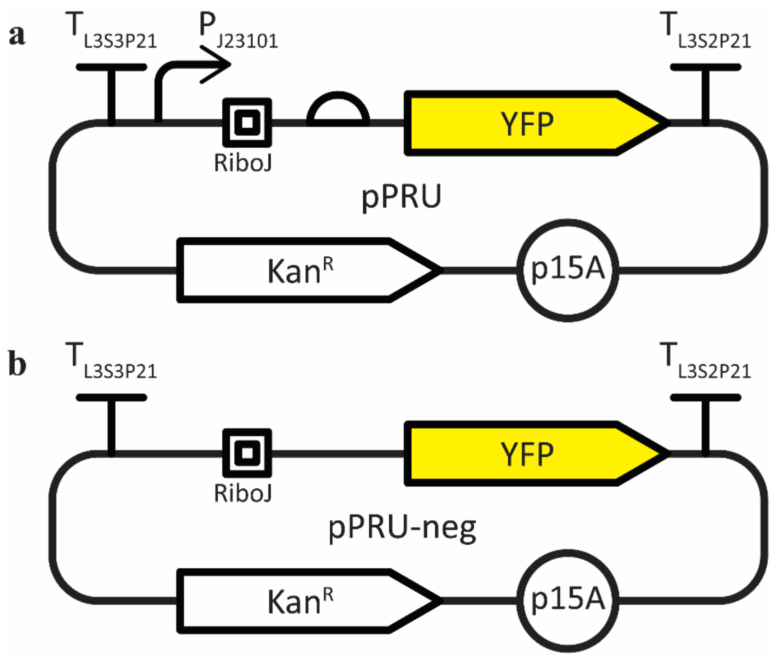
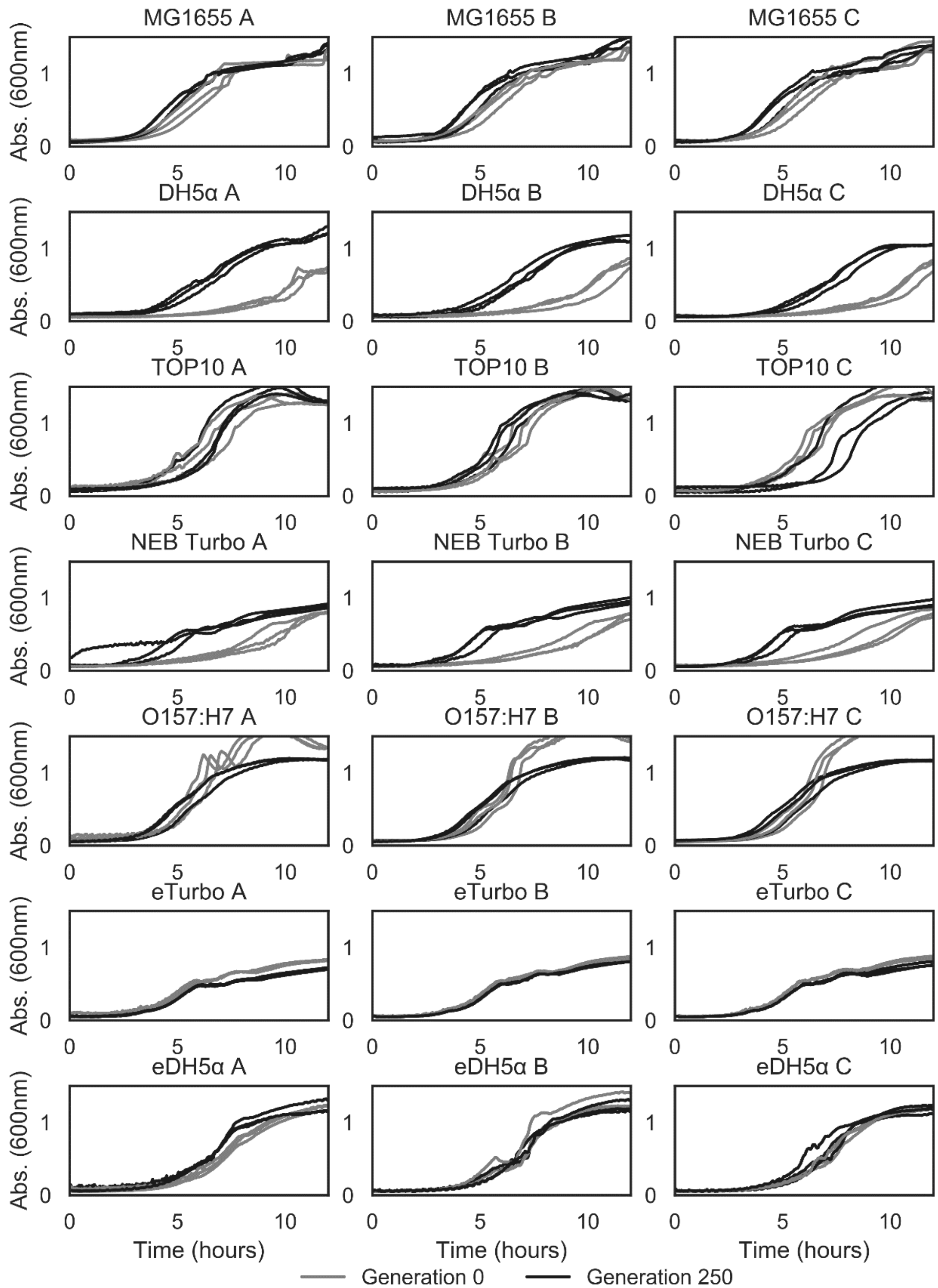
Improved stability of an engineered function using adapted bacterial strains

