Supplementary materials for:

A part toolbox to tune genetic expression in *Bacillus subtilis.*

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These supplementary materials contain:

-Supplementary methods.

-Supplementary Figures S1 to S11.

-DNA sequences of the EOU, the mkate2 protein, the BCDs, the spacers, the primers and G-Blocks used in this study.

These supplementary materials are accompanied by supplementary data files containing parts sequences and raw measurements data:

-Supplementary data file 1 contains sequences for all promoters, RBSs, and Ssra tags, as well as mean values and standard deviation of their relative activities, expressed in arbitrary units and in REUs and estimated GFP concentrations.

- Supplementary data file 2 contains raw data values from all cytometry experiments for parents promoters and RBSs, in exponential and stationary phase, expressed in arbitrary units and in REUs.

- Supplementary data file 3 contains raw data values from all cytometry experiments for promoter libraries, in exponential and stationary phase, expressed in arbitrary units and in REUs.

- Supplementary data file 4 contains raw data values from all cytometry experiments for RBS libraries, in exponential and stationary phase, expressed in arbitrary units and in REUs.

- Supplementary data file 5 contains raw data values from all cytometry experiments for SsrA tags libraries, in exponential and stationary phase, expressed in arbitrary units and in REUs.

- Supplementary data file 6 contains raw data values for cytometry experiments comparing context effect using GFP or mkate2 as a reporter, with and without BCD. Data are expressed in arbitrary units and in REUs.

- Supplementary data file 7 contains raw data values for plate-reader experiments comparing promoter activities between Montpellier and Jouy-en–Josas.

Supplementary methods

Bacillus subtilis integration protocol

Bacillus strain 168 was streaked on an LB agar plate. 5 mL of Medium A (0.2% ammonium sulfate, 1.4% dipotassium hydrogen phosphate, 0.6% potassium dihydrogen phosphate, 0.07% sodium citrate, 0.5% glucose, 0.02% magnesium sulfate heptahydrate, 0.2% yeast extract, and 0.025% casamino acids) was inoculated at 0.2-0.25 OD_{600nm} from fresh streaked plate and incubated at 37°C. After cessation of log growth (according to semi-log plot of OD_{600nm} over time) and 90 supplementary minutes, 0.5 mL of the culture was transferred into 4.5 mL of pre-warmed Medium B (0.2% ammonium sulfate, 1.4% dipotassium hydrogen phosphate, 0.6% potassium dihydrogen phosphate, 0.07% sodium citrate, 0.5% glucose, 0.08% magnesium sulfate heptahydrate, 0.1% yeast extract, 0.01% casamino acids, and 0.05% calcium chloride). After 90 min at 37°C, the culture should be highly competent. Competent cells were stored at -80°C with 1/10 v/v of glycerol, aliquoted at 0.5 mL in sterile tube and frozen with liquid nitrogen. For integration, tubes of 0.5 mL of competent cells were thaw at 37°C, 500 ng of DNA was added to the competent cells and incubated at 37°C for 30 minutes. 200 μL of LB was added to cells and cultures were incubated at 37°C for at least 30 minutes. Cells were plated on selective agar plated after spinning at 4 000 rpm during 2 minutes and removed of 600 μL of supernatant.

Plasmid constructions

For plasmid construction and amplification, *E. coli* NEB10β strain (NEB) was used. Transformation of plasmids was accomplished by electroporation. *E. coli* were made electrocompetent following the instruction manual of Bio-rad MicroPulser. Electroportations were realized adding 1 µL of DNA to 40 µL of competent cells and using Bio-Rad GenePulser and Ec1 program with 0.1 cm cuvette. SOC media was added after electric shock and cells were grown one hour at 37°C before plated on selective media. Antibiotics used were Ampicillin (100 µg/mL), Spectinomycin (100 µg/mL) and Erythromycin (0.5 µg/mL) (Sigma).

Design and construction of EOU (expression operating unit)

Two basic synthetic EOUs for gene expression were designed, one with sfGFP(sp) as reporter (47) and the other one with mKate2 (56) optimized for *B. subtilis* (with DNA2.0 algorithm). Both EOUs were composed of identical 40 base pair spacers design for Gibson Assembly (designed using the R2odna software, http://www.r2odna.com) to allow simple construction and switch of parts, of restriction enzyme sites for library constructions, of bidirectional and double terminators to insulate from genetic context (Bba_B0014 and Bba_B0015) and of parts for gene expression in *B. subtilis*, P_{veg} promoter mutated to add

AgeI restriction site, R0 RBS and mKate2(Bs) (simply named RFP from here) or sfGFP(sp) (simply named GFP from here). Cassettes named P_{veg}.RFP and P_{veg}.GFP were synthetised by DNA2.0 (Menlo Park, CA, USA).

P_{veg}.RFP and P_{veg}.GFP were cloned inside pDG1730 vector between position 636 to 676 using Gibson Assembly. pDG1730 and DNA2.0 fragments were PCR amplified with respectively P31/P32 and P33/P34 and ligations were performed using iso-thermal Gibson Assembly. After transformation using electroporation (Ampicillin selection), colony PCR verification (One Taq master mix and P16/P34 primers) and plasmid extraction, constructs were sequence for validation and named SG11 (RFP) and SG13 (GFP).

To construct SG29: P_{veg}.GFP with TSS and SG30: P_{REF}.GFP with TSS, part TSS was added to respectively SG13 and SG22 by Gibson Assembly with PCR amplification of vector by P162/P39 and of insert by P137/P34. SG22 was previously obtained by randomization of SG13 using P64 primer (see promoter libraries section).

Set of 10 promoters
For construction of the set of 10 promoters, 10 Gblocks were designed to replace P_{veg} promoters by the one of interest based on SG29. SG29 was PCR amplified with P109/P36 and ligations with Gblocks were performed using iso-thermal Gibson Assembly.

Set of 8 RBSs, with GFP and RFP as reporter
For construction of the set of 8 RBSs with GFP, primers composed of new RBSs and around 20bp homology sequence with GFP were designed. SG29 was PCR amplified as vector with P146/P39 and SG29 was PCR amplified with primers specific for each RBS (R1 to R7: P139 to P145 and R8: P225), ligations were performed using iso-thermal Gibson Assembly.

For construction of the set of 8 RBSs with RFP, SG29 was PCR amplified as vector with P146/P72 and SG11 was PCR amplified as insert with P34 and primers specific for each RBS (R0: P238, R1 to R7: P206 to P212). Ligations were performed using iso-thermal Gibson Assembly and constructions were sequenced using P33/P34 primers.

Set of 4sSsrA tags
For construction of a set of 4 classic SsrA tags, 4 Gblocks were designed to add SsrA degradation tags to SG13. SG13 was PCR amplified by P124/P39 and ligations with Gblocks were performed using iso-thermal Gibson Assembly.

Promoter, RBS and SsrA tag library construction

The various promoters, RBS and degradation tags libraries were generated by performing a PCR on the GFP gene using primers contain the regulatory region of interest

degenerated at strategic positions.

Promoter libraries

For the initial P_{veg} libraries (Figure S2A), SG13 was randomized by PCR amplification using P34 and the degenerated primers: P62 (full randomization), P63 (randomization of -35 box) or P64 (randomization of -10 box). For final P_{veg} libraries, SG29 was randomized by PCR amplification using P34 and the degenerated primers P213. Vectors and amplified fragments were digested by *Age*I and *Sph*I. For P_{serA} and P_{ymdA} libraries, SG36 (cassette with P_{serA} as promoter) and SG37 (P_{ymdA} as promoter) were randomized by PCR amplification using P34 and respectively P214 and P215 for design 1 (randomisation of -10 box), P341 and P342 for design 2 (randomisation of 6 nucleotides before -10 box). Vectors and amplified fragments were digested by *Bam*HI and *Sph*I.

