Supplementary Information

A System for the Continuous Directed Evolution of Biomolecules

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Supplementary Results

Calculation of phage generation time during PACE. We estimated the phage generation time using a steady-state replication model in which phage fitness is constant. One phage generation encompasses an infected cell exiting the lagoon, one of its progeny phage infecting an incoming cell, and that infected cell exiting the lagoon. To determine the average time required for one of the progeny phage to infect an incoming cell, we assumed that no progeny phage have yet infected an incoming cell and that cells entering the lagoon are instantaneously infected due to the high phage (5×10^{10} mL^{-1}) and cell (5×10^{8} mL^{-1}) concentrations relative to the adsorption coefficient of filamentous phage (3×10^{-11} mL^{1} min^{-1} $phage^{-1}$ $cell^{-1}$).[33]

Because lagoons operate in a steady state during most of a PACE experiment, the total phage and cell concentration does not change over time. Therefore, the progeny of a single cell exiting the lagoon infects exactly one cell entering the lagoon, on average, to achieve population replacement. A number of incoming cells equal to the total cell population will enter before one of the progeny phage achieves infection. Consequently, the average time from instantaneous cell exit to infection of a new cell by a progeny phage is 1/(dilution rate).

According to the dilution equation, solutes wash out at a rate of dC/dt = - (dilution rate) * (concentration). This gives a washout equation of C(t) = c1 * exp[-(dilution rate) * time]. This washout equation may be used as the probability density function prob [time] for the probability of a given cell washing out at a specific time after it enters the lagoon. To ensure that the probability of all currently resident cells washing out given infinite time is 1, we set c1 = 1 / (dilution rate). To calculate the probability <t> for a single cell, we integrate [t * prob[t], {t,0,infinity}]. The integral of [time * (dilution rate) * exp[-(dilution rate) * time], {t, 0, infinity}] = 1 / (dilution rate). Therefore, the average time from infection to washout is also equal to 1 / (dilution rate).

Summing the two halves of the phage replicative cycle, the average phage generation time in our model is equal to 2 / (dilution rate). At a dilution rate of 2.0 volumes/hour, as in the evolution towards T3 promoter recognition, the average phage undergoes 24 generations per 24 hours. At 2.5 volumes/hour, as in the initiation site evolution experiments, the average phage undergoes 30 generations per 24 hours. For the maximum observed dilution rate of 3.2 volumes/hour, the average phage undergoes just over 38 generations per 24 hours. In all cases the fastest replicating 1% of phage have undergone a significantly greater number of generations per 24 hours.

Mutagenesis. The mutagenesis plasmid (MP) encodes the DNA polymerase dominant negative proofreading subunit dnaQ926[20] and the error-prone repair DNA polymerase pol V (See the vector map in Supplementary Figure 2). The rapid expression of pol V from the MP may permit the use of alkylating mutagens which generate lesions that cannot otherwise be bypassed during DNA replication, as induction of the SOS pathway leading to pol V production requires longer than the average residence time of a host cell in the lagoon.[21] Both dnaQ926 and pol V are under the control of the araBAD operon and are expressed only when arabinose is added to the lagoon.

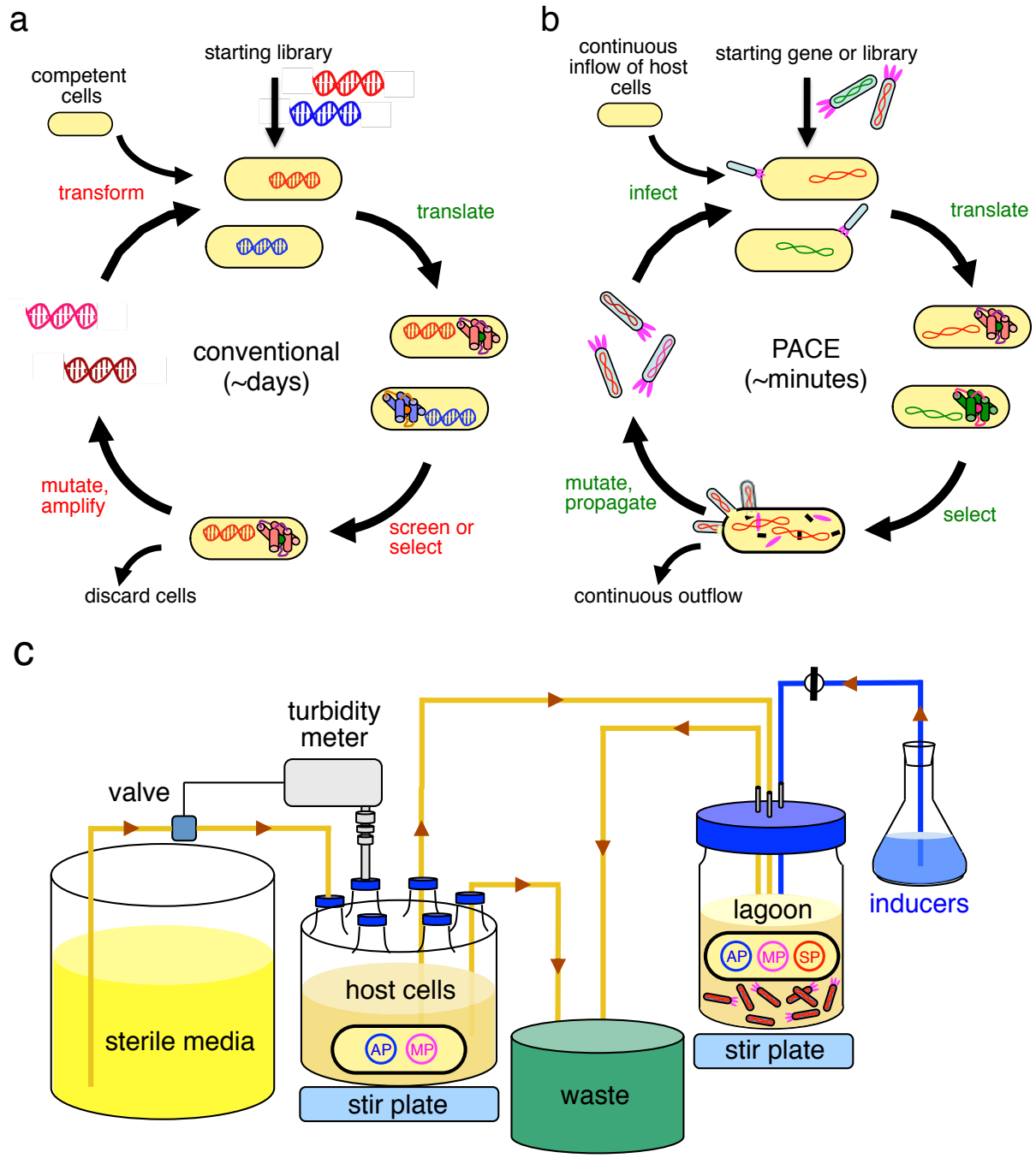
The basal mutation rate of replicating filamentous phage (5.3×10^{-7} substitutions/bp)[34] is sufficient to generate all possible single but not double mutants of a given library member in a 100 mL lagoon. For a target gene 1,000 base-pairs in length, a basal mutation rate of 5×10^{-7} yields 2.5×10^{7} base substitutions spread over 5×10^{10} copies of the gene in a 100 mL lagoon after one phage generation, easily covering all single point mutants but not all double mutants. Arabinose induction of the MP can increase the mutation rate to $\sim 5 \times 10^{-5}$, yielding $\sim 2.5 \times 10^{9}$ mutations spread over 5×10^{10} copies of the gene after one generation. The vast majority are single mutants which together comprise a target area of $\sim 2.5 \times 10^{12}$ base pairs, suggesting that some 1.2×10^{8} are double mutants, sufficient to cover all 9×10^{6} possible double mutants.

