

SUPPLEMENTARY METHODS

Comparison between cell viability measurements with OD600 or colony forming units methods

In this work, the quantitative assessment of cell lysis was performed by measuring over time the turbidity of liquid cultures through OD600 readings. Cell viability can also be determined by plating-out samples taken from time points from the growing cultures and counting the colonies that form (colony forming units – CFU). As measurement of cell viability is an important characteristic in this work, OD600 and CFU methods were compared. TOP10 bearing pLC-T4LysHSL were grown in selective LB, incubated in the microplate reader and lysis was induced with HSL, as described in the Methods section of the paper. Cultures were taken from the wells immediately before lysis induction (intact cells) and after 3 hours from induction (lysed cells). These cultures were properly diluted in LB and 100 μ l were spread on three selective LB agar plates for each culture. The plates were incubated at 37°C for about 16 hours and then CFUs were counted, averaged and multiplied by the dilution factor to yield the CFUs per ml of the culture.

This experiment was performed in exponential phase (t=0 hours) or late stationary phase (t=20 hours) and three independent experiments were performed for both growth phases.

The OD600 vs CFU data for intact cells and lysed cells were fitted with a linear regression, yielding an identical regression coefficient of $5E+8$, with $R^2 > 0.95$ (data not shown). Since the linear fitting gives identical coefficients when cells are intact or lysed, OD600 can be effectively used to monitor the cell viability over time in all the experiments performed in this work. In this way, the real-time detection of cell growth dynamics in several bacterial samples can be achieved with a very thick time grid (few minutes) by using a microplate reader and then the lysis entity, which we have defined, can be easily computed as reported in the Methods section.

Computation of the percentage of extracellular RFP molecules in protein release assays

As described in the Methods section of the paper, the fluorescence of lysed pellet and supernatant of TOP10-rfp-lys and TOP10 bearing pHc-RFP were analyzed after 125 minutes from HSL induction. The lysed pellet was in a 400 μ l final volume (200 μ l of LB + 200 μ l of 2X lysis buffer) and the fluorescence of 200 μ l was measured. Fluorescence background (i.e. the lysed pellet of TOP10 with pLC-T4Lys⁻ non fluorescent culture) was subtracted and the resulting value was multiplied by 2 to obtain the total amount of RFP in the pellet.

In the supernatant analysis, 100 μ l of supernatant were mixed with 100 μ l of 2X lysis buffer and the fluorescence of the resulting 200 μ l was measured. Background (i.e. the fluorescence of pLC-T4Lys⁻ supernatant) was subtracted and the obtained value was multiplied by 30, because 100 μ l of supernatant were analyzed, while the total supernatant volume was 3 ml. Finally, for each culture, the percentage of extracellular RFP was computed as: $100 * F_s / (F_s + F_p)$, where F_s and F_p are the volume-corrected fluorescence values of supernatant and lysed pellet respectively. Supplementary Table 1 shows the raw and processed data for each described step.

SUPPLEMENTARY RESULTS

Sequence analysis of the used BioBrick™ parts

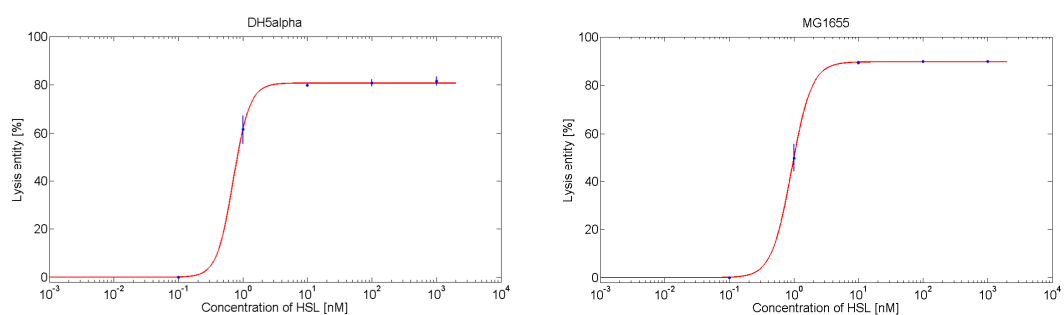
BBa_K112808 sequencing, performed on the DNA submitted to the Registry, showed discrepancies when compared to its submitted nucleotide sequence: there was a silent point mutation in the holin gene (c70a); the transcriptional terminator at the end of the part was actually BBa_K112710 and not BBa_B0010; the alignment showed several gaps in the DNA scars between the basic parts. However, the regulatory parts and the amino acid sequences of the translated genes were correct and the lysis assays showed that BBa_K112808 is fully functional. BBa_K098995 showed discrepancies as well: the DNA scar between the *cIts* gene and its RBS was not present; there were two point mutations in the *cIts* gene (g198a and g351a of cIts) resulting in two amino acid substitutions (A67T and E117K respectively), but they do not appear to affect the heat-induction capability of this part, as shown in the paper.