RBS libraries

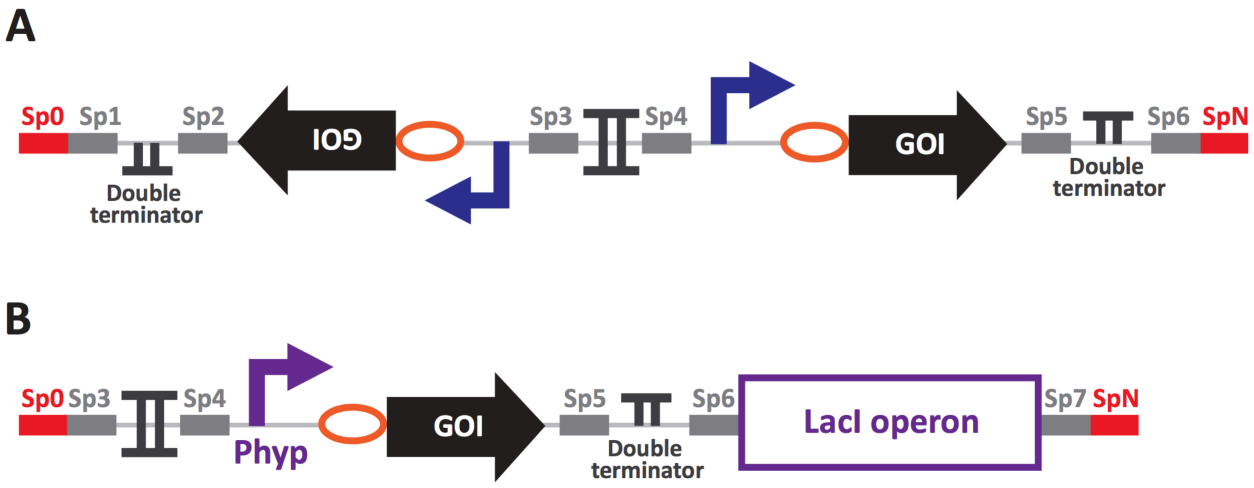
For RBS libraries, SG29 (cassette with R0 as RBS), SG45 (R1 as RBS) and SG47 (R2 as RBS) were randomized by PCR amplification using P34 and the degenerated primers respectively: P220, P221 and P222. Vectors and amplified fragments were restricted by NheI and SphI.

SsrA tag libraries

For SsrA tag libraries, SG13 was randomized by PCR amplification using P33 and P51 degenerated primer. Vectors and amplified fragments were restricted by *Nhe*I and *Sph*I.

Supplementary figures

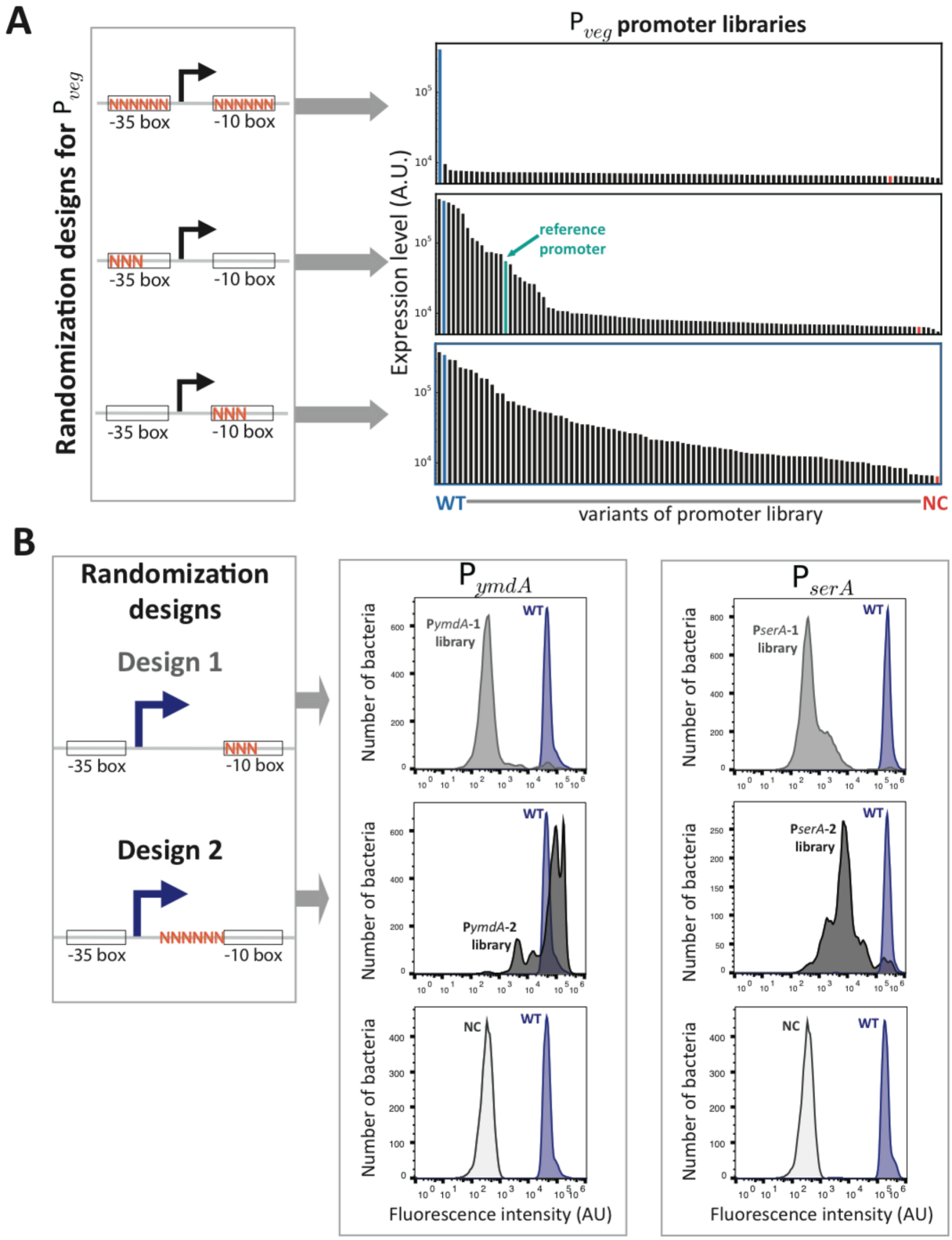
Figure S1: Expression operating unit design for expression of two genes and for inducible expression of gene.



(A) Architecture of our standardized and modular double expression operating unit (dEOU) based on the same design than EOU. The dEOU is composed of two sets of standard regulatory elements (promoter, RBS, GOI) positioned in opposite direction two maximize insulation between the two cassettes. Spacers (SpX) of 40 bp designed to facilitate for one-step isothermal assembly enable simple construction and switching of parts.

(B) Architecture of expression operating unit for inducible expression with example of $P_{hyperspank}$ promoter expressed under regulation of LacI repressor with induction by IPTG. Repressor operon is placed in 3' of the EOU, between spacer Sp6 and Sp7.

Figure S2: Engineering promoter libraries: different parent sequences and randomisation designs result in libraries with various distribution of expression level.

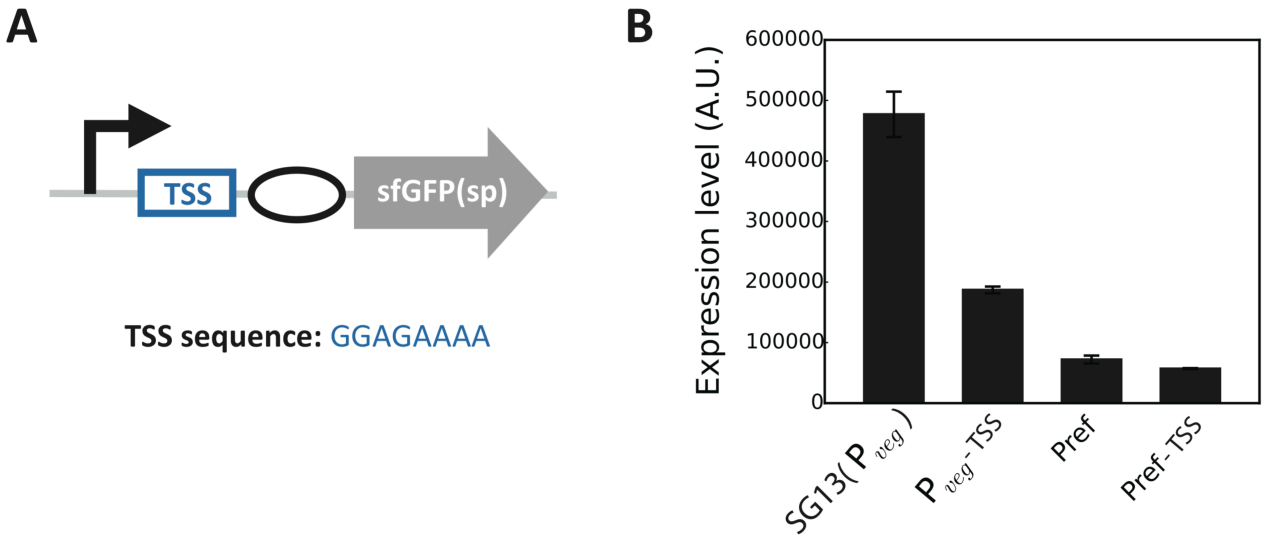


(A) Based on P_{veg} sequences (without TSS), we tested 3 promoter libraries designs (left panel); randomisation of -35 box and -10 box, randomisation of 3 nucleotides within the -10 box and randomisation of 3 nucleotides within the -35 box. Distribution of expression levels for each library (right panel) corresponds to the measurement of fluorescence

intensity over OD on a plate reader (see methods for details). The selected reference promoter obtained from the -10 box randomisation library is highlighted in pale blue.

