Supporting information

Supplementary Figures

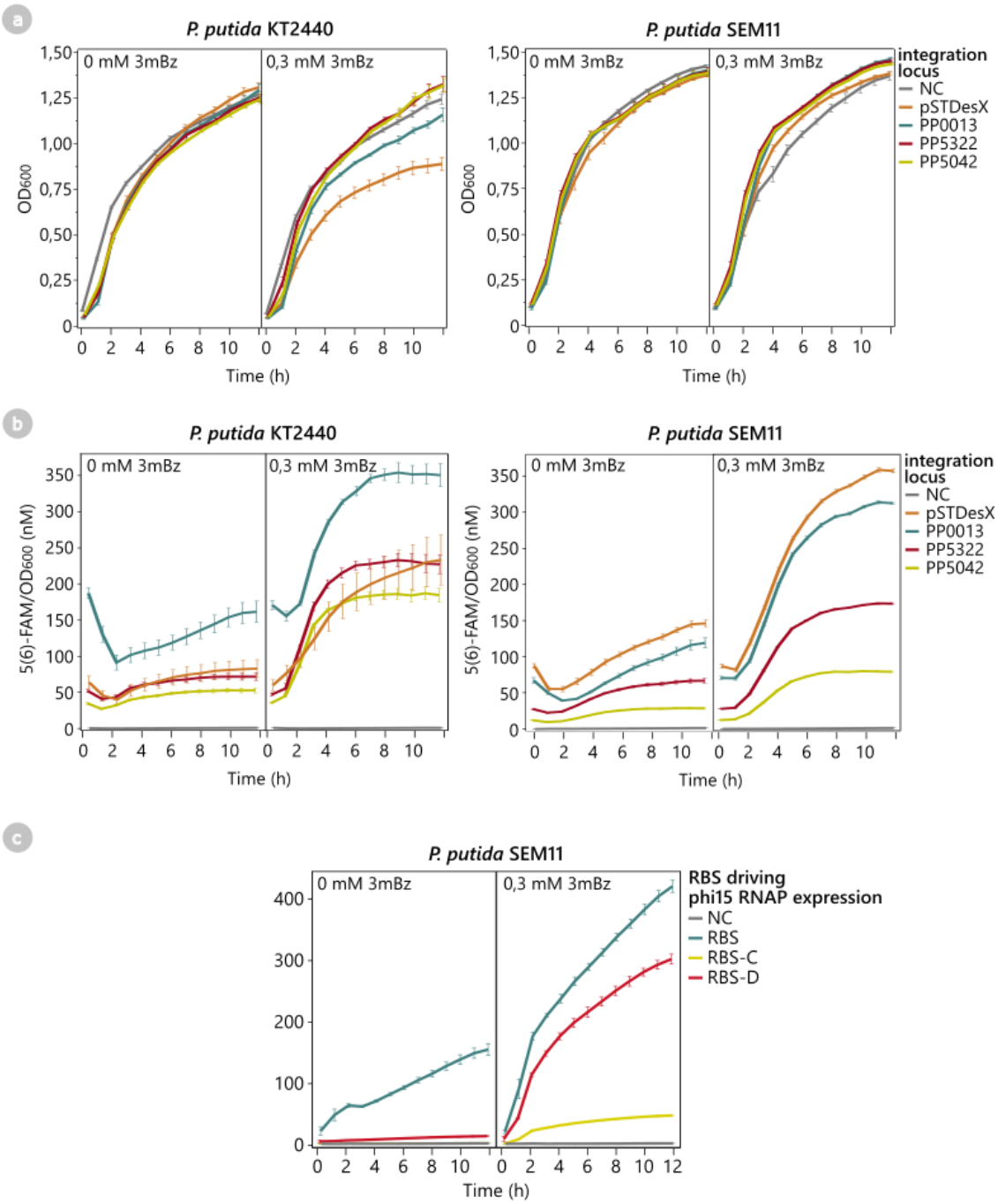


Figure S1: Genomic integration of XylS/Pm::phi15rnap in P. putida *KT2440 and SEM11 does not impact cell growth. a and b) The genomic locus where phi15rnap is integrated has a significant influence on the fluorescent intensity, but does not impact cell growth .* P. putida *KT2440 and SEM11 strains without phi15rnap (NC), vector-borne expression of phi15rnap (pSTDesX), or phi15rnap integrated in one of three different genomic loci (PP0013, PP5322 and PP5042) were induced with 0.3 mM 3mBz at OD_{600} 0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12 h. Datapoints and error bars represent the mean OD_{600} (a) and 5(6)-FAM/OD_{600} (b) values and standard error of four biological replicates. c) A weaker RBS and start codon to drive phi15rnap expression improves the dynamic range of the phi15 expression system.* P. putida *SEM11 strains without phi15rnap (NC) or phi15rnap integrated in PP0013 with three different RBSs (original RBS, RBS-C and RBS-D) were induced with 0.3 mM 3mBz at OD_{600} 0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12h. Datapoints and error bars represent the mean OD_{600} and 5(6)-FAM/OD_{600} values and standard error of four biological replicates.*