Characterization of the HSL-inducible lysis device in high-copy plasmid

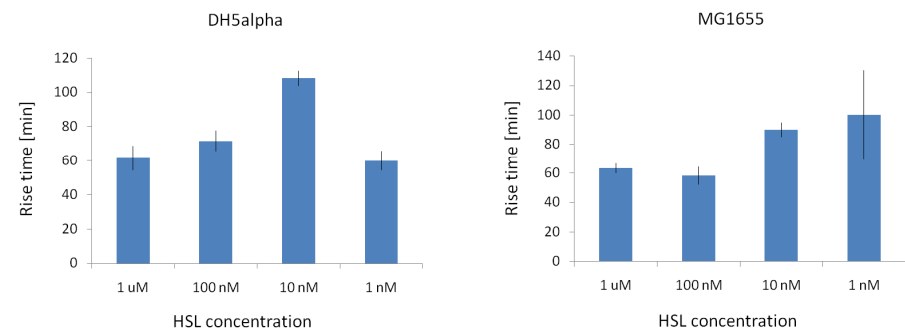
Lysis was assayed in a 96-well microplate by measuring the OD600 dynamics of TOP10 bearing HSL-inducible lysis device pHC-T4LysHSL, induced with HSL and uninduced. Induced and uninduced TOP10 bearing the promoterless lysis device pHC-T4Lys⁻ were used as negative controls. On 10 experiments carried out in separate days, in half of them bacteria did not lyse upon induction (data not shown). Lysis occurred in exponential and early stationary phases, but never in late stationary phase. Lysis behavior in high copy plasmid had a high variability between different experiments carried out in separate days, in fact lysis entity in exponential phase and early stationary phase was $58.6 \pm 7.5\%$ and $42.2 \pm 9.9\%$ respectively. TOP10 bearing the HSL-inducible lysis device pHC-T4LysHSL also showed a very high and variable doubling time (69 ± 6 min), probably due to the toxicity of the lysis proteins expressed by the leakage activity of *lux* promoter present in high copy number, while the negative control pHC-T4Lys⁻ grew faster (doubling time of 45 ± 4.7 min). In pHC-T4LysHSL cultures that failed to lyse, the doubling time was 47.4 ± 2.1 min, suggesting that mutations caused by selective pressure had occurred and the original culture had been replaced by a mutant culture with higher fitness. Finally, as resulted for the HSL-inducible lysis device in low copy plasmid induced with HSL 100 nM, lysis occurred after 15 min from the induction in all the growth phases and mutants arose after about 2-3 h from the lysis start (data not shown). All the described quantitative results are summarized in Supplementary Table 2.

Supplementary Figure 1 – Transfer function, rise time and delay time of the HSL-inducible lysis device in low copy plasmid in early stationary phase in microplate reader for DH5alpha and MG1655 *E. coli* strains, grown at 37°C in LB medium. Lysis entity of bacteria with pLC-T4LysHSL induced with different concentrations of HSL in early stationary phase at OD600~0.7 (DH5alpha) and OD600~1 (MG1655) (A). The experimental data (circles) were fitted with a Hill function (line, $V_{max}=80.7$, $K_{50}=0.7$, $n=3.4$ for DH5alpha and $V_{max}=90$, $K_{50}=0.9$, $n=2.8$ for MG1655). For each concentration, the rise time from 10% to 90% (B) and the delay time before the OD600 drop after induction (C) are also reported. Error bars represent the 95% confidence interval of the estimated mean.