(B) Two other parent promoters: P_{serA} and P_{ymdA} were used for engineering promoter libraries. We tested 2 designs (left panel): randomisation of 3 nucleotides within the -10 box and randomisation of 6 nucleotides between the -35 and -10 box. Right panel, distribution of expression level for the 4 libraries (2 promoters parent sequences and 2 randomisation designs for each of them). Histograms are obtained by measurement of the fluorescence intensity (in arbitrary units) of the pool of variants by flow-cytometry after integration in *B. subtilis*. For details, see method.

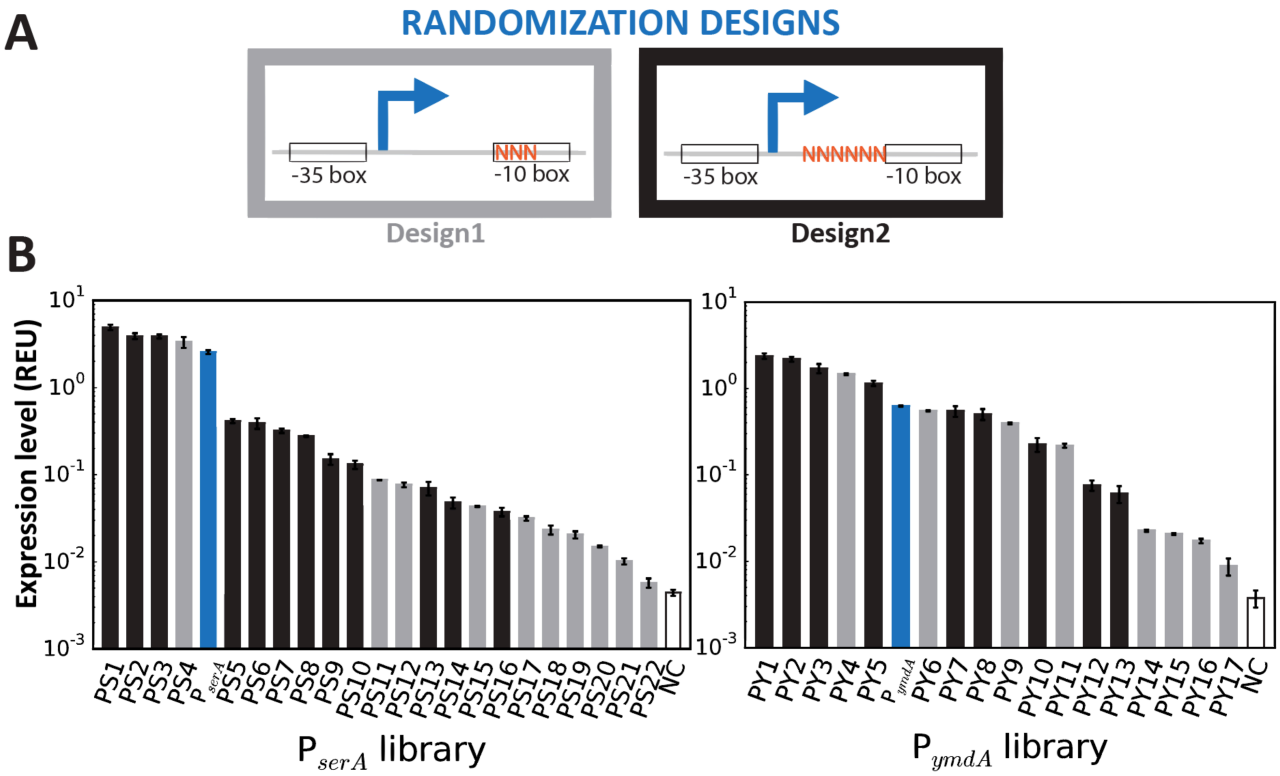
Figure S3: Effect of TSS part on promoter efficiency.



(A) A standard TSS sequence was placed between promoters and ribosome binding sites. We chose the 8 nucleotides after the promoter of *fbaA* gene of *B. subtilis*.

(B) Expression level of 2 promoters with and without the standard TSS part; SG13 (corresponding to the P_{veg} promoter without the TSS part), P_{veg} (TSS), the reference promoter P_{REF} (a variant of P_{veg}), and P_{REF} (TSS). Constructs were cloned on our modular cassette (R0 was used as a RBS). Expression levels are in arbitrary unit (A.U.) and correspond to the fluorescence intensity measured flow-cytometer obtained over 3 independent experiments performed on exponential phase (3 replicates per experiments). Error-bars correspond to standard deviation between the 3 experiments.

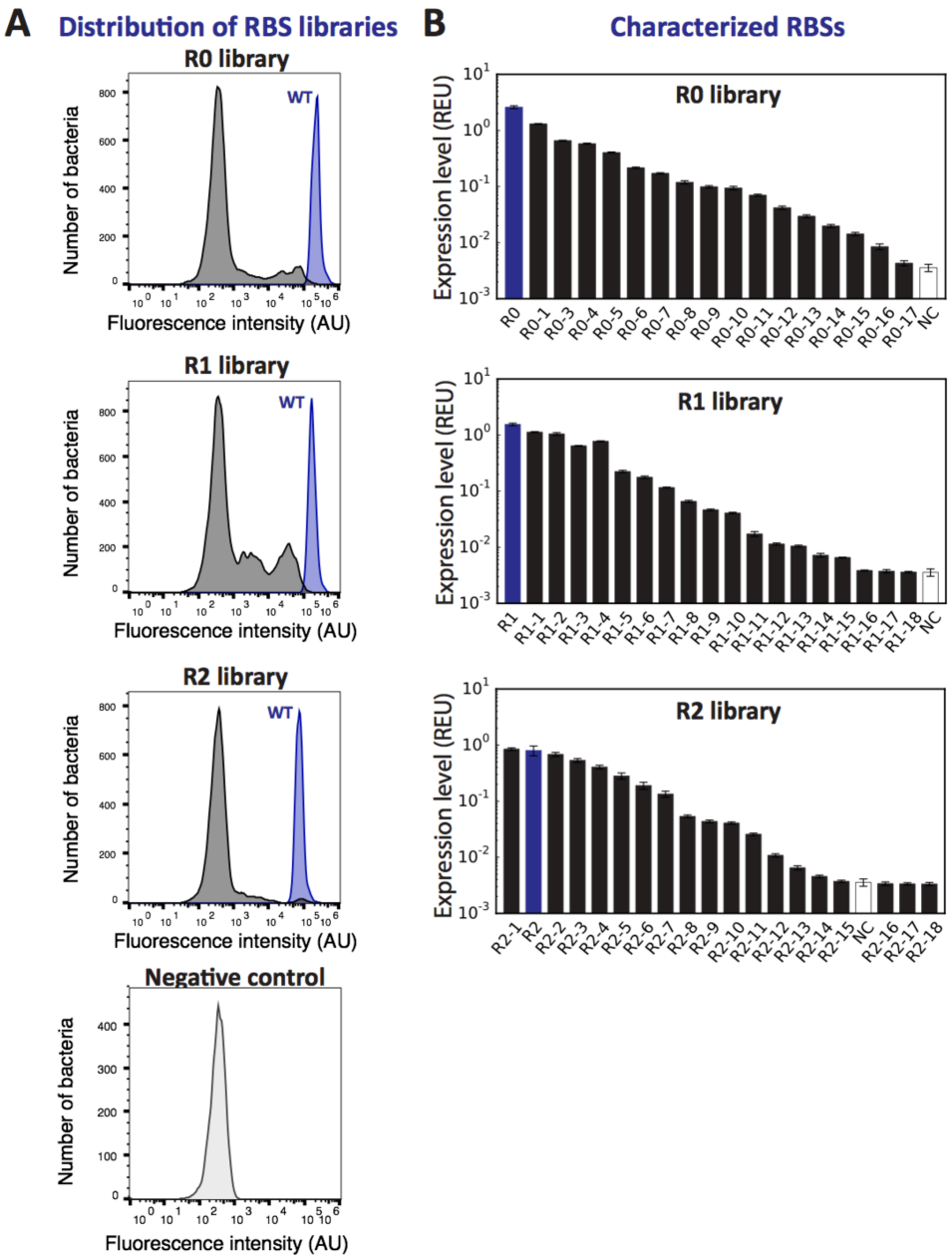
Figure S4: Fully characterised P_{serA} and P_{ymdA} variants and comparison of the two different randomisation designs.



