zeocin
NJM 797	C31.final.321.ΔmutS:zeocin.ΔλRed	*0TAG, ΔmutS:zeo, tolC in WT position, lacZ-*	SDS, Zeocin

Figure S1 – Plaque images from relative titer assays, Related to Figure 1. (A) Close-up and (B) uncropped images from viral relative titer assays. (C) Plates with phage λ at high multiplicity of infection. A control plate with only *ΔUAGΔRF1* cells forming a bacterial lawn, a plate *ΔUAGΔRF1* cells challenged with 10^6 viral particles also forms a bacterial lawn, while a plate of *ΔUAGΔRF1* cells challenged with 10^8 viral particles is clear of bacterial growth with exception of scattered colonies.

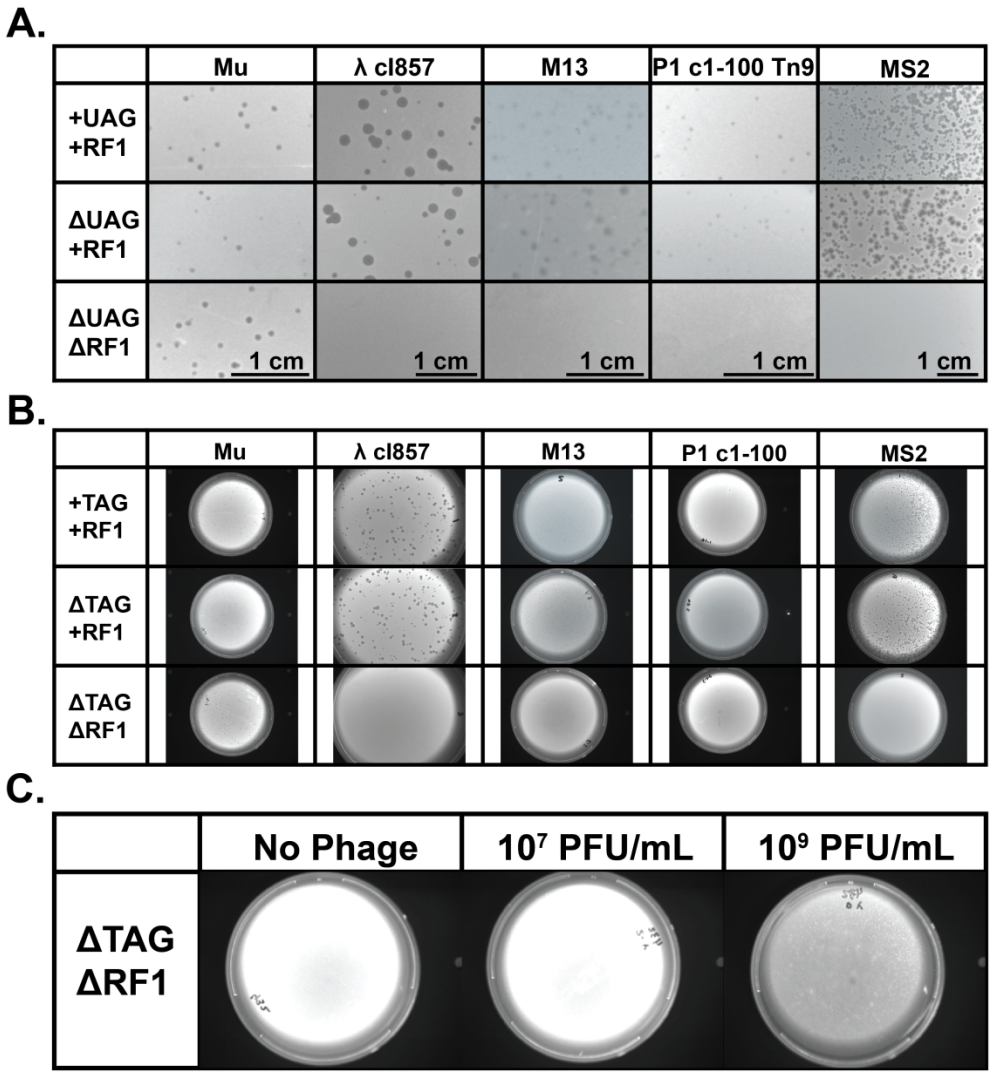


Figure S2 - Adhesion and progeny per cell differs between F-independent and F-dependent phages, Related to Figure 1. (A) A schematic of viral infection demonstrating adhesion and progeny per cell. Viral adhesion occurs on cell surface receptors, leading to infection and production of viral progeny in the cell. (B) λ cI857, (C) M13, (D) MS2 adhesion and (E) P1 c1-100 adsorption (left y-axis) and progeny per cell (right y-axis). Data are mean with standard deviation, n=3. P-values are denoted as follows: * is P ≤ 0.05, ** is P ≤ 0.01, *** is P ≤ 0.001, and **** is P ≤ 0.0001.