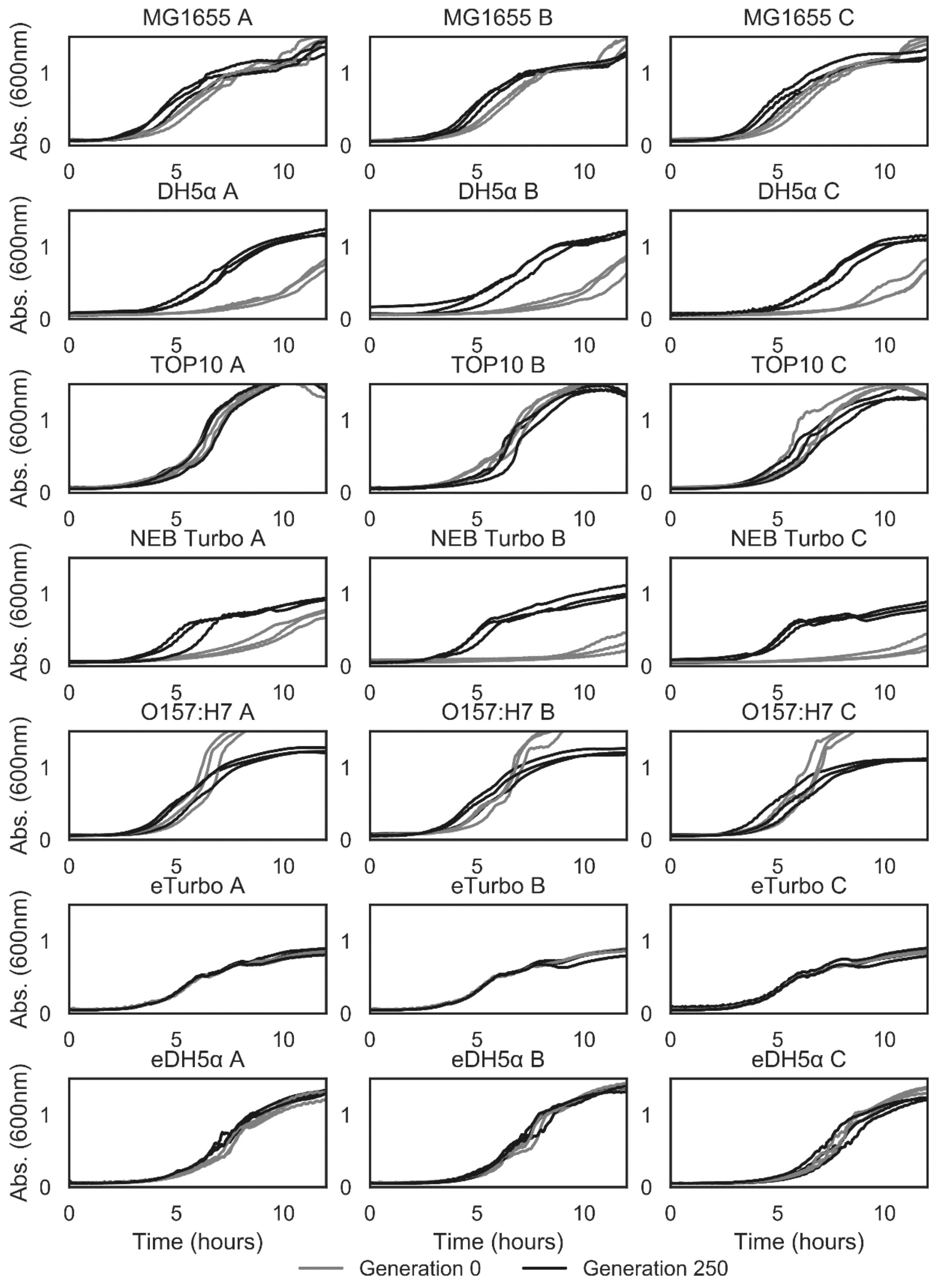
Supplementary Figure 1: Maps of plasmids used in this study. (a) Plasmid pRPU encoded fluorescence function into *E. coli*. Plasmid pRPU contains the YFP coding DNA sequence (CDS) under control of the constitutive promoter P_{J23101}. The mRNA transcript from P_{J23101} is insulated with the riboJ insulator and terminated downstream of the YFP CDS with the transcriptional terminator $T_{L3S2P21}$. The plasmid contains the p15A origin of replication and the kanamycin resistance marker. (b) Plasmid pRPU-neg, used for competitive fitness assays, was constructed by inactivated the P_{J23101} promoter and corresponding RBS.