(A) Two different randomisation designs, design 1: randomisation of 3 nucleotides within the -10 box (grey) and design 2: 6 nucleotides between the -35 box and the -10 box (black).

(B) (C) Characterisation of over 10 variants per design for P_{serA} libraries **(B)** and P_{ymdA} libraries **(C)**. Expression levels were measured by flow-cytometry and expressed in REU. Data correspond to the mean of 3 independent experiments performed in triplicates and error-bars correspond to standard deviation over these 3 experiments. Grey bars design variants engineered following the design 1 and black bars design variants engineered following the design 2.

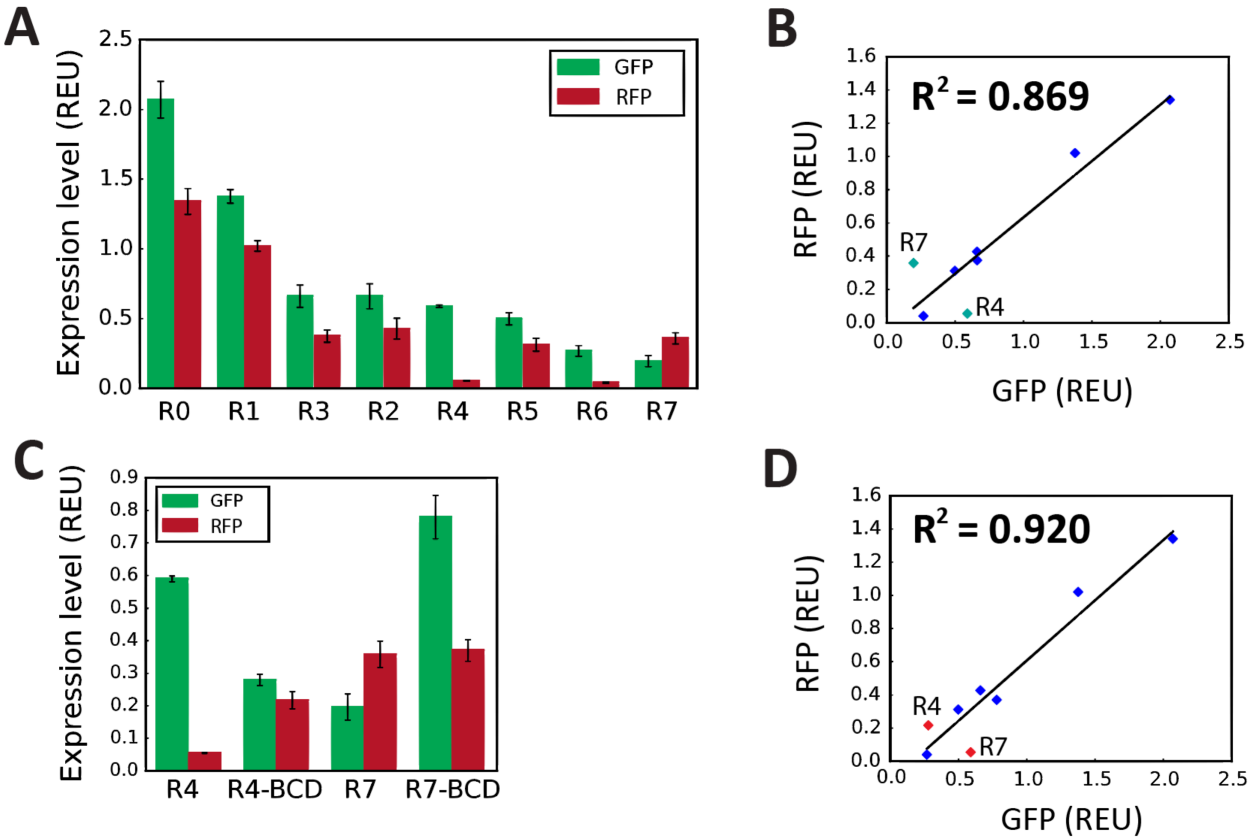
Figure S5: Distribution of expression levels and full set of characterised variants for the 3 RBS libraries.



(A) Distribution of expression level for the 3 RBS libraries (R0, R1, R2) and the negative control stain (without GFP) as a background fluorescence control. Histograms are obtained by flow-cytometer measurement of fluorescence intensity (in arbitrary unit) of the pool of variants after integration in *B. subtilis*. Blue histogram corresponds to the wild-type RBS sequence and the black histogram to the full library.

(B) Full set of characterised variants for each library. Expression levels were measured by flow-cytometry and expressed in REU. Data correspond to the mean of 3 independent experiments performed in triplicates and error-bars correspond to standard deviation over these 3 experiments.

Figure S6: Effect of reporter sequences on translation efficiency and insulation of this effect using bicistrons.



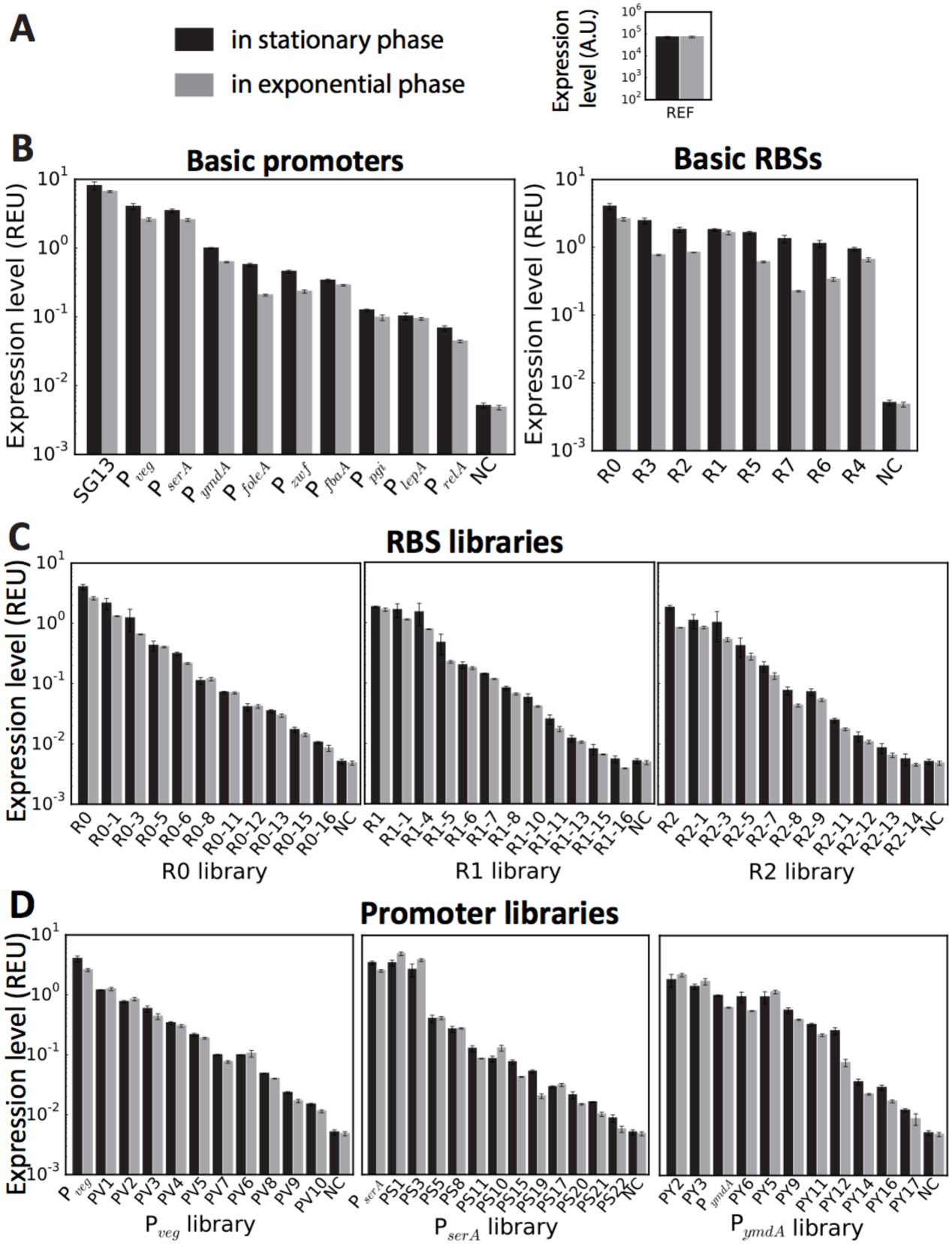
(A) Characterisation of 8 RBSs with two different reporters: sfGFP(sp) (named GFP) and mKate2(Bs) (named RFP). Expression levels were measured by flow-cytometry and expressed in REU. Data correspond to the mean of 3 independent experiments performed in triplicates and error-bars correspond to standard deviation over these 3 experiments. The reference constructs used were P_{REF} with corresponding reporter.