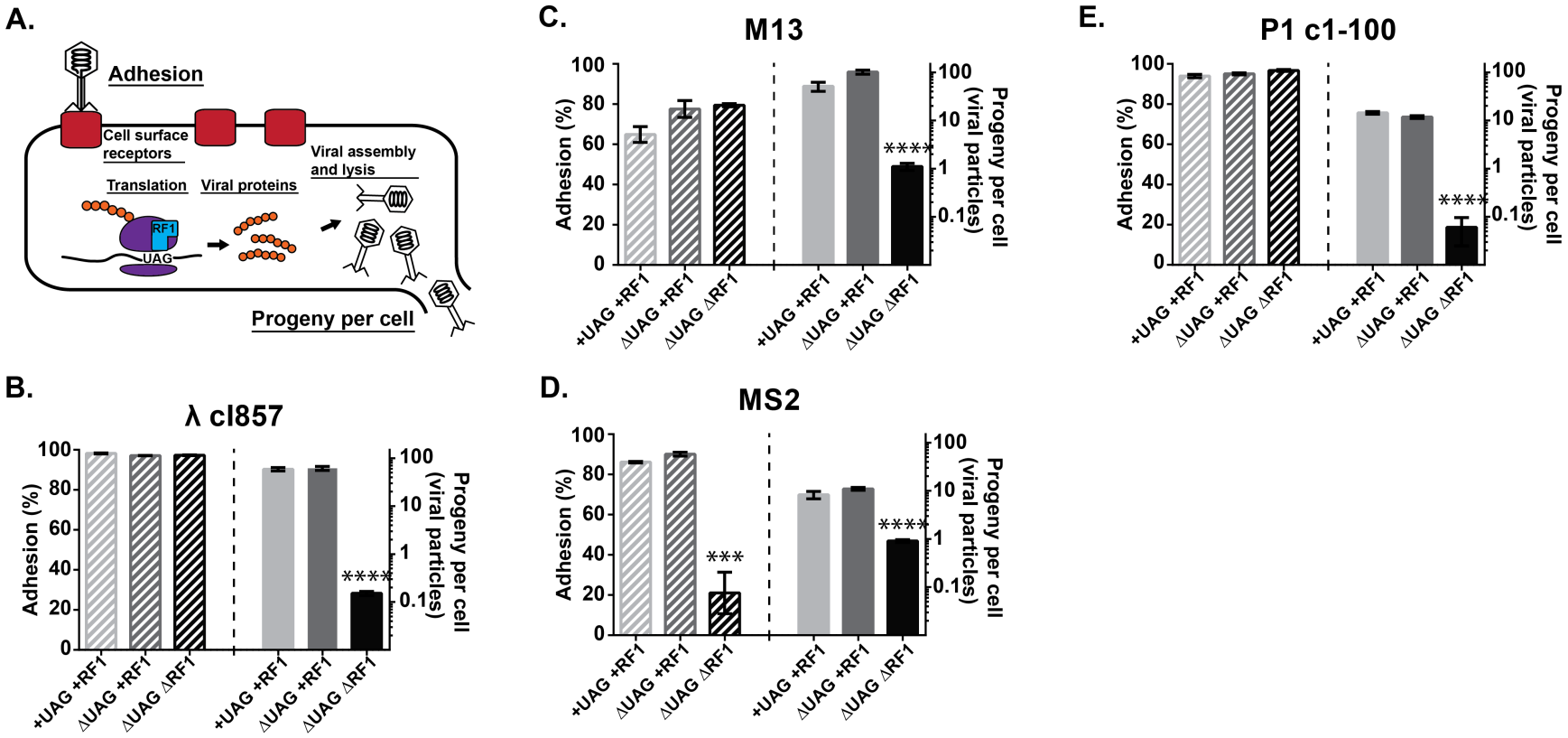


Figure S3 – Recoding UAG codons in viruses restores the ability to infect *ΔUAGΔRF1* hosts, Related to Figure 1. (A) Schematic showing the strategy to recode phage λ cI857 using multiplex automated genome engineering (MAGE). (B) Close-up and (C) uncropped images from viral relative titer assays using λ variants with the stop codons of indicated genes recoded from UAG to UAA. (D) Close-up and (E) uncropped images from viral relative titer assays using M13 wild-type and recoded variants infecting *ΔUAGΔRF1* hosts with wild-type or partially recoded *pF.*