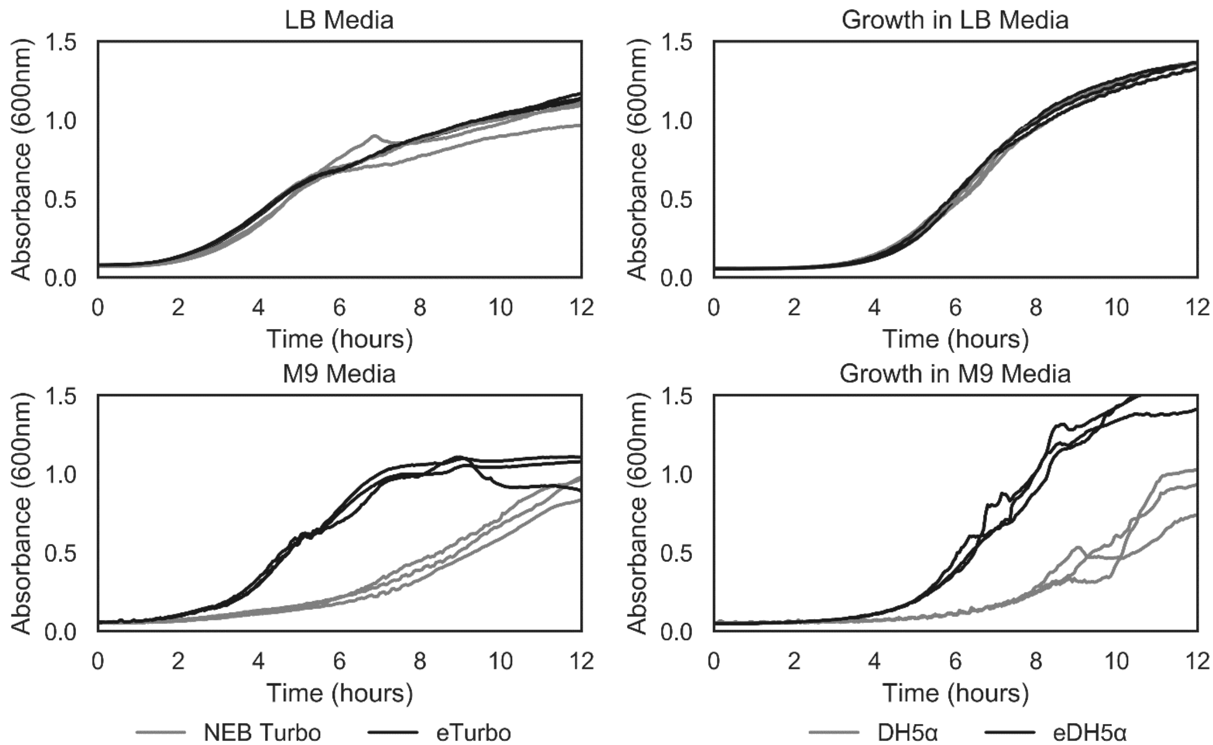
Supplementary Figure 2: Growth curves of *E. coli* strains at generation 0 and generation 250 of stability assays. *E. coli* strains containing pRPU and grown in M9 media with kanamycin. Growth curves were measured from samples of each culture at generation 0 (gray curves) and generation 250 (black curves). Each biological replicate (A, B, C) was measured in technical triplicate. Some curves overlap.