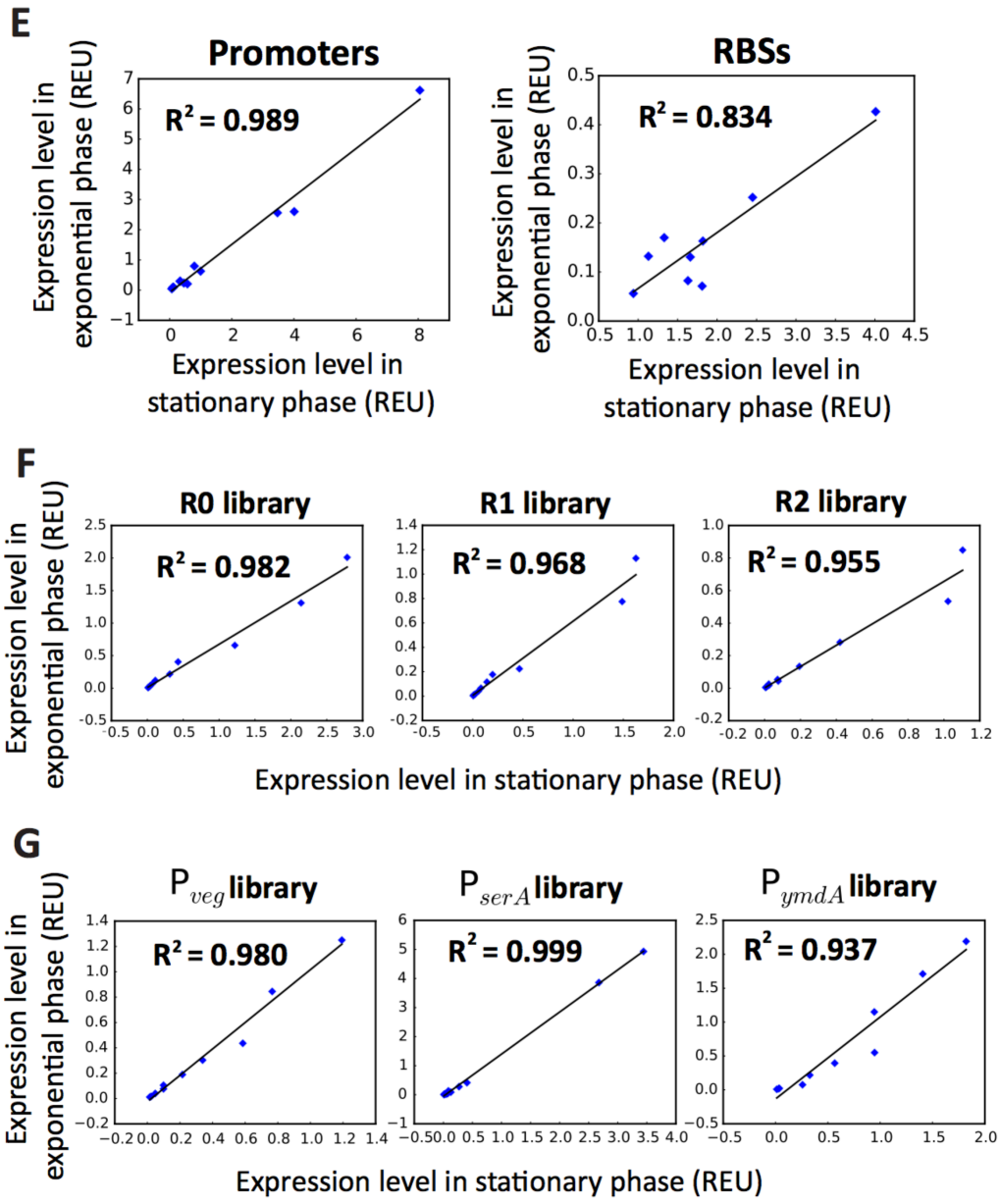
(B) Expression level of RBSs with RFP over expression level of RBSs with GFP in relative expression unit. A linear correlation was found with a coefficient of determination of 0.898. The two ribosome binding sites with the worse correlation are R4 and R7 (green dots).

(C) For R4 and R7, a bicistronic design was used to decouple translation initiation from putative context effects arising from interactios between the RBS and the reporter coding sequence. Expression levels were measured by flow-cytometry and expressed in REU. Data correspond to the mean of 3 independent experiments performed in triplicates and error-bars correspond to standard deviation over these 3 experiments.

(D) Expression level of RBSs coupled with RFP over expression level of RBSs coupled with GFP in REU with R4-BCD and R7-BCD (red dots) instead of mono-cistron constructs. A linear correlation was found with a coefficient of determination of 0.920. Full measurements data are available in supplementary data file 6.

Figure S7: Comparison of promoter and RBS strengths between stationary and exponential phase.

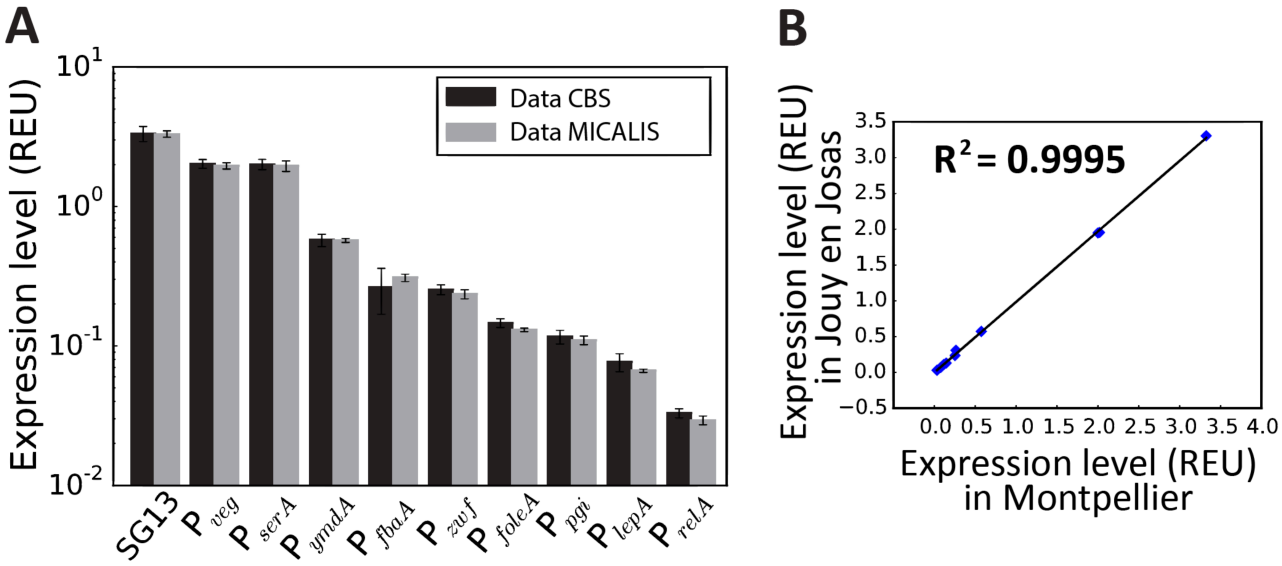




Characterisation of promoter and RBS libraries was performed on exponential and stationary phases. Expression levels were measured by flow-cytometry and expressed in REU. Data correspond to the mean of 3 independent experiments performed in triplicates and error-bars correspond to standard deviation over these 3 experiments. Grey bars correspond to exponential phase and black bars to stationary phase. **(A)** Expression level of the reference construct in absolute unit in exponential and stationary phase. **(B) (C) (D):** Expression levels of basic promoters and RBSs in exponential and stationary phase expressed in REU **(B)**, RBS libraries **(C)** and promoter libraries **(D)**.