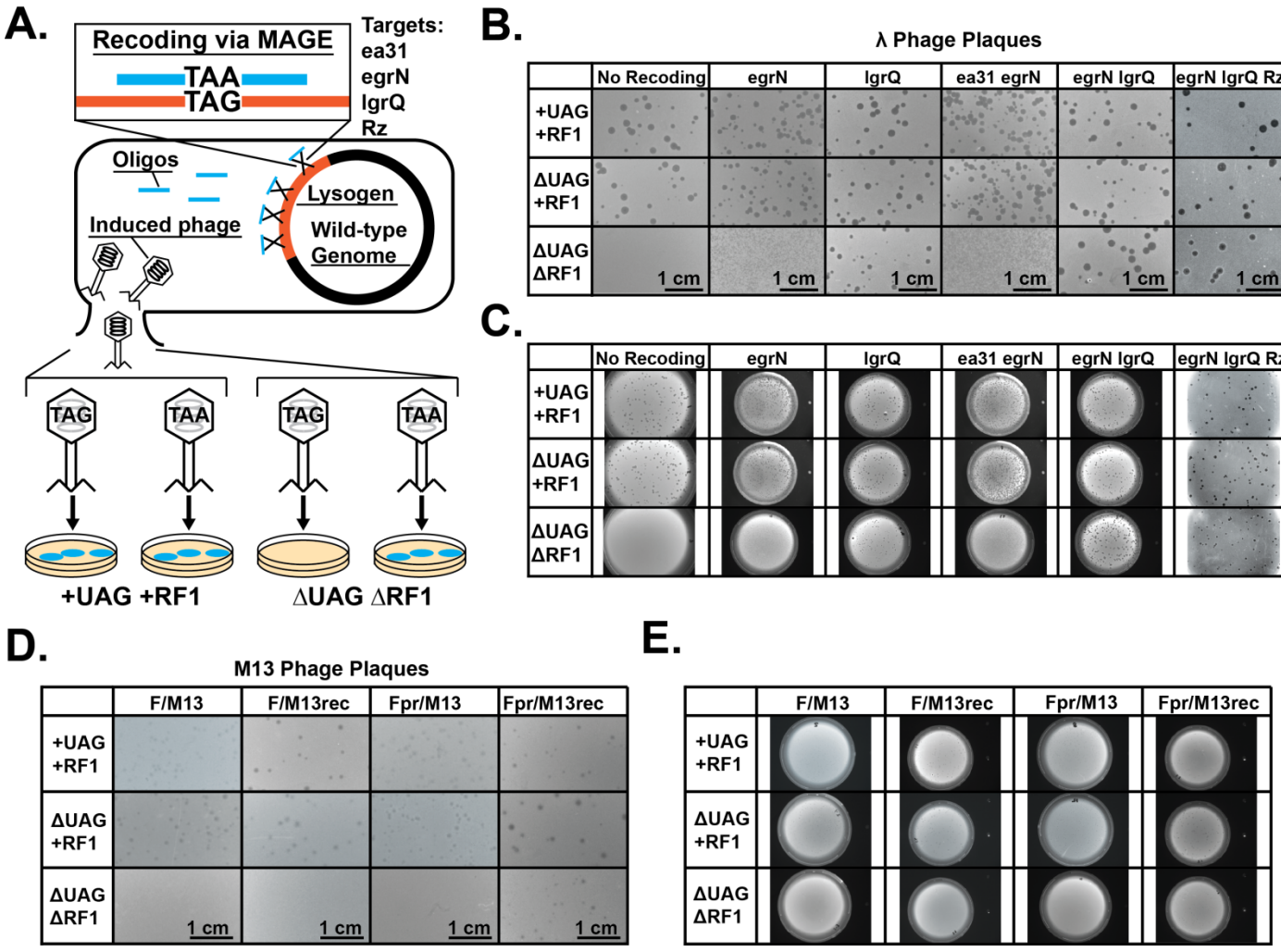