Mutational analysis of T3-evolved variants. Mutational analysis (Supplementary Table 2) of the PACE-evolved T7 RNAP genes revealed basic structure-activity relationships among mutant clones (Fig. 3d). The N748D mutation known to enable recognition of the T3 base at the -11 position[21] appeared in isolates of both lagoons within 48 hours after shifting to the full T3 promoter (Fig. 3d). Two other known mutations observed during PACE include E222K, a specificity broadener,[22] and K98R, a change to the corresponding amino acid in T3 RNAP that directly contacts the -15 and -16

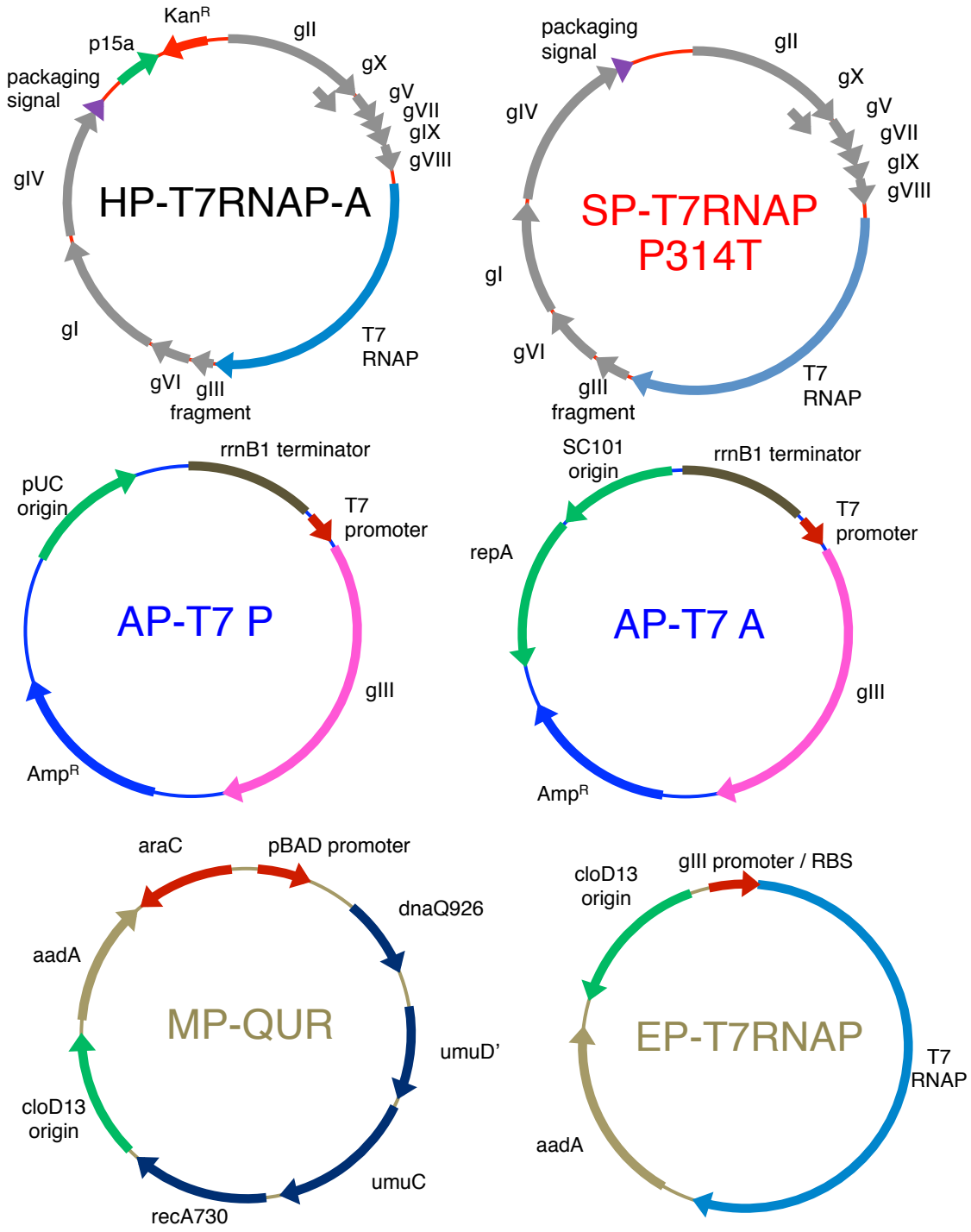
bases altered in the T3 promoter.[28] These two mutations were never observed in the same clone, suggesting mutual exclusivity or similar functional effects.

Mutational analysis of iC$_6$-evolved variants. Mutational analysis (Supplementary Table 2) revealed that the H524N and A827V mutations were conserved among all 12 sequenced clones, presumably arising very early during PACE. In addition, all clones contained either K577M or both C125R and S128R. Several clones contained all five mutations, including the most active clone assayed, suggesting that both observed sets of mutations increase activity and are mutually compatible. Of the five mutations, only C125R and S128R are predicted to be capable of contacting and stabilizing the newly initiated RNA, though A827V is in close proximity to the active site.

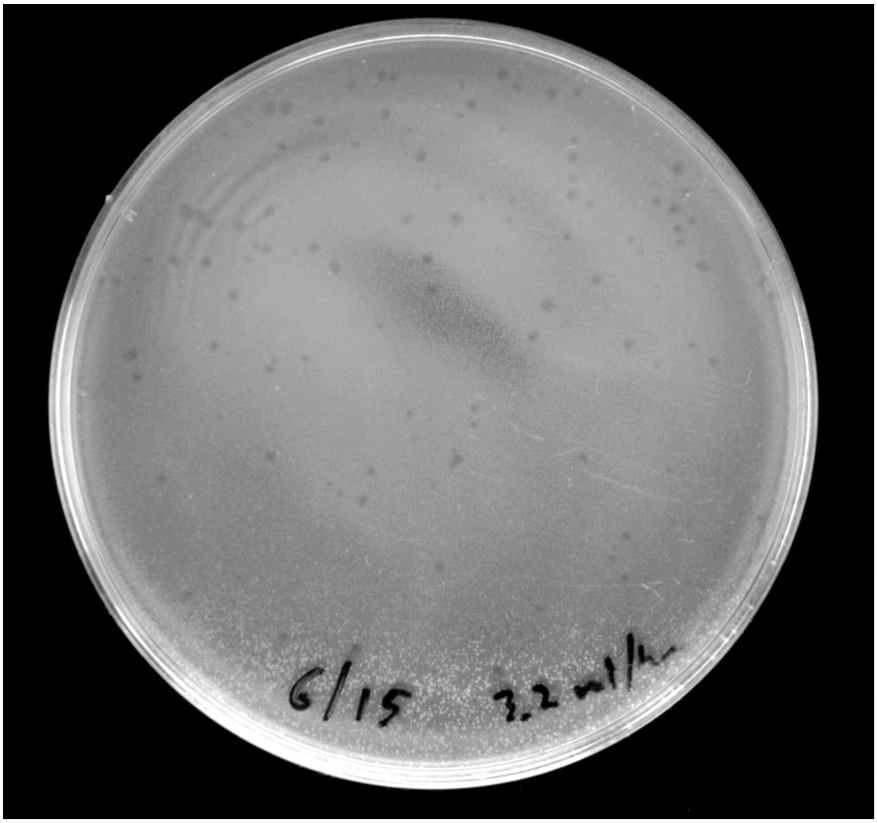
Supplementary Figures and Legends



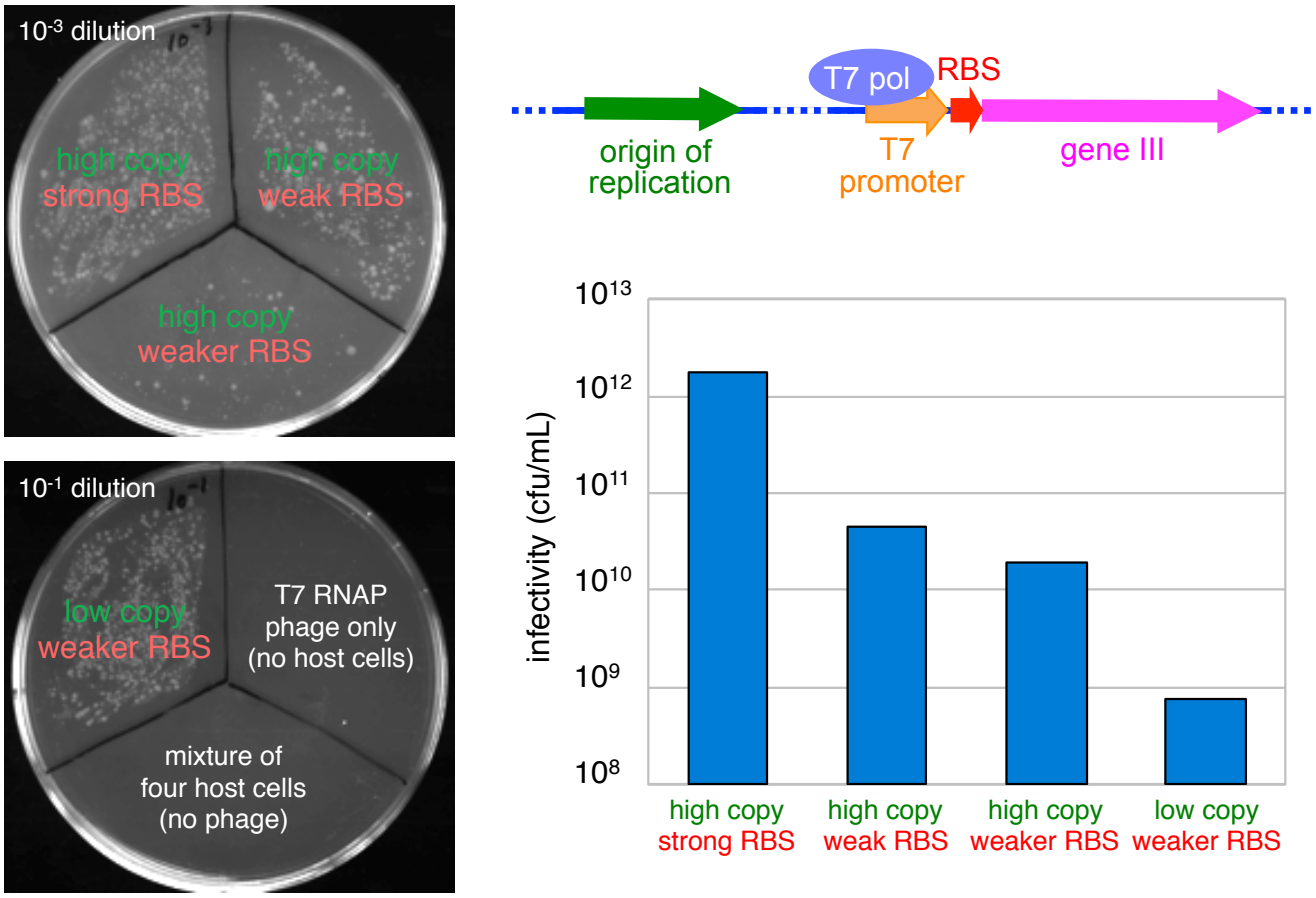
Supplementary Figure 1. Directed evolution cycles in (a) conventional directed evolution in cells and (b) phage-assisted continuous evolution (PACE). Steps in the evolution cycle that typically require the intervention of the researcher are shown in red; those that do not are shown in green. (c) Schematic of the PACE apparatus. Host *E. coli* cells maintained at constant cell density continuously flow through a lagoon vessel (along with optional chemical inducers) containing phage at dilution rates of ~1.0-3.2 lagoon volumes per hour.