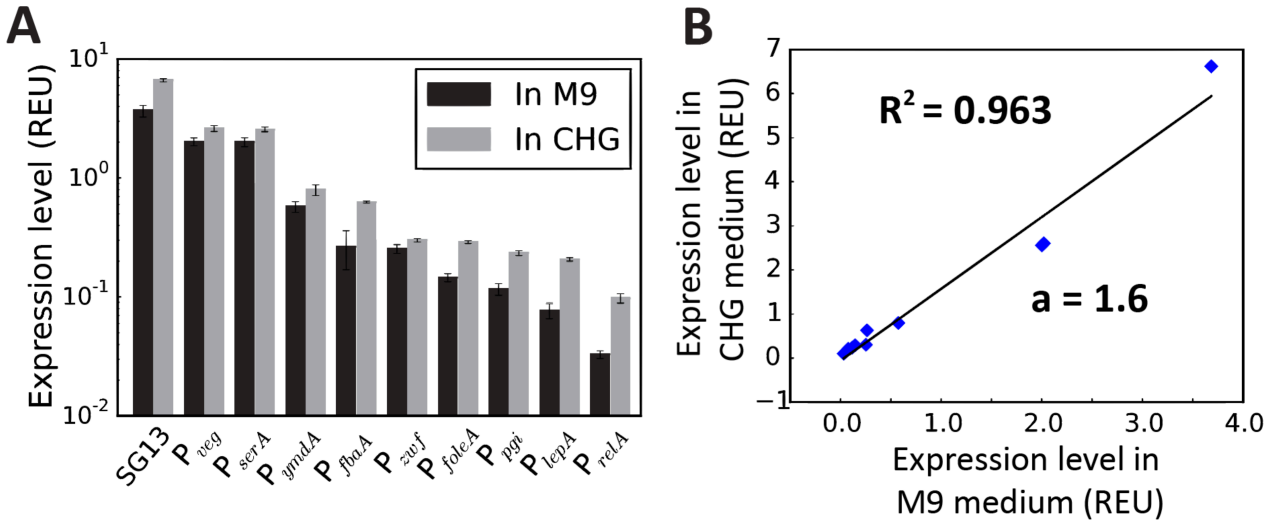
(E) (F) (G): Expression level in exponential phase over expression level in stationary phase in relative expression units for promoters and RBSs sets **(E)**, RBS libraries **(F)** and promoter libraries **(G)**. Linear correlation were performed for each construction sets and a coefficient of determination between 0.83 and 0.999 were found.

Figure S8: Measurement of parts activities in 2 different laboratories.



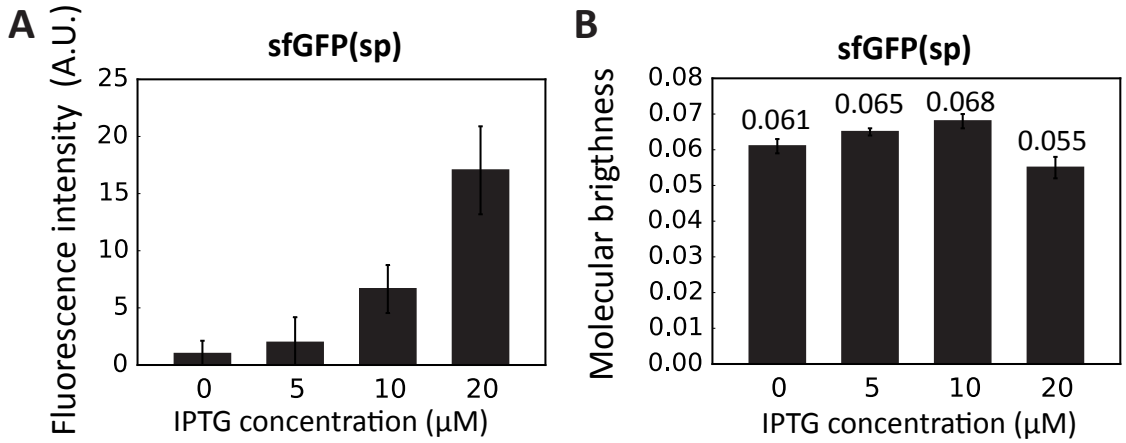
(A) Expression levels in relative expression unit correspond to GFP/OD for 3 kinetic experiments on a plate reader (3 replicates per experiment) in CHG medium (more details in methods). Experiments were performed in parallel in 2 different laboratories with 2 different experimenters. Error-bars correspond to standard deviation over the three experiments. SG13 correspond to the P_{veg} promoter without the +1/+8 part **(B)** Correlation of expression level between both laboratories in REU with coefficient of determination of 0.9995. Full library measurements data are available in supplementary 7.

Figure S9: Correlation of gene expression levels between two medium conditions: M9 and CHG.



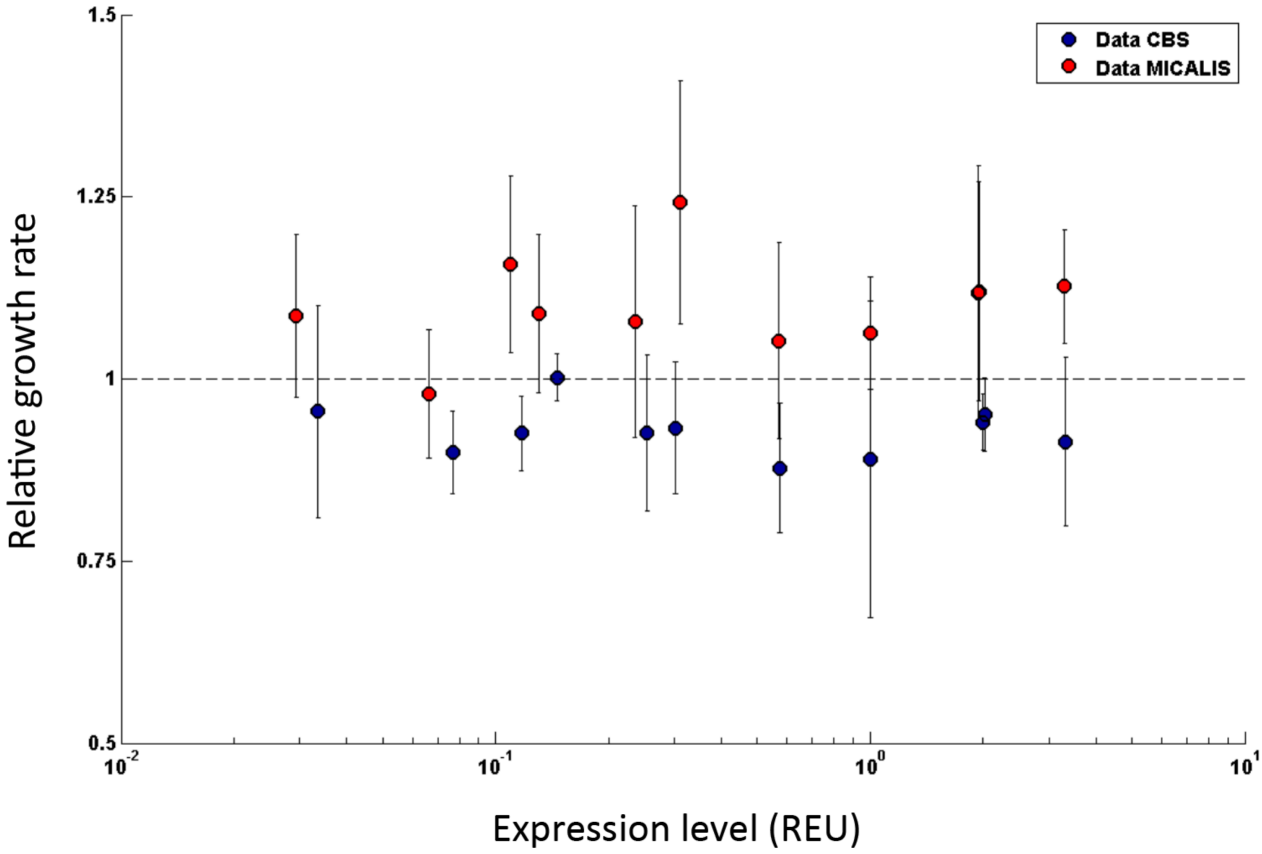
(A) Expression level in REU of the basic promoter set measured by flow-cytometer in exponential phase in M9 (black bars – Figure 2) and measured on a plate-reader in CHG media (grey bars – Figure S8). SG13 correspond to the P_{veg} promoter without the +1/+8 part **(B)** Correlation between experiments on M9 using flow-cytometer and on CHG using plate-reader. Linear correlation with coefficient of determination of 0.963 and director coefficient of 1.6.