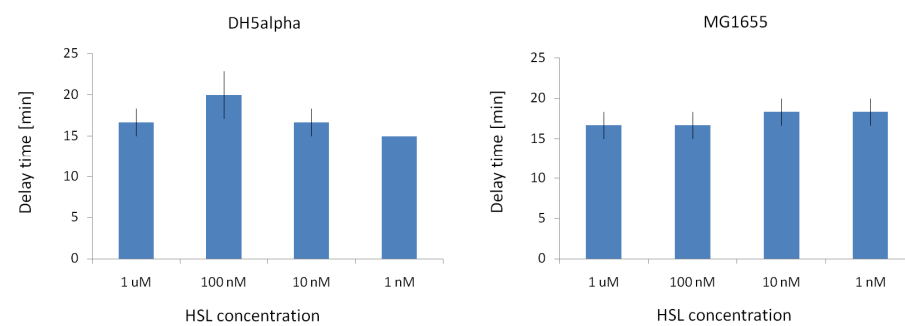
A)



B)

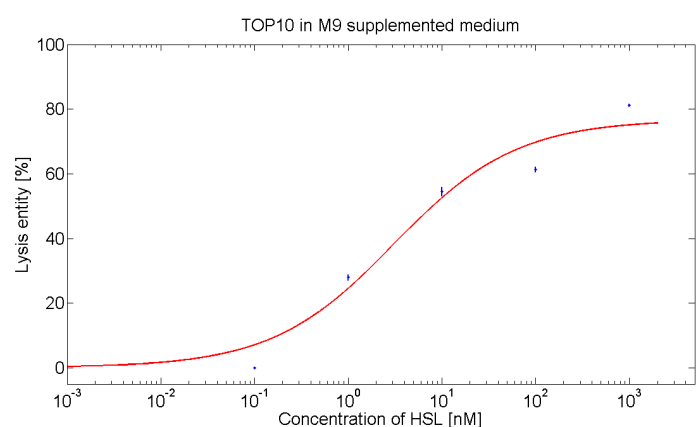


C)

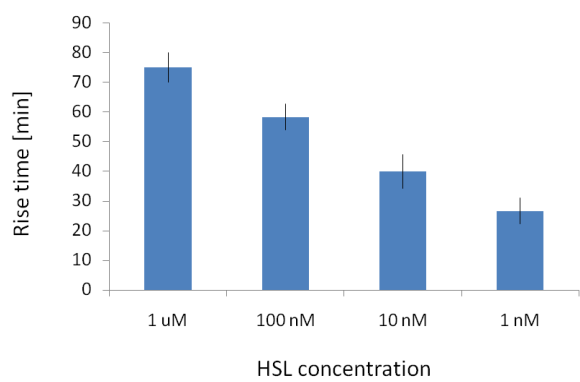


Supplementary Figure 2 – Transfer function, rise time and delay time of the HSL-inducible lysis device in low copy plasmid in early stationary phase in microplate reader for TOP10 strain, grown at 37°C in M9 supplemented medium. Lysis entity of TOP10 with pLC-T4LysHSL induced with different concentrations of HSL in early stationary phase at OD600~0.46 (A). The experimental data (circles) were fitted with a Hill function (line, $V_{max}=76.7$, $K_{50}=3.1$, $n=0.66$). For each concentration, the rise time from 10% to 90% (B) and the delay time before the OD600 drop after induction (C) are also reported. Error bars represent the 95% confidence interval of the estimated mean.