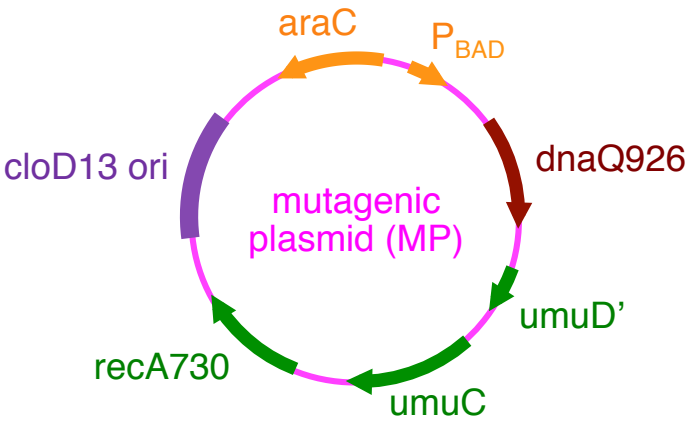
Supplementary Figure 2. Vector maps of plasmids used in PACE experiments. Helper phage HP-T7RNAP was used for the discrete infection assays in Fig. 3. Continuous propagation on host cells bearing accessory plasmid AP-T7 P for 48 hours yielded selection phage SP-T7RNAP P314T, the starting point for subsequent PACE experiments. Accessory plasmid AP-T7 A is the lower-copy version of AP-T7 P used to increase selection stringency. AP-T7 R and AP-T7 S are identical to AP-T7 P except for the gIII ribosome-binding site as detailed in the Plasmids section of the Methods. The arabinose-inducible mutagenesis plasmid MP-QUR was used for all mutagenesis and PACE experiments. The expression plasmid EP-T7RNAP was used to assay transcriptional activity in cells. See Supplementary Table 1 for additional information about all vectors used in this work.

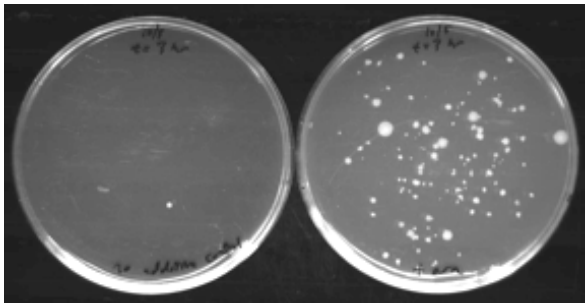


Supplementary Figure 3. Continuous phage propagation persists at a lagoon flow rate of 3.2 volumes per hour. A plaque assay was performed after continuous overnight dilution of SP encoding wild-type T7 RNA polymerase propagating on host cells containing an AP with a high-copy wild-type T7 promoterat a flow rate of 3.2 lagoon volumes per hour. The presence of plaques containing T7 phage indicates that the phage replication rate is sufficient to withstand this dilution rate.



Supplementary Figure 4. Selection stringency can be controlled by varying the gene III RBS and the accessory plasmid copy number. Selection phage encoding T7 RNAP were paired with accessory plasmids containing a strong, weak, or weaker ribosome binding site with a high-copy colE1 or a very low-copy SC101 origin. Phage infectivity diminished with decreasing RBS strength and with decreasing copy number, reflected in lower phage titers.





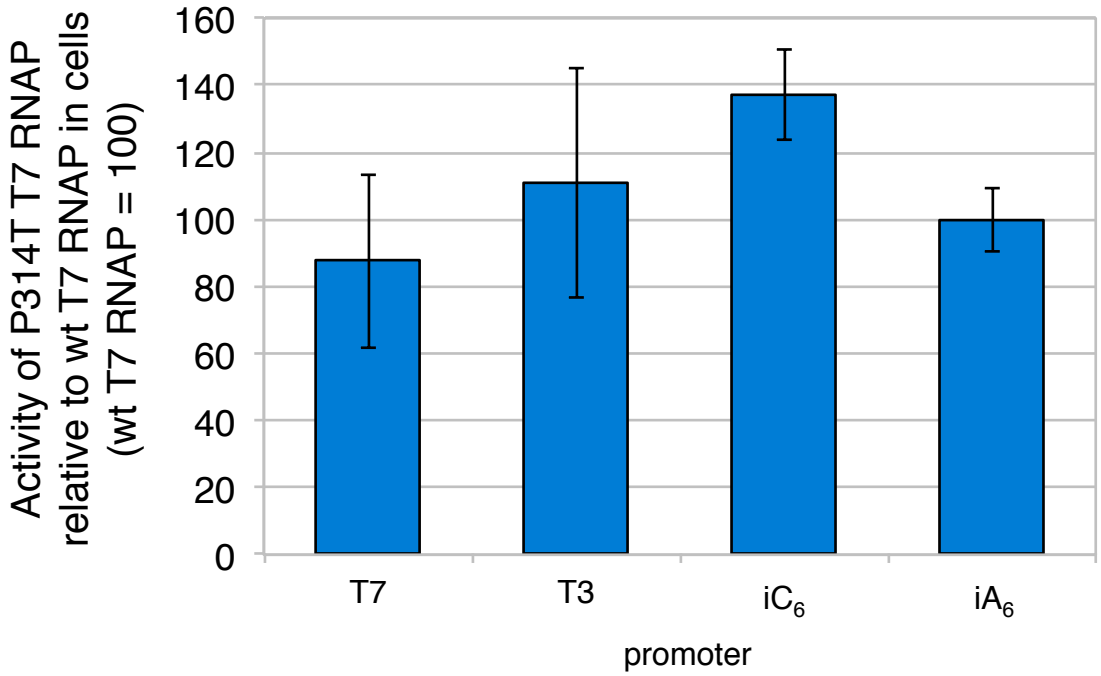
– arabinose + arabinose

observed mutations (*n* = 72)