Figure S10: Characterisation of sfGFP(sp) using 2-photon microscope and number and brightness method.



(A) (B) Characterisation of the molecular brightness of the sfGFP(sp) at different concentrations of IPTG using 2p sN&B method. sfGFP(sp) expression was induced at different levels using pHyperspank promoter and IPTG concentration from 0 to 20 µM. Fluorescence intensity **(A)**, number of GFP per excitation volume and molecular brightness **(B)** were determined at each IPTG concentration. Error bars correspond to cell-to-cell variation (experiments were performed once).

Figure S11: Growth rate for B. subtilis strains with various GFP expression level.



Relative growth rate correspond to growth rate of strains with a specific expression level in REU over growth rate of the negative control. Data represented correspond to the characterisation of 10 strains with different promoters in 2 different laboratories: CBS and Micalis (see Fig S8). Error-bars correspond to standard deviation over the three experiments.

DNA sequences

Expression Operating Unit (example with Pveg.R0.sfGFP(sp))

Sp0-Sp3-B0014-Sp4-**PVEG**-TSS-**NheI**-**R0**-sfGFP(sp)-Sp5-**SphI**-Sp5'-B0015-Sp6-**SpN**

CTCGGATACCCTTACTCTGTTGAAAACGAATAGATAGGTTAAGGAACGGTTATTTCTGCGTAGATCTATCTTACACAGCA
TCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGAGAGAATATAAAAAGCCAGATTATTAATCCG
GCTTTTTTATTATTTAGGCAACTGAAACGATTCGGATCCTGTATTACTATTCTTA**AATTTTGTCAAAATAATTTTATTGA**
CAACGTCTTATTAACGTTGATACCGGTTAAATTTTATTTGACAAAAATGGGCTCGTGTTGTACAATAAATGTGGAGAAAA
GCTAGCGATTAACTAATAAGGAGGACAAAC**ATG**TCAAAAGGAGAAGAACTTTTTACAGGTGTAGTACCTATCTTGGTTGA
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CACGGTATGGACGAACTTTATAAATAA**ACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG**GCATGCTAAAAGT
CTCGTAAAGCGTTCTATCAATAACCCGTTGGTG**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTT**
CGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTA
TACCGTCTCAGAATCGGCCGTGAACAATAAAATAGTTTCGGT**ATTATTGACCACTTCCGAGTAGAATCGTGCTTCAGTAA**
GA

Phyp.R0.sfGFP(sp).LacI_operon

Sp0-Sp3-B0014-Sp4-**PHYP**-**NheI**-**R0**-sfGFP(sp)-Sp5-**SphI**-Sp5'-B0015-Sp6-LacIoperon-Sp7-**SpN**

CTCGGATACCCTTACTCTGTTGAAAACGAATAGATAGGTTAAGGAACGGTTATTTCTGCGTAGATCTATCTTACACAGCA
TCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGAGAGAATATAAAAAGCCAGATTATTAATCCG
GCTTTTTTATTATTTAGGCAACTGAAACGATTCGGATCCTGTATTACTATTCTTA**CTCGAGGGTAAATGTGAGCACTCAC**
AATTCATTTTGCAAAAGTTGTTGACTTTATCTACAAGGTGTGGCATAATGTGTGTAATTGTGAGCGGATAACAATTGCTA
GCGATTAACTAATAAGGAGGACAAAC**ATG**TCAGAACTAATCAAAGAGAATATGCACATGAAGCTGTACATGGAAGGAACG
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GTCGGCTGAAAGATCGTACGTACCAATTATTGTTTCGTGATTGTTCAAGCCATAACACTGTAGGGATAGTGGAAAGAGTG
CTTCATCTGGTTACGATCAATCAAATATTCAAACGGAGGGAGACGATTTTGATGAAACCAGTAACGTTATACGATGTCGC

AGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAA
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TTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCT
GGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGG
AAAGCGGGCAGTGATAATAAAAGGTCCCGTCTGAACTTACTGTGAATTCGACTA**ATTATTGACCACTTCCGAGTAGAATC**
GTGCTTCAGTAAGA

mKate2(Bs)

ATGTCAGAACTAATCAAAGAGAATATGCACATGAAGCTGTACATGGAAGGAACGGTAAACAATCATCATTTCAAATGT
ACAAGCGAGGGTGAGGGGAAGCCTTATGAAGGGACACAAACCATGCGGATTAAAGCAGTCGAGGGCGGACCCCTT
CCGTTTGCCTTCGATATCTTGGCTACGAGCTTTATGTATGGGTCGAAAACATTTATCAATCACACGCAGGGGATTCCA
GACTTTTTCAAACAAAGTTTTCCGGAAGGCTTTACGTGGGAACGTGTGACCACGTATGAAGATGGCGGCGTCTTAAC
AGCTACACAAGATACATCTTTACAAGACGGATGCTTGATATACAACGTTAAGATTCGCGGTGTTAACTTTCCGTCAAA
CGGACCTGTTATGCAGAAGAAAACCCTGGGCTGGGAAGCGTCAACAGAAACACTCTATCCAGCCGACGGTGGACTT
GAGGGCCGTGCCGATATGGCTCTTAAACTCGTGGGCGGTGGCCATCTGATTTGCAATCTTAAAACTACTTATCGGTC
CAAAAAGCCGGCGAAGAATTTGAAAATGCCTGGAGTATACTACGTTGATAGACGATTAGAAAGGATTAAAGAAGCAG
ACAAAGAAACTTATGTAGAGCAGCATGAAGTCGCAGTGGCGAGATATTGTGATTTACCGTCTAAACTGGGACATCGC
TAA

BCDs

BCD-4

RBS0-first start codon-RBS4-second start codon

GGGCCCAAGTTCACTTAAGATTAACTAATAAGGAGGACAACAACAATGAAAGCAATTTTCGTACTGAAtgacatgaaagg
aagtatttgatAATG

BCD-7

RBS0-first start codon-RBS7-second start codon

GGGCCCAAGTTCACTTAAGATTAACTAATAAGGAGGACAACAACAATGAAAGCAATTTTCGTACTGAAggtgggaaggag
gaactactAATG

Spacer sequences

Spacer names	Spacer sequences
sp0	CTCGGATACCCTTACTCTGTTGAAAACGAATAGATAGGTT
sp1	TGCTCGTAGTTTACCACGGATACAGACAGTGATAATCTTA
sp2	AGATTACTACTGATAACCACTGTTGATTGGGATACCCGTA
sp3	AAGGAACGGTTATTTCTGCGTAGATCTATCTTACACAGCA
sp4	AGGCAACTGAAACGATTCGGATCCTGTATTACTATTCTTA
sp5	ACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG
sp5’	TAAAAGTCTCGTAAAGCGTTCTATCAATAACCCGTTGGTG
sp6	CCGTCTCAGAATCGGCCGTGAACAATAAAATAGTTTCGGT
sp7	TAATAAAAGGTCCCGTCTGAACTTACTGTGAATTCGACTA
spN	ATTATTGACCACTTCCGAGTAGAATCGTGCTTCAGTAAGA