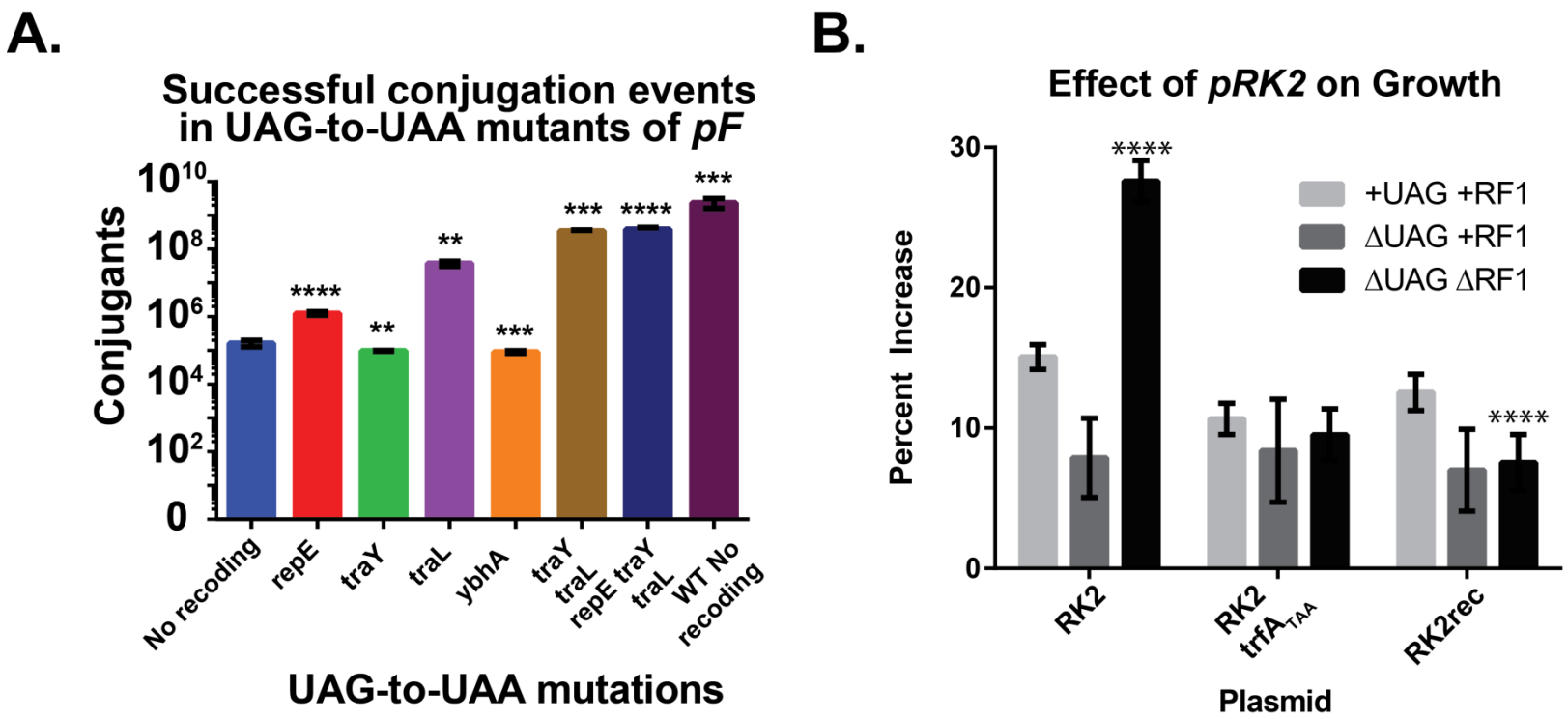