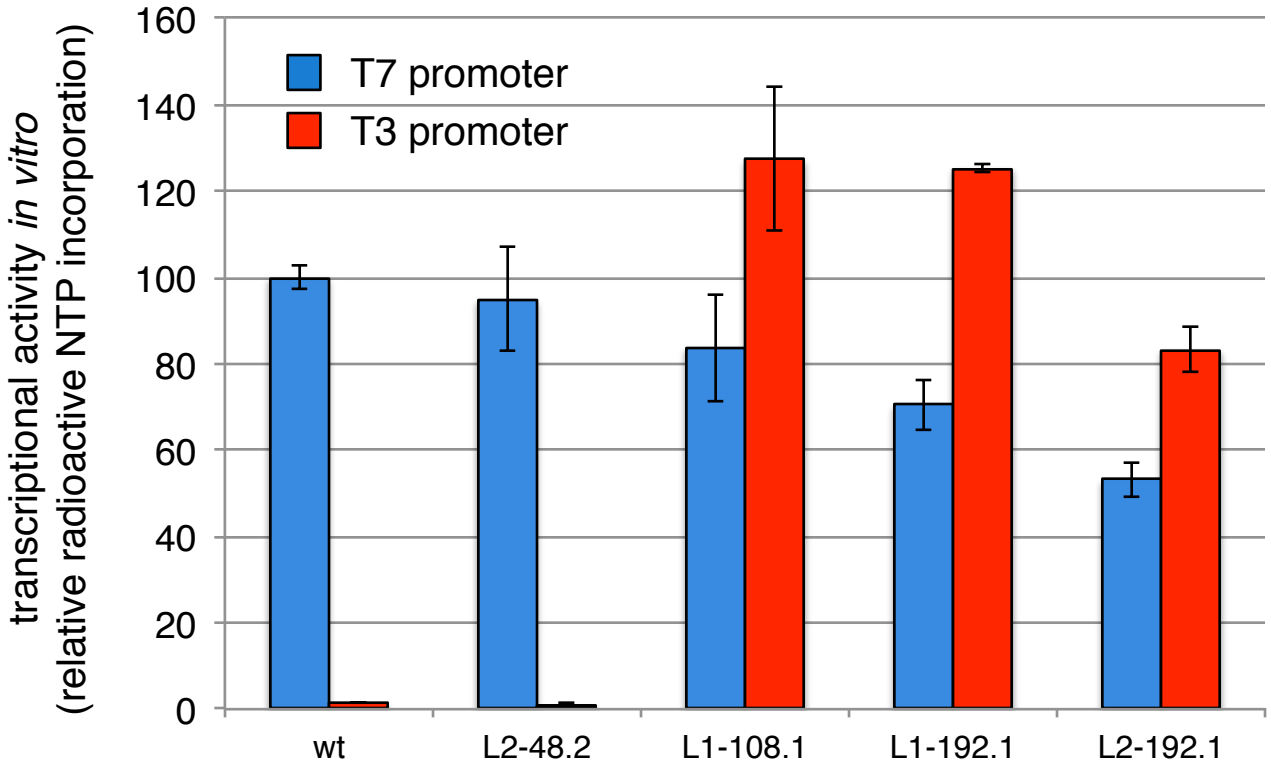
G/C to A/T: 43%
A/T to G/C: 12%
G/C to T/A: 15%
A/T to C/G: 8%
G/C to C/G: 7%
A/T to T/A: 10%

	# mutant colonies after 3 hours	
	– arabinose	+ arabinose
	1	114
	2	204
	9	861
	1	60
total:	13	1239

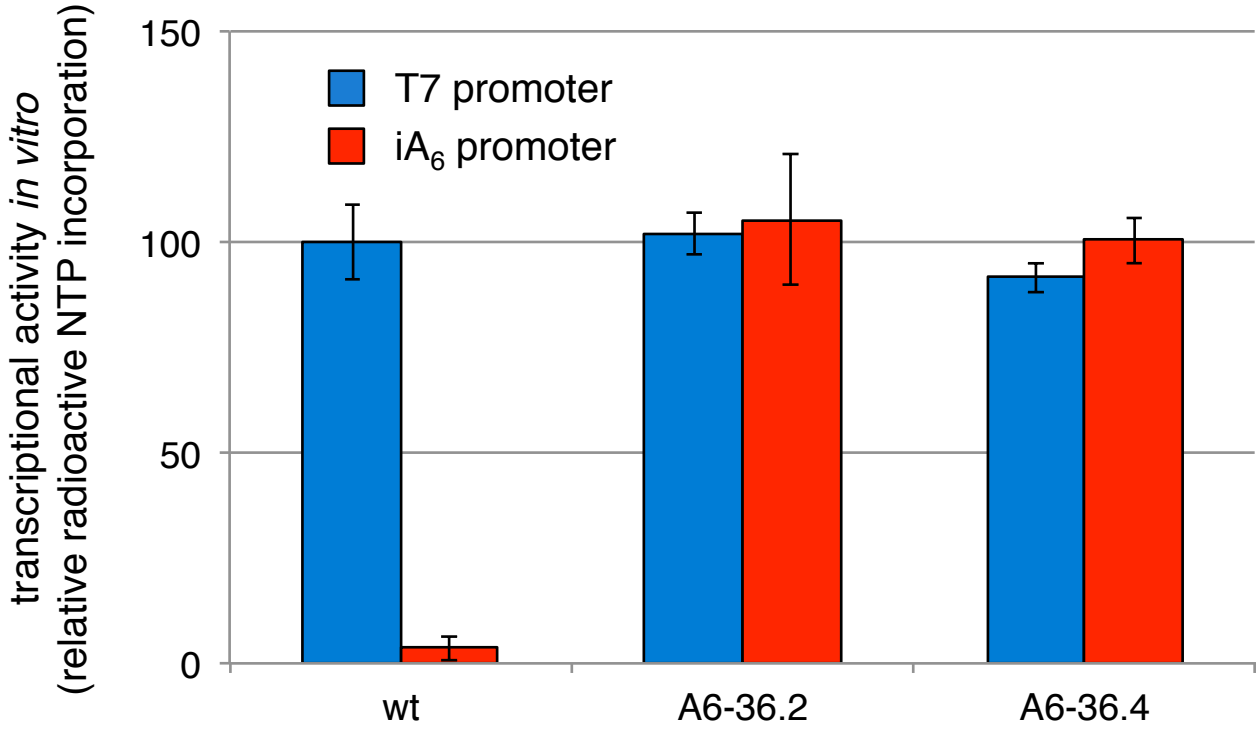
Supplementary Figure 5. Structure and function of the mutagenesis plasmid (MP). Induction with arabinose increases the frequency of inactivating mutations in a phage-encoded *lacI* gene by ~100-fold over four independent experiments. All types of transitions and transversions were observed, with a bias towards G/C to A/T transitions.



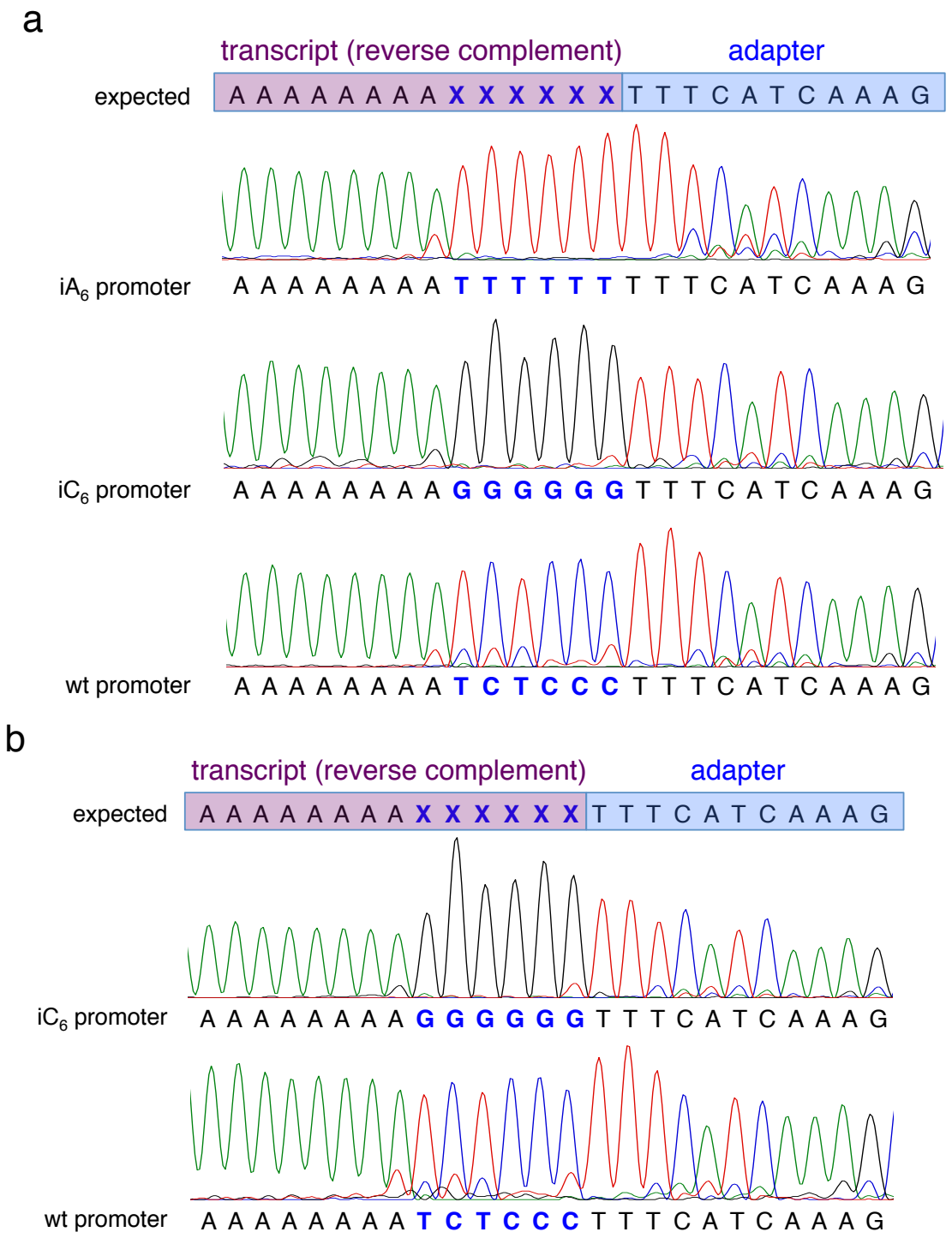
Supplementary Figure 6. Transcriptional activity of the starting T7 RNAP harbouring a P314T mutation is similar to that of wild-type polymerase when assayed on T7, T3, iC_6, and iA_6 promoters in cells. Each bar shows the activity of the P314T mutant RNAP relative to wild-type T7 RNAP (defined as 100) on the same promoter. Note that absolute transcriptional activity levels differ dramatically between bars; only relative levels comparing P314T to wild-type T7 RNAP for each promoter are shown. Error bars represent the standard deviation of at least three independent assays.