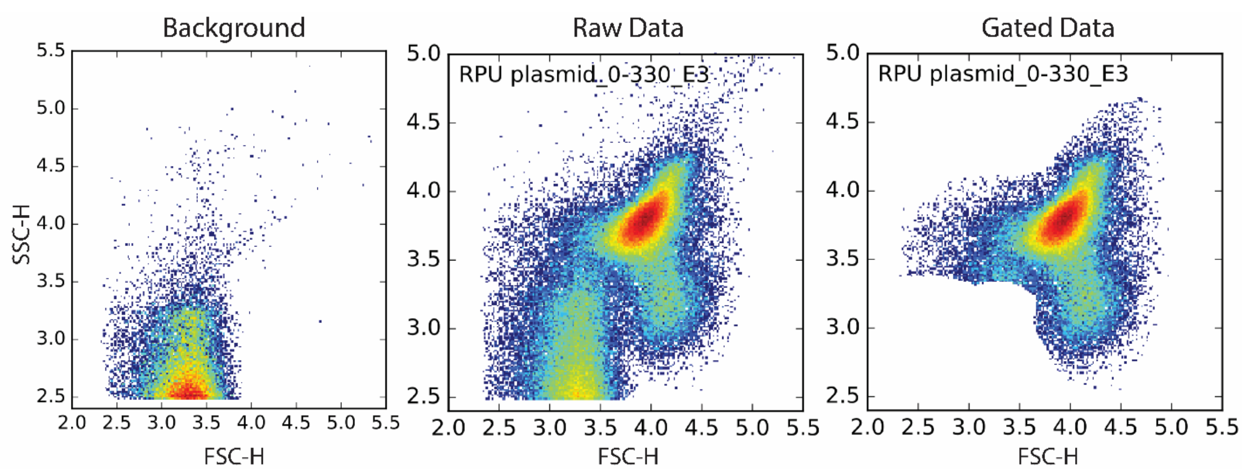
Supplementary Figure 3: Growth curves of *E. coli* strains at generation 0 and generation 250 of competitive fitness assays. *E. coli* strains containing pRPU and grown in M9 media with kanamycin. Growth curves were measured from samples of each culture at generation 0 (gray curves) and generation 250 (black curves). Each biological replicate (A, B, C) was measured in technical triplicate. Some curves overlap.



Supplementary Figure 4: Growth curves show increased growth rates after adapting *E. coli* strains to M9 media. Growth curves of NEB Turbo (left column) and DH5α (right column) before adaptation (gray curves) and after 100 generations of adaptation (black curves). Growth curves were measured in LB media (upper plots) and M9 media (lower plots). Measured in biological triplicate.



Supplementary Figure 5: Automated gating to isolate single cell event in flow cytometry. Cytometry data was gated by automating the subtraction of background events. Left, control blank sample to identify background events. Center, raw data from cytometer containing background and cell events. Right, gated results show cell events with background events removed.



Supplementary table 1: Variability (geometric standard deviation, σ_g) of YFP expression from pRPU in different strains of *E. coli*.

Strain	Variability (σ_g)
MG1655	1.14
O157:H7	1.20
TOP10	1.06
DH5α	1.34
eDH5α	1.16
NEB Turbo	1.12
eTurbo	1.08

Supplementary table 2: Growth rate (as doublings per hour) with standard deviation of seven *E. coli* strains at generation 0 (μ_0) and generation 250 (μ_{250}) of the stability assays and competitive fitness assays.