Primer sequences

Primer numbers	Primer sequences
16	gccgcgatttccaatgaggtta
31	caacagagtaagggtatccgagcgatcagaccagtttttaatttgtgtg
32	gagtagaatcgtgcttcagtaagaggcgattttcgttcgtgaatac
33	CTCGGATACCCTTACTCTGTTGAAAAC
34	TCTTACTGAAGCACGATTCTACTCGG
36	TGCTGTGTAAGATAGATCTACGCAG
39	ACTTTATCTGAGAATAGTCAATCTTCGGAAATC
40	CACCTGGGATTTCCGAAGATTGAC
51	GGATCGGAgcatgcTTAAHNAHNAHNAGCAACATTTTGATTAAATGAATTTGTTTTGCCTGCtttataaagttcgtccataccgtgagtg
62	ACGTTGATACCGGTTAAATTTTATNNNNNNAAAATGGGCTCGTGTTGNNNNNNaaatgtgctagcgattaactaataaggagg
63	ACGTTGATACCGGTTAAATTTTATNNNACAAAAATGGGCTCGTGTTGTATAATaaatgtgctagcgattaactaataaggagg
64	ACGTTGATACCGGTTAAATTTTATTTGACAAAAATGGGCTCGTGTTGNNNAATaaatgtgctagcgattaactaataaggagg
71	cgttttcaacagagtaagggtatccgag
72	attattgaccacttccgagtagaatcgtg
109	gattaactaataaggaggacaaacatgtc
124	TTTATAAAGTTCGTCCATACCGTGAGTGATACC
137	ggttaaattttatttgacaaaaatgggctcgtgttgtacaataaatgtggagaaaagctagcgattaactaataaggaggacaaac
139	ggttaaattttatttgacaaaaatgggctcgtgttgtacaataaatgtggagaaaagctagcgattaactaataaggaggacaaac
140	ggctcgtgttgtacaataaatgtggagaaaagctagcggtgggaaggaggggttcgacatgtcaaaaggagaagaacttttacagg
141	ggctcgtgttgtacaataaatgtggagaaaagctagcggtgggaaggaggaactactatgtcaaaaggagaagaacttttacagg
142	ggctcgtgttgtacaataaatgtggagaaaagctagcggtgggaaggaggacattcgacatgtcaaaaggagaagaacttttacagg
143	ggctcgtgttgtacaataaatgtggagaaaagctagcaaaggaggtgatgacatgtcaaaaggagaagaac

ttttacagg

144 ggctcgtgttgtacaataaatgtggagaaaagctagcgctcttaaggaggattttagaatgtcaaaaggag
aagaacttttacagg

145 ggctcgtgttgtacaataaatgtggagaaaagctagctgacatgaaaggaagtatttgaaaatgtcaaaag
gagaagaacttttacagg

146 gctagctttctccacatttattgtac

160 gattaactaataaggaggacaaacatgtcagaactaatc

161 gattagttctgacatgtttgtcctccttattagttaatc

162 catttattgtacaacacgagc

206 ggctcgtgttgtacaataaatgtggagaaaagctagcggtgggaaggaggtgatccaatgtcagaactaat
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207 ggctcgtgttgtacaataaatgtggagaaaagctagcggtgggaaggaggggttcgacatgtcagaacta
atcaaagagaatatgcac

208 ggctcgtgttgtacaataaatgtggagaaaagctagcggtgggaaggaggaactactatgtcagaactaat
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210 ggctcgtgttgtacaataaatgtggagaaaagctagcaaaggaggtgatgacatgtcagaactaatcaaag
agaatatgcac

211 ggctcgtgttgtacaataaatgtggagaaaagctagcgctcttaaggaggattttagaatgtcagaactaa
tcaaagagaatatgcac

212 ggctcgtgttgtacaataaatgtggagaaaagctagctgacatgaaaggaagtatttgaaaatgtcagaac
taatcaaagagaatatgc

213 ACGTTGATaccggttaaattttatttgacaaaaatgggctcgtgttgNNNaataaatgtggagaaaagcta
gcgattaac

214 CGTTGATggatcctgtattactattcttaactgcgtcaatacacgttgacactcttttgagaatatgtNNN
attatcagggagaaaagctagcgattaac

215 CGTTGATggatcctgtattactattcttagttaagatggcaagcttgacaagtatttccgacacattNNNa
atgaagttggagaaaagctagcgattaac

220 GTGATCCAgctagcgattaactaataaNNNNNNcaaacatgtcaaaaggagaagaacttttacagg

221 GTGATCCAgctagcGGTGGAANNNNNNTGATGACatgtcaaaaggagaagaacttttacagg

222 GTGATCCAgctagcgctcttaNNNNNNattttagaatgtcaaaaggagaagaacttttacagg

225 ggctcgtgttgtacaataaatgtggagaaaagctagcGGTGGAAAGGAGGTGATGACatgtcaaaaggaga
agaacttttacagg

238 ggctcgtgttgtacaataaatgtggagaaaagctagcgattaactaataaggaggacaaacatgtcagaac
taatcaaagagaatatgc

341 CGTTGATggatcctgtattactattcttaactgcgtcaatacacgttgacactcttttgNNNNNNtgttaa
attatcagggagaaaagct

342 CGTTGATggatcctgtattactattcttagttaagatggcaagcttgacaagtatttcNNNNNNatttaca
atgaagttggagaaaagct

343 aggcaactgaaacgattcggatcctgtattactattcttaggagaaaagctagcgattaactaataaggag
gac

423 ctgtaaaaagttcttctccttttgacatgctagcacatttattgtacaacacg

424 cgtgttgtacaataaatgtgctagcatgtcaaaaggagaagaacttttacag

Gblock sequences

Gblock names	Gblock sequences
ssrA_LAA	CAGCAGCAGGTATCACTCACGGTATGGACGAACTTTATAAAGCAGGTAAGACTAATTCATTTAATC AAAATGTTGCTCTTGCAGCATAAACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG
ssrA_LDD	CAGCAGCAGGTATCACTCACGGTATGGACGAACTTTATAAAGCAGGTAAGACTAATTCATTTAATC AAAATGTTGCTCTTGATGATTAAACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG
ssrA_AAV	CAGCAGCAGGTATCACTCACGGTATGGACGAACTTTATAAAGCAGGTAAGACTAATTCATTTAATC AAAATGTTGCTGCTGCTGTTTAAACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG
ssrA_ASV	CAGCAGCAGGTATCACTCACGGTATGGACGAACTTTATAAAGCAGGTAAGACTAATTCATTTAATC AAAATGTTGCTGCTAGTGTTTAAACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG
ssrA_LVA	CAGCAGCAGGTATCACTCACGGTATGGACGAACTTTATAAAGCAGGTAAGACTAATTCATTTAATC AAAATGTTGCTTTAGTTGCTTAAACTTTATCTGAGAATAGTCAATCTTCGGAAATCCCAGGTG
Gblock_fbaA	ctcggatacccttactctgttgaaaacgaatagataggttaaggaacggttatttctgcgtagatc tatcttacacagcatcacactggctcaccttcgggtgggcctttctgcgtttatatactagagaga gaatataaaaagccagattattaatccggctttttttattatttaggcaactgaaacgattcggatc ctgtattactattcttaaatcatgtcattatgttgccgatttgtcgaaaagttggtatcctagtta tggagaaaagctagcgattaactaataaggaggacaaacatgtcaaaaggagaagaacttttaca ggtgtagtacctatcttggttg
Gblock_zwf	ctcggatacccttactctgttgaaaacgaatagataggttaaggaacggttatttctgcgtagatc tatcttacacagcatcacactggctcaccttcgggtgggcctttctgcgtttatatactagagaga gaatataaaaagccagattattaatccggctttttttattatttaggcaactgaaacgattcggatc ctgtattactattcttaaaaagggcttaaatgtttgctttcgttgaattttagatttaaaatgaag gggagaaaagctagcgattaactaataaggaggacaaacatgtcaaaaggagaagaacttttaca ggtgtagtacctatcttggttg
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Gblock_serA	ctcggatacccttactctgttgaaaacgaatagataggttaaggaacggttatttctgcgtagatc tatcttacacagcatcacactggctcaccttcgggtgggcctttctgcgtttatatactagagaga gaatataaaaagccagattattaatccggctttttttattatttaggcaactgaaacgattcggatc ctgtattactattcttaactgcgtcaatacacgttgacactcttttgagaatatgttaaattatca gggagaaaagctagcgattaactaataaggaggacaaacatgtcaaaaggagaagaacttttaca ggtgtagtacctatcttggttg
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Gblock_relA	ctcggatacccttactctgttgaaaacgaatagataggttaaggaacggttatttctgcgtagatc tatcttacacagcatcacactggctcaccttcgggtgggcctttctgcgtttatatactagagaga gaatataaaaagccagattattaatccggctttttttattatttaggcaactgaaacgattcggatc ctgtattactattcttaacttctgctctttacatctttcgtttttttcttgataataaactacaat aggagaaaagctagcgattaactaataaggaggacaaacatgtcaaaaggagaagaacttttaca ggtgtagtacctatcttggttg
Gblock_folEA	ctcggatacccttactctgttgaaaacgaatagataggttaaggaacggttatttctgcgtagatc tatcttacacagcatcacactggctcaccttcgggtgggcctttctgcgtttatatactagagaga gaatataaaaagccagattattaatccggctttttttattatttaggcaactgaaacgattcggatc ctgtattactattcttagggcattcactttgcttttagcggggcatatgtgctagaatcgaaatta

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Gblock_lepA ctcggataccttactctgttgaaaacgaatagataggttaaggaacggttatttctgcgtagatc
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