Supplementary Figure 7. *In vitro* activity of purified T7 RNAP mutants from lagoons 1 and 2. Transcriptional activity was measured *in vitro* using a standard radioactive nucleotide incorporation assay.[30] Error bars represent the standard deviation of at least three independent assays.

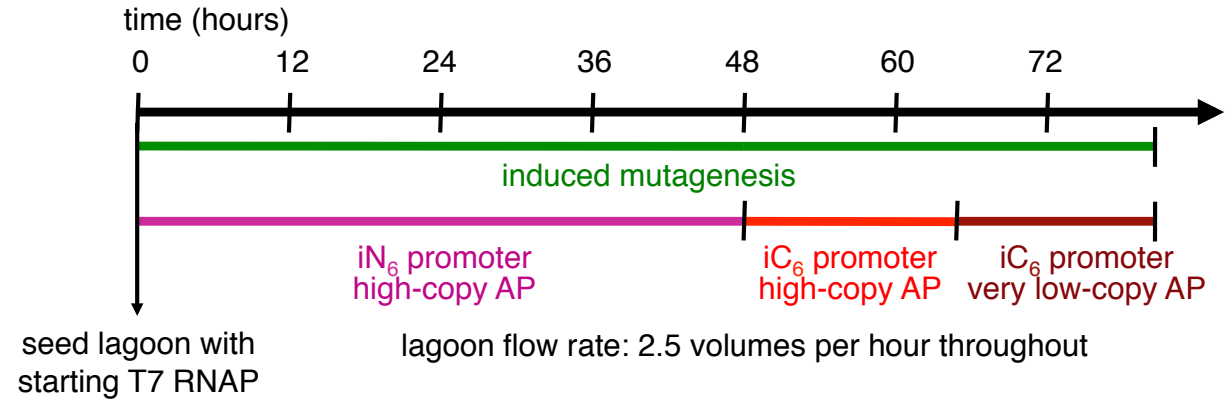


Supplementary Figure 8. *In vitro* activity of purified T7 RNAP mutants evolved to recognize the iA_6 promoter. Transcriptional activity was measured *in vitro* using a standard radioactive nucleotide incorporation assay.[30] Error bars represent the standard deviation of at least three independent assays.



Supplementary Figure 9. RNA sequences of transcription products from evolved polymerase enzymes. (a) DNA sequence chromatograms from RACE analysis of the C6-80.9 evolved polymerase transcribing either the iC_6template (upper trace) or the wild-type template (lower trace). (b) DNA sequence chromatograms from RACE analysis of the A4-36.4 evolved polymerase transcribing the iA6 template (upper trace), the iC_6template (middle trace) or the wild-type template (lower trace). Initiation began at +1 with template-encoded bases rather than non-templated nucleotides, demonstrating that A4-36.4 is capable of initiating with GTP, ATP, or CTP. See Methods for experimental details.

a



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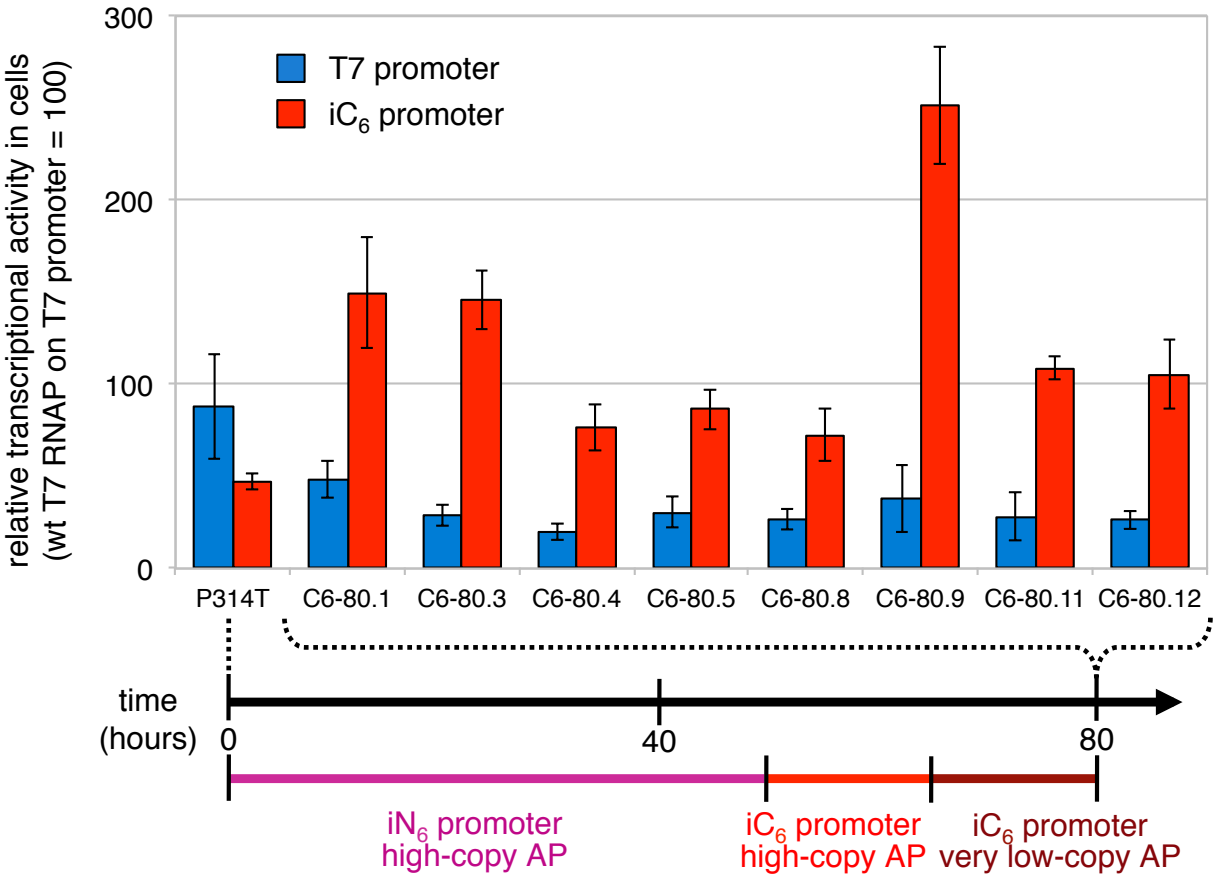
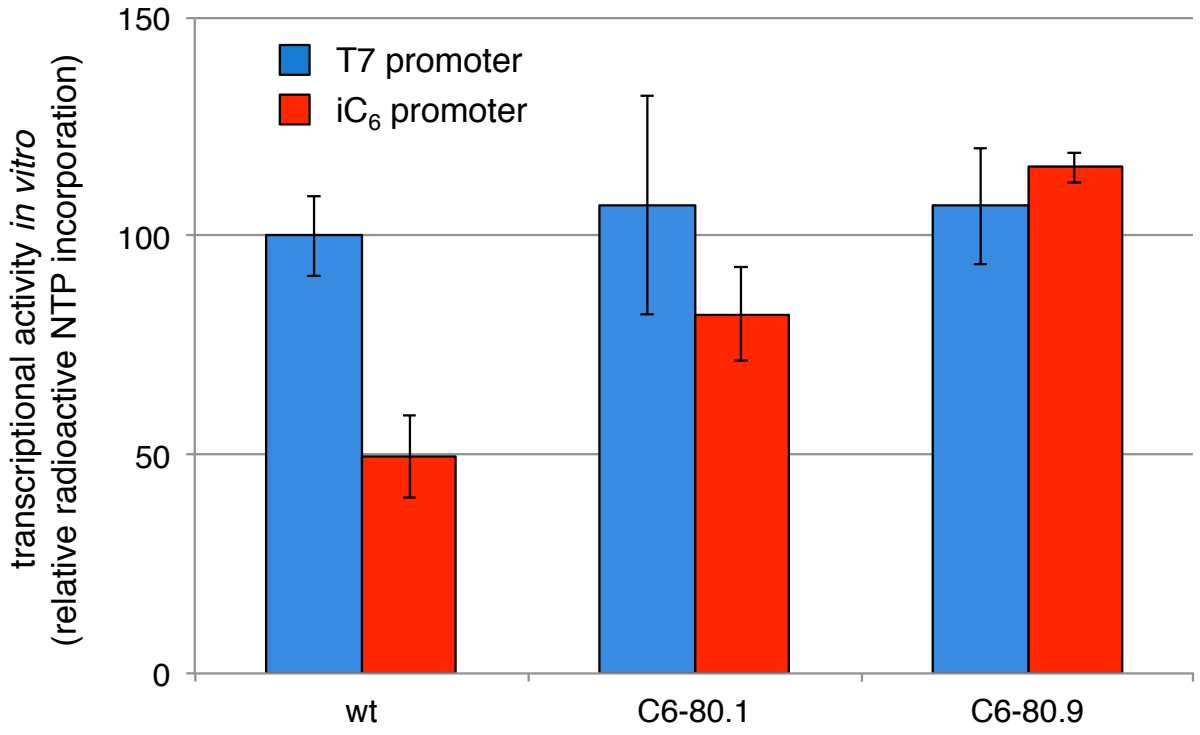
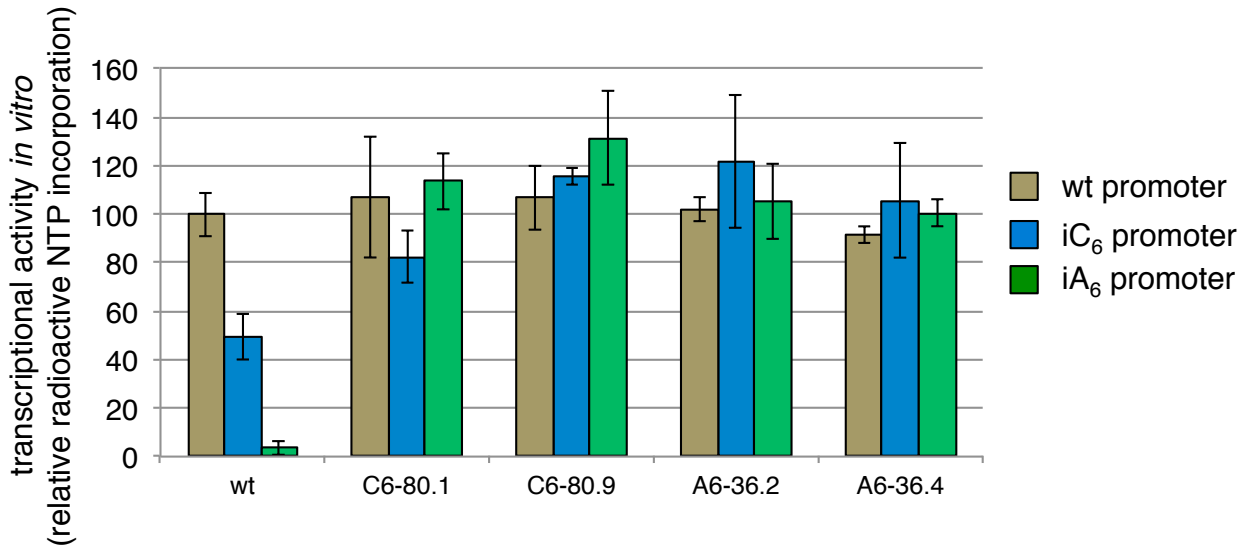