	Stability Assay							
	μ_0	μ_{250}	N_0	N_{250}	μ_0	μ_{250}	N_0	N_{250}
MG1655	1.36±0.08	1.65±0.11	9	8	1.26±0.14	1.52±0.17	8	9
O157:H7	1.47±0.12	1.60±0.06	9	9	1.52±0.07	1.60±0.06	9	9
TOP10	1.15±0.22	1.27±0.24	9	9	1.25±0.14	1.25±0.15	9	9
DH5α	0.71±0.09	1.26±0.12	9	9	0.77±0.08	1.15±0.15	9	9
eDH5	1.16±0.23	1.12±0.16	9	8	1.17±0.03	1.36±0.10	9	9
NEB Turbo	0.65±0.06	1.26±0.08	9	8	0.63±0.07	1.25±0.08	9	9
eTurbo	1.12±0.10	1.13±0.05	9	9	1.04±0.08	1.14±0.04	9	9

Supplementary table 3: Growth rate (as doublings per hour) with standard deviation of NEB Turbo and eTurbo in LB and M9 growth media. The change in growth rate (Δμ) calculated using $\mu_{eTurbo}/\mu_{NEB\ Turbo}$.

	Growth Rate (hr^{-1})		
Media	NEB Turbo	eTurbo	Δμ
LB	1.87±0.19	1.92±0.23	1.03±0.16
M9	0.62±0.08	1.01±0.16	1.63±0.34

Supplementary Table 4: Growth rate (as doublings per hour) with standard deviation of DH5α and eDH5α in LB and M9 growth media. The change in growth rate (Δμ) calculated using $\mu_{eDH5\alpha}/\mu_{DH5\alpha}$.

	Growth Rate (hr^{-1})		
Media	DH5α	eDH5α	Δμ
LB	1.63±0.07	1.54±.04	0.94±0.05
M9	0.70±0.11	1.23±.10	1.75±0.31

Supplementary Table 5: Minimum Information Standard for Engineered Organism Experiments (MIEO).

MIEO Category	**Factor**	**Level**	**Supplier Part**
Media components (M9)	KH2PO4	3 g/L	BD (248510)
	Na2HPO4	6.78 g/L	BD (248510)
	NaCl	0.5 g/L	BD (248510)
	NH4Cl	1.0 g/L	BD (248510)
	D-glucose	0.721 g/L	Sigma (G8270)
	Casamino acids	2 g/L	Calbiochem(2240)
	CaCl2	0.011 g/L	Sigma (21115)
	MgSO4	0.241 g/L	Sigma (83266)
	Vitamin B1 (Thiamine)	3.40 x 10-4 g/L	Sigma-Aldrich (T4625)
Media properties	pH	7.4	
	LB-Miller	25g/L	Fisher BioReagents (BP1426)
	Bacto-agar	15g/L`	BD (214010)
Container geometry	Type	"Culture" tube	Falcon (352059)
	Container shape	Round	
	Container bottom	Round	
	Container volume	14 mL	
	Fill volume	3mL	
	Cover	Snap cap	
Container shaking	Shaking speed	200 rpm	
	Shaking diameter	2.5 cm	
	Shaking mode	Orbital	
Time	Growth time	24h	
Environment	Temperature	37°C	
	Relative humidity	Not measured	
Selective Agents	Antibiotic type	Kanamycin	gibco (11815-32)
	Antibiotic concentration	50 ug/mL	
Inoculum	Type	Single colony	
	Concentration at inoculation	N/A	
	Age of inoculum at inoculation	N/A	

Passage	Volume of inoculum	3µL	
	Age of inoculum at inoculation	24 hours	
	Concentration at inoculation	10^-3	
Flow Cytometry	Device	Cytometer	
	Device	Autosampler	
	PBS		Invitrogen (AM9625)
	Focusing Fluid		Attune Focusing Fluid (4488621)
	Calibration Beads		Spherotech (RCP-30-5A)
	Chloramphenicol		Sigma(C1919)

pRPU Plasmid Sequence:

L3S3P21 – red text
P_{J23101} – green highlighted text
RiboJ – gray highlighted text
RBS – green emboldened text
eYFP – yellow highlighted text
$T_{L3S2P21}$ – gray highlighted red text
P15A – cyan highlighted text
Kanamycin resistance – underlined text

>plasmid pRPU
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