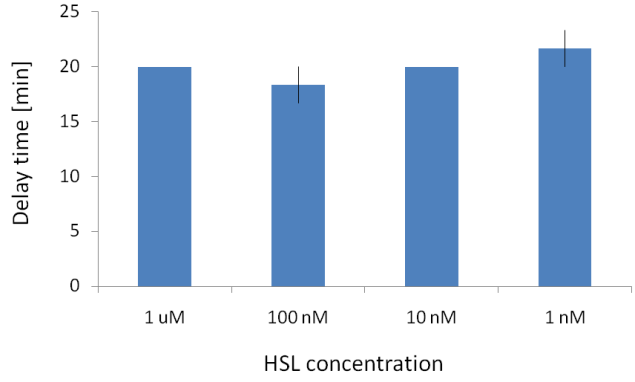
A)



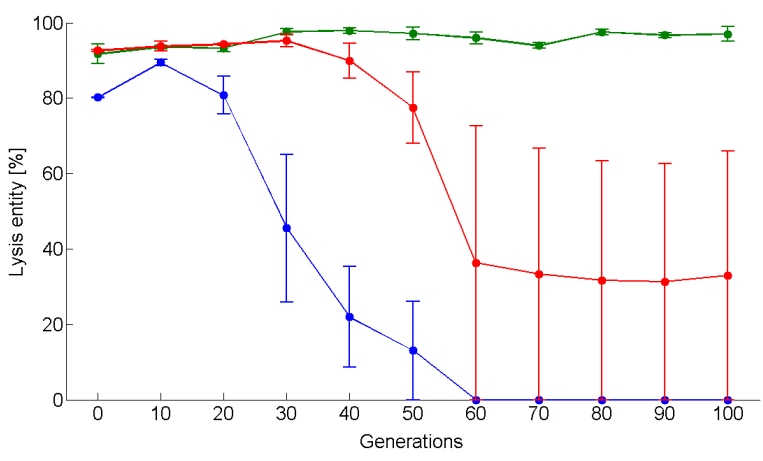
B)



C)



Supplementary Figure 3 – Evolutionary stability of pLC-T4LysHSL over 100 generations in different *E. coli* strains grown in selective LB medium. TOP10 (blue line), DH5alpha (red line) and MG1655 (green line) bearing pLC-T4LysHSL were propagated for 100 generations and every 10 generations the lysis entity was assayed. Here, these values are reported with error bars indicating the standard error of the mean, measured on three bacterial populations evolved in parallel for each strain.



Supplementary Table 1 – Raw and processed RFP fluorescence data in protein release assays.

Raw RFP fluorescence data are reported for lysed pellets and supernatants of the assayed cultures. Resulting values after data processing (i.e. background subtraction, volume correction and percentage of extracellular RFP computation) are also reported.

Culture		Raw fluorescence [RFU]			
		replicates:	#1	#2	#3
TOP10-rfp-lys	supernatant		515	517	517
	lysed pellet		16641	17483	18598
TOP10-rfp-lys + HSL	supernatant		1779	1907	1679
	lysed pellet		1216	1280	1116
TOP10 with pHC-RFP	supernatant		497	484	495
	lysed pellet		16369	13264	19060
TOP10 with pHC-RFP + HSL	supernatant		479	476	476
	lysed pellet		14711	16479	16638
TOP10 with pLC-T4Lys ⁻ (background fluorescence)	supernatant		133		
	lysed pellet		156		
Fluorescence after background subtraction [RFU]					
		replicates:	#1	#2	#3
TOP10-rfp-lys	supernatant		382	384	384
	lysed pellet		16485	17327	18442
TOP10-rfp-lys + HSL	supernatant		1646	1774	1546
	lysed pellet		1060	1124	960
TOP10 with pHC-RFP	supernatant		364	351	362
	lysed pellet		16213	13108	18904
TOP10 with pHC-RFP + HSL	supernatant		346	343	343
	lysed pellet		14555	16323	16482
Fluorescence corrected by volume [RFU]					
		replicates:	#1	#2	#3
TOP10-rfp-lys	supernatant		11460	11520	11520
	lysed pellet		32970	34654	36884
TOP10-rfp-lys + HSL	supernatant		49380	53220	46380
	lysed pellet		2120	2248	1920
TOP10 with pHC-RFP	supernatant		10920	10530	10860
	lysed pellet		32426	26216	37808
TOP10 with pHC-RFP + HSL	supernatant		10380	10290	10290
	lysed pellet		29110	32646	32964
Percentage of extracellular RFP molecules [%]					
		replicates:	#1	#2	#3
TOP10-rfp-lys			25.79338	24.94911	23.7997
TOP10-rfp-lys + HSL			95.8835	95.94721	96.0248
TOP10 with pHC-RFP			25.19264	28.65618	22.3145
TOP10 with pHC-RFP + HSL			26.28514	23.9659	23.7897

Supplementary Table 2 – Quantitative characterization of TOP10 bearing the HSL-inducible lysis device in high copy plasmid pHC-T4LysHSL grown at 37°C in microplate. Induction was carried out with HSL 100 nM. Mean lysis entity and lysis delay after the induction are reported together with their standard error for the different growth phases. The doubling time of the uninduced lysis device and its negative control are reported too. The doubling time of the induced cultures which failed lysis is also shown.