(Supplementary Figure 10 continues on the next page).

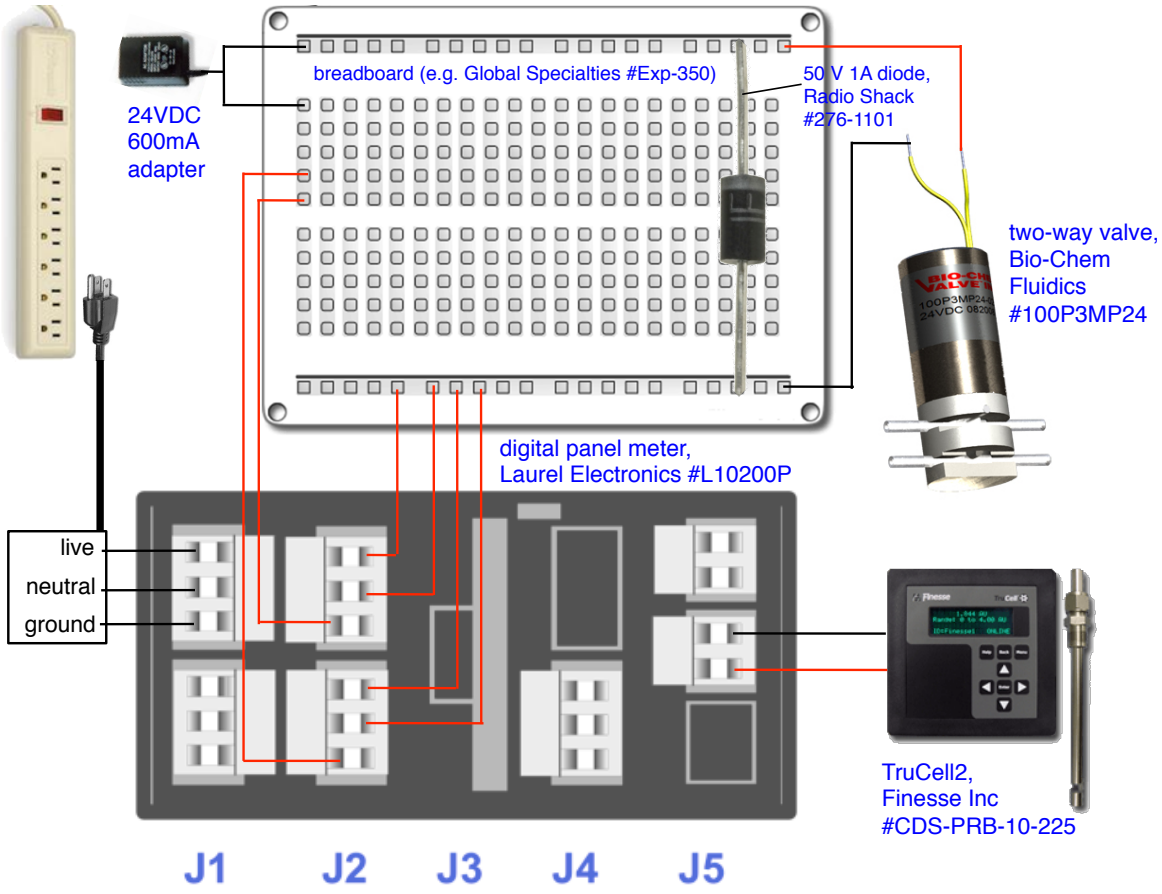
C



Supplementary Figure 10. Evolution of T7 RNAP variants that initiate transcription with C. (a) PACE schedule. (b) Activity in cells on the T7 and iC_6 promoters of T7 variants isolated after 80 hours of PACE. Assays were performed as described in Fig. 3b. (c) *In vitro* activity of a selection of purified T7 RNAP variants assayed in (b). Transcriptional activity was measured *in vitro* using a standard radioactive nucleotide incorporation assay. Error bars represent the standard deviation of at least three independent assays.



Supplementary Figure 11. *In vitro* transcription activity of T7 RNAP mutants evolved to initiate on the $iC_6$and iA_6promoters assayed on wild-type, iC_6, and iA_6 promoters. Transcriptional activity was measured in vitro using a standard radioactive nucleotide incorporation assay. All four variants assayed exhibit the ability to initiate transcription with wild-type-like efficiency on wild-type, iC_6, and iA_6 promoters. Error bars represent the standard deviation of at least three independent assays.



Supplementary Figure 12. Schematic illustrating the electrical system controlling the flow of fresh media in response to the cell density. The valve controlling turbidostat media inflow is opened and closed by a programmable digital panel meter processing cell density information from the TruCell2 probe.