Figure S4 – Recoding conjugative plasmids restores conjugation and propagation in *ΔUAGΔRF1* hosts, Related to Figure 2. (A) Quantifying successful conjugation events for partially-recoded *pF* variants from *ΔUAGΔRF1* compared to conjugation of an unrecoded *pF* from *+UAG+RF1* as a positive control. "WT" is a wild-type (+UAG+RF1) cell's conjugation efficiency. (B) Percent increase in doubling time of cells containing wild-type or recoded *pRK2* variants (x-axis) with standard and alternate genetic codes. Data are mean with standard deviation, n=3. P-values are denoted as follows: * is P ≤ 0.05, ** is P ≤ 0.01, *** is P ≤ 0.001, and **** is P ≤ 0.0001.



SUPPLEMENTAL METHODS

Strains and media

All bacteria used in studies are *Escherichia coli* MG1655 derivatives with *mutS* replaced by *zeo*^{R} as described previously (Lajoie et al., 2013). To prevent confounding effects of recombineering proteins in assays, we replaced the lambda red cassette with the wild-type *bioAB* amplified from MG1655 using recombineering (Sharan et al., 2009) in all strains except donor strains of **Figure 2**. Strains used in viral titer assays and as donors in quantifying conjugation efficiencies are also *ΔtolC*. A full list of genotypes can be found in **Table S4**.

We performed all phage assays in Tryptone-KCl (TK) media as described previously (Jaschke et al., 2012; Valentine et al., 2002). For phages P1*vir* and P1c1-100, we added 10 mM $CaCl_2$ to TK media to increase phage binding. For phages M13 and MS2, we added 30 μg/mL kanamycin to ensure maintenance of *pF*, which encodes the pili that these phages use as surface receptors. We performed all conjugation assays in LB Lennox with pH 7.5.

Phages and conjugative plasmids

We obtained phages Mu *ts*, λ cI857, T5, M13, and P1*vir* from Dr. John Wertz at the Yale CGSC. Phage MS2 was a generous gift from Paul Turner at Yale University, and phage P1c1-100 was a generous gift from Dr. Małgorzata Łobocka at the Warsaw University of Life Sciences SGGW. To prevent confounding effects from lyosgeny, all lysogenic phages have inducible or constitutive lytic pathways. Phages Mu, λ, and P1 c1-100 contain temperature-sensitive lytic repressors, resulting in lysis when incubated at 37°C. Phage P1*vir* carries a deletion of the c1 gene, resulting in obligate lysis. We propagated all phages by mixing virus with 300 μL of mid-log (OD_{600}=0.5) *+UAG+RF1* host in 3 mL of TK soft agar and pouring onto TK solid agar plates. After incubation overnight at 37 °C, we extracted virus by scraping soft agar from plate into 3mL of TK media, vortexed for 10 seconds, then centrifuging at 3300 rcf for 10 min to separate agar and supernatant and passed supernatant through Costar 8160 SpinX filters. We stored all viral stocks at 4 °C and determined titers by infecting *+UAG+RF1* with serially-diluted phage using the agar overlay method described above.