	<u>TOP10 with pHC-T4LysHSL</u>		
	Exponential phase	Early stationary phase	Late stationary phase
Lysis entity [%]	58.6±7.5 ^b	42.2±9.9 ^c	0 ^a
Lysis delay after induction [min]	15 ^b	15 ^c	-
Doubling time [min]	69±6 ^b		
Doubling time when lysis failed [min]	47.4±2.1 ^b		
Doubling time of negative control [min]	45±4.7 ^d		

^a measured on 3 independent experiments

^b measured on 5 independent experiments

^c measured on 2 independent experiments

^d measured on 10 independent experiments

Supplementary Table 3 – Quantitative characterization of pLC-T4LysHSL and pLC-T4LysHeat in DH5alpha and MG1655 *E. coli* strains grown in LB or in TOP10 grown in M9 supplemented medium. Induction was carried out with HSL 100 nM. Mean lysis entity and lysis delay after the induction are reported for the different growth phases together with their standard error, measured on 3 independent experiments for the different growth phases. The doubling time of the uninduced lysis device and its negative control are reported too. As reported in the paper, the doubling times were measured at 37°C in experiments involving pLC-T4LysHSL, while they were measured at 30°C in experiments involving pLC-T4LysHeat. Negative controls were pLC-T4Lys⁻, in the same strain, in the same medium and at the same growth temperature as the inducible lysis device.

DH5alpha with pLC-T4LysHSL in LB medium

	Exponential phase (OD600=0.18)	Early stationary phase (OD600~0.29)	Late stationary phase (OD600~1.6)
Lysis entity [%]	88.3±1.1	79±0.5	39.7±0.5
Lysis delay after induction [min]	26.6±1.6	15	20
Doubling time [min]	54.6±0.4		
Doubling time of negative control [min]	41.8±1.8		

MG1655 with pLC-T4LysHSL in LB medium

	Exponential phase (OD600=0.16)	Early stationary phase (OD600~0.94)	Late stationary phase (OD600~2.3)
Lysis entity [%]	93±0.6	85± 0.6	37.7± 0.5
Lysis delay after induction [min]	20	20	20
Doubling time [min]	43±1		
Doubling time of negative control [min]	32.3±0.5		

TOP10 with pLC-T4LysHSL in M9 supplemented medium

	Exponential phase (OD600=0.13)	Early stationary phase (OD600~0.46)	Late stationary phase (OD600~1.4)
Lysis entity [%]	77.7±0.5	43.9±0.4	38.4±2.2
Lysis delay after induction [min]	30	18.3±0.85	20
Doubling time [min]	87.3±2.9		
Doubling time of negative control [min]	93.4±3.8		

DH5alpha with pLC-T4LysHeat in LB medium

Lysis entity in exponential phase [%] (OD600=0.3)	83.4±4.3
Lysis delay after induction [min]	43.3±2.9
Doubling time [min]	79.1±5.5
Doubling time of negative control [min]	88.4±2.3

MG1655 with pLC-T4LysHeat in LB medium

Lysis entity in exponential phase [%] (OD600=0.34)	82.6±1.3
Lysis delay after induction [min]	41.6±2.9
Doubling time [min]	46.8±1.7
Doubling time of negative control [min]	46.7±1.3

TOP10 with pLC-T4LysHeat in M9 supplemented medium

Lysis entity in exponential phase [%] (OD600=0.19)	46.3±0.8
Lysis delay after induction [min]	62±3.7
Doubling time [min]	192.4±2.1
Doubling time of negative control [min]	160.8±11.1