Supplementary Table 1. Plasmids used in this work.

PACE plasmid	Class	Source	Notes
HP-T7RNAP-A	Selection phage	This work	VCSM13-derived; T7RNAP replaces gIII
SP-T7RNAP P314T	Selection phage	This work	Evolved from HP-T7-A; no longer Kan^R
AP-T7 P	Accessory	This work	High-copy T7RNAP accessory
AP-T7 A	Accessory	This work	Very low-copy T7RNAP accessory
AP-T7T3 P	Accessory	This work	AP-T7 P: hybrid promoter
AP-T3 P	Accessory	This work	AP-T7 P: T3 promoter
AP-T3 A	Accessory	This work	AP-T7 A: T3 promoter
AP-T7-iN6 P	Accessory	This work	AP-T7 P: N6 transcript start
AP-T7-iC_6 P	Accessory	This work	AP-T7 P: C_6 transcript start
AP-T7-iC_6 A	Accessory	This work	AP-T7 A: C_6 transcript start
AP-T7-iA_6 P	Accessory	This work	AP-T7 P: A_6 transcript start
AP-T7-iA_6 A	Accessory	This work	AP-T7 A: A_6 transcript start
MP-QUR	Mutagenesis	This work	+ara -> dnaQ926, DNA pol V
Other plasmids			
AP-T7 R	Accessory	This work	Weak RBS
AP-T7 S	Accessory	This work	Weaker RBS
EP-T7RNAP	Expression plasmid	This work	For in-cell activity quantification
HPdOd3	Helper	This work	For phagemid infection assays
AP-RNAP α-LGF2	Accessory	This work	2-hybrid accessory
SP-Gal11p-Zif268	Selection phagemid	This work	2-hybrid phagemid
AP-RZH3	Accessory	This work	Recombinase accessory
SP-HinHZ	Selection phagemid	This work	Recombinase *phagemid
SP-Cre	Selection phagemid	This work	Control phagemid
VCSM13-lacI	Assay phage	This work	For loss-of-function mutagenesis assay
pJC137	Assay plasmid	This work	Selects for loss of *lacI* repressor function
pT7-911Q	Expression plasmid	V. D'Souza	T7 RNAP expression/purification

Supplementary Table 2. Genotypes of assayed T7 RNAP variants

Target	Clone	Amino acid changes	Silent mutations (bp)
T3	L1-48.1	I4M G175R Y178H E222K M267I E484A G542V L699I	t2239c g2274a t2376c
T3	L1-48.2	I4M G175R H176L Y178H E484A G542V L699I	g2274a
T3	L1-48.3	I4M G175R E222K E356D G542V L699I	g1440a c1587t
T3	L1-108.1	I4M G175R E222K Q239R G542V N748D	a378g c2238a
T3	L1-108.2	I4M G175R E222K Q239R G542V N748D	c2238a
T3	L1-108.3	I4M G175R E222K Q239R G542V N748D	t1725c c2238a
T3	L1-192.1	I4M E63V S128R G175R E222K Q239R G542V N748D	c2238a t2376a
T3	L1-192.2	I4M E63G S128R G175R E222K D351A G542V N748D	a1020g c2238a t2376a
T3	L2-48.1	N165S G542V N601S E775K	c327t g957a
T3	L2-48.2	N165S A354S L360P K450R G542V E775K	
T3	L2-48.3	N165S G542V K577E E775K	c583t c2265t
T3	L2-108.1	I4M T76N Y178H F182L D208Y G542V N748D E775K L864F	
T3	L2-108.2	I4M T76N Y178H F182L D208Y G542V N748D E775K L864F	
T3	L2-108.3	N165S L196F E222K G280C M401T P451T G542V N748D E775K	c2265t
T3	L2-192.1	I4M D66Y G175R E222K G280C G542V N748D E775K L864F	c1182a t1446c
iC_6	C6-80.1	A65E C125R S128R M267V H524N A827V	g537a c762a g915a g1236a g1380a
iC_6	C6-80.3	C125R S128R Y385C H524N K577M A827V D851N	c762a a807g g1236a g1380a g1599t g2631a
iC_6	C6-80.4	H524N K577M Q754R A827V	a231g c726a a807g g1236a g1380a g2631a
iC_6	C6-80.5	A383V H524N K577M A827V	c762a a807g g1236a t1275g g1380a g2631a

iC_6	C6-80.8	C125R S128R H524N K577M K713E A827V	c762a a807g g1236a g1380a g2631a
iC_6	C6-80.9	C125R S128R H524N K577M A827V	c762a a807g g1236a g1380a g2631a
iC_6	C6-80.11	A7T V64D C125R S128R D388Y H524N A827V	c762a a807g g1236a g1380a g2631a
iC_6	C6-80.12	C125R S128R N410S H523L H524N K577M A827V	a696g c762a a807g t858c g1197a c1269a g1431a a1926g t2626c
iA_6	A6-36.1	K93T H300R S397R S684Y	
iA_6	A6-36.2	K93T G198V T243N G259D H300R S397R S684Y	g585a
iA_6	A6-36.3	K93T S397R E565K S684Y	t1284c
iA_6	A6-36.4	K93T A136T S228A S397R S684Y	t2052c
iA_6	A6-36.5	K93T S228A S397R S684Y	t2052c

Supplementary Table 3. Complete list of reagents, equipment, and suppliers.

Turbidostat	Source	Catalog #	Purpose
BioProbe flask, 0.5 L	Bellco Glass	1965-97005	Turbidostat, small
BioProbe flask, 1 L	Bellco Glass	1965-97001	Turbidostat, medium
BioProbe flask, 3 L	Bellco Glass	1965-97003	Turbidostat, large
Corning Scholar 171 magnetic stirrer	Thermo Fisher Scientific	11-497-22	Stirs turbidostats
Corning PC-240 magnetic stirrer	Thermo Fisher Scientific	114973C	Stirs large turbidostats
GL32 probe holder	Bellco Glass	1965-97010	Holds cell density probe
GL45 septa	Bellco Glass	C139-545SS	Port access
GL32 septa	Bellco Glass	C139-532SS	Port access
GL45 open caps	Bellco Glass	C139-545HTSC	Port access
GL32 open caps	Bellco Glass	C139-532HTSC	Port access
Large autoclavable venting filter 6-10mm	VWR	28137-652	Turbidostat venting
24VDC 3-way valve	Bio-Chem Fluidics	100P3MP24-05S	Controlling media flow
Masterflex L/S economy variable drive	Cole Parmer, Inc	07554-80	Fluid transfer
L/S 8-channel, 3-roller pump head	Cole Parmer, Inc	07519-05	Fluid transfer
Masterflex L/S small cartridges	Cole Parmer, Inc	07519-80	Fluid transfer
Tygon two-stop pump tubing, L/S 14	Cole Parmer, Inc	06416-14	Fluid transfer
Tubing,pharmed,2.79mm ID,100'	Cole Parmer, Inc	95809-48	Fluid transfer
Male luer with lock ring x 1/8" hose barb, PP, 25/pk	Cole Parmer, Inc	45503-04	Fluid transfer
Female luer x 1/8" hose barb adapter, PP, 25/pk	Cole Parmer, Inc	45500-04	Fluid transfer
Nalgene jerrican waste container	Thermo Fisher Scientific	2240	Waste container
Needle, blunt disposable	VWR	BD305180	Fluid addition
20L carboys w/handle	VWR	16101-109	Media vessel
Polyvent filling/venting closure	VWR	16225-229	Media cap
Needle, blunt end 18Gx6”	VWR	20068-682	Waste withdrawal

Electrical equipment	**Source**	**Catalog #**	**Purpose**
TruCell2 cell density meter	Finesse, Inc.	CDS-PRB-10-225	Density monitoring
L10200P digital panel meter	Laurel Electronics	L10200P	Valve control
Experimentor 350 solderless breadboard	Global Specialties	EXP-350	Valve control
1N4001 Micromini Silicon Diode, 50V 1A	Radio Shack	276-1101	Valve control
UL-recognized hookup wire	Radio Shack	278-1224	Wiring
Germicidal UV lamp	American Air & Water	SM-36-2GR	Sterilization