We obtained the *pF* from the Yale CGSC (NCBI Accession AP001918.1, GI 8918823) and added *kan*^{R} from plasmid *pZE21* for antibiotic selection. We used the *pRK2* described in Isaacs et al. (2011), which is a derivative of the *pRK2* described in Pansegrau et al. (1994) carrying *bla*^{R} instead of *kan*^{R}. The complete nucleotide sequence for the plasmid is available in NCBI database (Accession L27758.1, GI 508311). Plasmids were maintained in cells by addition of either kanamycin (for *pF*, 30 μg/mL) or tetracycline (for *pRK2,* 12 μg/mL).

Quantifying Viral Adhesion

To quantify viral adhesion, we diluted a known titer of virus such that 1-10 μL of virus could be added to 100 μL of mid-log cells at an MOI of 0.15. One aliquot of diluted virus was added to cells and plated immediately to verify viral titer, while remaining aliquots were added to 100 μL of mid-log cells at an MOI of 0.15. We then incubated cultures with shaking at 37 °C for 10 minutes for all viruses except M13, which we incubated for 20 min. After incubation, we chilled cultures on ice at 4 °C and centrifuged for 2 minutes at 14,000 rpm to remove cells and adhered phage, and then plated 100-fold dilutions of supernatant using the methods described above for viral relative titers to quantify non-adhered phage. We calculated percent adsorption by subtracting the number of non-adhered phage in supernatant from total viral particles in titer, or (% adsorption) = [(total # viral particles)-(# viral particles in supernatant)]/ (total # viral particles)*100.

Quantifying Viral Progeny per Cell

To quantify progeny per cell, we diluted virus such that 1-10 µL of virus could be added to 1mL of mid-log cells at an MOI of 0.15. One aliquot of diluted virus was added to cells and plated immediately to verify viral titer, while remaining aliquots were added to 100 µL of mid-log cells at an MOI of 0.15. After addition of virus, we incubated cultures with shaking at 37 °C for 10 minutes and then placed cultures on ice at (n + 10 minutes), where n = the time to lysis for the phage. We then serially diluted cultures 100-fold and plated in the same manner as viral relative titers. Using these viral titers, we calculated progeny per cell by dividing progeny after one viral reproductive cycle by the starting number of viral particles, or (progeny per cell) = (# viral particles after one reproductive cycle)/ (# viral particles infected on cells).

Quantifying number of successful conjugation events

We used conjugation conditions described previously (Ma et al., 2014). Briefly, we grew cultures of donor and recipient cells to late log in antibiotics selecting for plasmid or recipient and then rinsed and resuspended in media to remove antibiotics. We then concentrated cells and normalized to OD_{600}=20 by doing 100-fold dilution and normalizing to OD_{600}=0.2, then mixed donors and recipients in 1:1 ratio and spotted onto prewarmed LB Lennox agar plates in 2x 20uL and 6x 10uL pattern. We incubated plates at 37 °C for 2 hours (*pF*) then rinsed cells from the plate, diluted serially 10-fold, and plated on plates containing antibiotic selecting for recipient strain with the conjugative plasmid, incubated plates overnight at 37°C, and counted the number of patched colonies that grew.

Doubling time assays for strains carrying *pRK2* variants

Cells were grown to confluency overnight in LB Lennox, pH 7.5 with Tetracycline (12 µg/mL), then diluted 100-fold into fresh media with Tetracycline in a 96-well flat-bottomed plate in triplicate. This plate was placed in a plate reader (Synergy HT) using a program with the following conditions: 34 °C, 24 hour kinetic run, OD_{600} reads every 10 minutes with continuous shaking. Doubling times were calculated as described previously (Rovner et al., 2015).

PCR and Sequencing

A complete list of primers used in this study and their purpose is available in the **Oligonucleotides** Excel spreadsheet. PCR reactions for DNA assembly were performed with Kapa 2G HiFi HotStart Readymix (KK2602). PCR reactions for sequencing were performed with Kapa 2G HotStart ReadyMix with dye (KK5609).

SUPPLEMENTARY REFERENCES

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