Lagoons	**Source**	**Catalog #**	**Purpose**
Pyrex 100 mL bottles	VWR	16157-103	Lagoon vessel
Pyrex 1 L bottle	VWR	16157-191	Arabinose supplement
GL45 septa	Bellco Glass	C139-545SS	Port access
GL45 open caps	Bellco Glass	C139-545HTSC	Port access
Thermo Variomag Poly 15 magnetic stirrer	VWR	89030-746	Stirring
Needle, blunt disposable	VWR	BD305180	Fluid addition
Needle, blunt end 18Gx6”	VWR	20068-682	Waste withdrawal
Autoclavable 0.2 um filters	VWR	28137-650	Venting
L/S brushless programmable drive	Cole Parmer, Inc	07550-50	Fluid transfer
L/S 8-channel pump head for microbore tubing	Cole Parmer, Inc	07534-08	Fluid transfer
Microbore two-stop tube sets, silicone; 2.06 mm ID.	Cole Parmer, Inc	06421-42	Lagoon to waste
Microbore two-stop tube sets, silicone; 1.42 mm ID.	Cole Parmer, Inc	06421-34	Turbidostat to lagoon
Microbore two-stop tube sets, silicone; 0.82 mm ID.	Cole Parmer, Inc	06421-26	Supplement to lagoon
Male luer with lock ring x 1/16" hose barb, PP, 25/pk	Cole Parmer, Inc	45503-00	Fluid transfer
Male luer with lock ring x 3/32" hose barb, PP, 25/pk	Cole Parmer, Inc	45503-02	Fluid transfer
Male luer with lock ring x 1/8" hose barb, PP, 25/pk	Cole Parmer, Inc	45503-04	Fluid transfer
Female luer x 1/16" hose barb adapter, PP, 25/pk	Cole Parmer, Inc	45500-00	Fluid transfer
Female luer x 3/32" hose barb adapter, PP, 25/pk	Cole Parmer, Inc	45500-02	Fluid transfer

Female luer x 1/8" hose barb adapter, PP, 25/pk	Cole Parmer, Inc	45500-04	Fluid transfer
Tubing,pharmed,0.89mm ID,100ft	Cole Parmer, Inc	95809-26	Fluid transfer
Tubing,pharmed,1.42mm ID,100ft	Cole Parmer, Inc	95809-34	Fluid transfer
Tubing,pharmed,2.06mm ID,100ft	Cole Parmer, Inc	95809-42	Fluid transfer

Turbidostat Media	**Source**	**Catalog #**	**Purpose**
Potassium phosphate dibasic, 50 kg	VWR	EM-PX1570-20	Turbidostat media
Potassium phosphate monobasic, 10 kg	United States Biological	P5110	Turbidostat media
Ammonium sulfate, 5 kg	United States Biological	A1450	Turbidostat media
Tween 80	VWR	100511-562	Turbidostat media
Glucose, 10 kg	United States Biological	G3050	Turbidostat media
Sodium citrate dihydrate, 5 kg	United States Biological	S5001	Turbidostat media
Casamino acids, 10 kg	United States Biological	C2080	Turbidostat media
L-leucine	United States Biological	L2020-05	Turbidostat media
Magnesium sulfate, anhydrous	Sigma Aldrich	246972	Turbidostat media
Carbenicillin	Gold Biotechnology	C-103-100	Turbidostat media
Spectinomycin	Gold Biotechnology	S-140-25	Turbidostat media
Tetracycline HCl	Gold Biotechnology	T-101-25	Turbidostat media
Nalgene 500 mL filter unit, 0.2 um pore size	VWR	450-0020	Turbidostat media
L-arabinose	Gold Biotechnology	A-300-1	Inducing mutagenesis

Standard Media	**Source**	**Catalog #**	**Purpose**
2xYT, 10 kg	United States Biological	T9200	Standard culture
LB broth Miller, 10 kg	United States Biological	L1520	Standard culture
Kanamycin	Gold Biotechnology	K-120-25	Standard culture
Chloramphenicol	Gold Biotechnology	C-119-5	Standard culture
X-Gal	Gold Biotechnology	X4281C	Standard culture

PCR	**Source**	**Catalog #**	**Purpose**
Oligonucleotides	Integrated DNA Technologies	N/A	Cloning
HotStartPhusion II DNA polymerase	New England Biolabs	F-549L	PCR
dNTPs	Bio-Rad	170-8874	PCR
*Dpn*I	New England Biolabs	R0176L	Template removal
MinElute PCR purification kit	Qiagen	28006	PCR cleanup

Isothermal assembly and cloning	**Source**	**Catalog #**	**Purpose**
Phusion DNA polymerase	New England Biolabs	F-530-L	Isothermal assembly
Taq DNA ligase	New England Biolabs	M0208L	Isothermal assembly
T5 DNA exonuclease	Epicentre Biotechnologies	T5E4111K	Isothermal assembly
TempliPhi, 500 rxns	GE Healthcare	25-6400-50	Isothermal assembly
Nicotinamide adenine dinucleotide (NAD)	Sigma Aldrich	N8410	Isothermal assembly
PEG-8000	Sigma Aldrich	9510	Isothermal assembly

Activity assays	**Source**	**Catalog#**	**Purpose**
Falcon Microtest 96-well OptiLux plates	BD Biosciences	353948	Fluorescence assays
M5 plate reader	Molecular Devices		Fluorescence assays
4-methylumbelliferyl-beta-D-galactopyranoside (MUG)	Gold Biotechnology	MUG1	Fluorescence assays
Misonix CL4 ultrasonic convertor	Misonix		Protein purification
Ni-NTA spin columns	Qiagen	31014	Protein purification
Amicon Ultra-0.5 30K concentration columns	Millipore	UFC503096	Protein purification
NuPage 4-12% gel	Invitrogen	NP0323BOX	Protein quantification
rNTPs	Jena Biosciences	NU-1014L	T7 transcription

[α-^{32}P]ATP	Perkin-Elmer	BLU003X250UC	Phosphorimaging
Ribonuclease T1	Ambion	AM2283	Leader cleavage
Criterion 5% TBE-urea gel	Bio-Rad	345-0086	Phosphorimaging
Criterion 10% TBE-urea gel	Bio-Rad	345-0088	Phosphorimaging
Criterion 15% TBE-urea gel	Bio-Rad	345-0089	Phosphorimaging
Typhoon Trio	GE Healthcare	63-0055-87	Phosphorimaging
Phosphor screen	GE Healthcare	63-0035-44	Phosphorimaging

RACE experiments	**Source**	**Catalog#**	**Purpose**
Turbo DNase	Ambion	AM2239	Template removal
Calf intestinal phosphatase	New England Biolabs	M0290L	Triphosphate removal
T4 polynucleotide kinase	New England Biolabs	M0201L	Phosphate addition
T4 RNA Ligase I (ssRNA Ligase)	New England Biolabs	M0204L	Ligation
*Mly*I	New England Biolabs	R0610S	Cut T7 promoter
*Hin*fI	New England Biolabs	R0155S	Cut T7 promoter
Superscript III reverse transcriptase	Invitrogen	18080093	Reverse transcription

Bacterial strains	**Source**	**Catalog#**	**Purpose**
Mach1 chemically competent cells	Invitrogen	C862003	Cloning
NEB Turbo chemically competent cells	New England Biolabs	C2984H	Cloning
PirPlus DH10βF'DOT cells	Thermo Fisher	MBC1249	Infection assays

Source	**Headquarters**
Bellco Glass	Vineland, NJ
Thermo Fisher Scientific	Waltham, MA
VWR	Pittsburgh, PA
Bio-Chem Fluidics	Boonton, NJ
Cole Parmer, Inc	St Louis, MO
Finesse, Inc.	San Jose, CA
Laurel Electronics	Santa Clara, CA
Global Specialties	Wallingford, CT
American Air & Water	Hilton Head Island, SC
United States Biological	Marblehead, MA

Sigma Aldrich	St Louis, MO
Gold Biotechnology	St Louis, MO
Integrated DNA Technologies	Coralville, IA
Bio-Rad	Hercules, CA
Qiagen	Valencia, CA
Epicentre Biotechnologies	Madison, WI
GE Healthcare	Piscataway, NJ
BD Biosciences	Franklin Lakes, NJ
Perkin-Elmer	Waltham, MA
Misonix	Farmingdale, NY
Millipore	Billerica, MA
Ambion	Austin, TX

Methods References and Supplementary References

29 Martin, C. T. & Coleman, J. E. Kinetic analysis of T7 RNA polymerase-promoter interactions with small synthetic promoters. *Biochemistry* **26**, 2690-2696 (1987).

30 Gibson, D. G. *et al.* Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods* **6**, 343-345 (2009).

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

32 Ichetovkin, I. E., Abramochkin, G. & Shrader, T. E. Substrate recognition by the leucyl/phenylalanyl-tRNA-protein transferase. Conservation within the enzyme family and localization to the trypsin-resistant domain. *J Biol Chem* **272**, 33009-33014 (1997).

33 Tzagoloff, H. & Pratt, D. The initial steps in infection with coliphage M13. *Virology* **24**, 372-380 (1964).

34 Drake, J. W. A constant rate of spontaneous mutation in DNA-based microbes. *Proc Natl Acad Sci USA* **88**, 7160-7164 (1991).