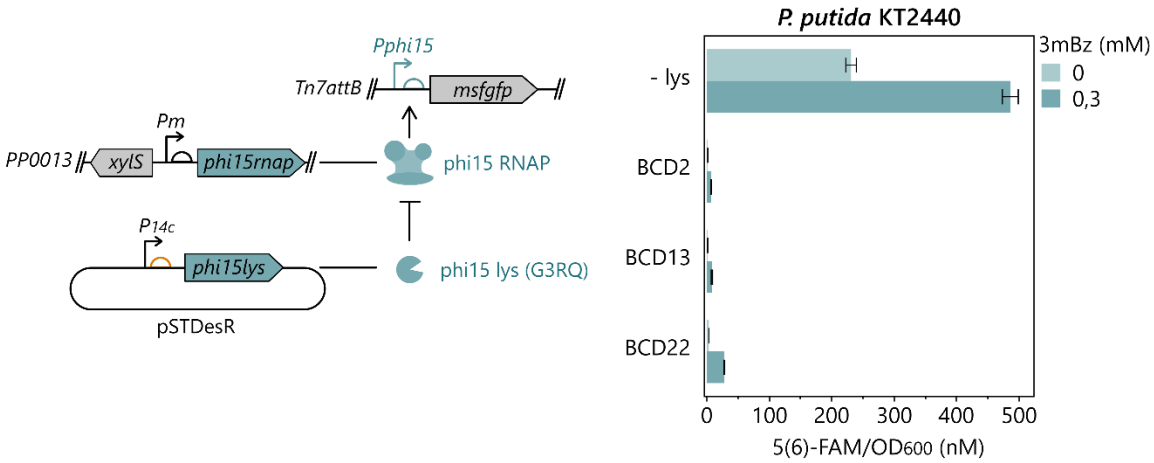


Figure S2: Selection of the RBS for expression of phi15 lysozyme G3RQ. P. putida *KT2440 PP0013::phi15rnap(RBS) without lysozyme (-lys) and with pSEVA42·phi15lysozyme(G3RQ) with different RBSs (BCD2, BCD13 or BCD22) were induced with 0.3 mM 3mBz at* OD_{600} *0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12 h. Datapoints and error bars represent the mean* OD_{600} *and 5(6)-FAM/*OD_{600} *values and standard error of four biological replicates after 12h of induction.*

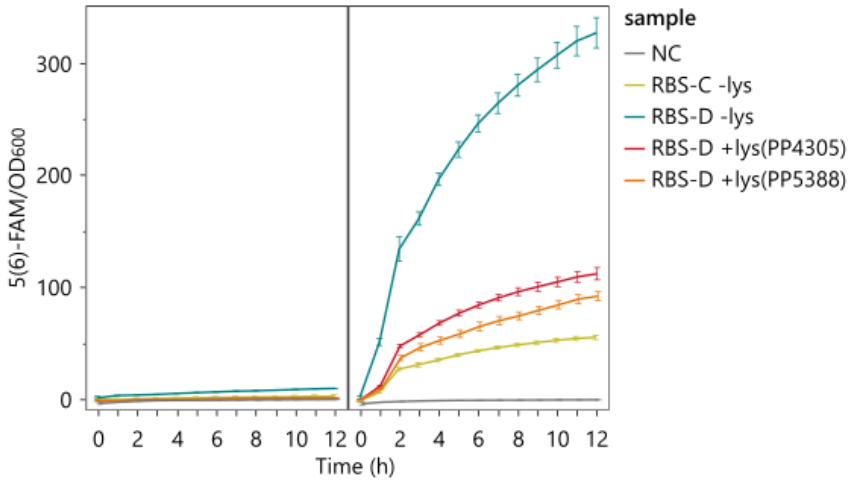


Figure S3: Genomic integration of P_{14c}-BCD22-phi15lys(G3RQ) *in* P. putida *SEM11 improves the dynamic range of the phi15 expression system. The phi15 lysozyme (G3RQ) reduces basal expression and improves the dynamic range of the phi15 expression system.* P. putida *SEM11 without phi15rnap (NC), with the phi15rnap in PP0013 with either RBS-C (RBS-C -lys) or RBS-D (RBS-D -lys) and with the phi15 lysozyme (G3RQ) in locus PP4305 or PP5388 (RBS-D +lys(PP4305) and RBS-D +lys(PP5388) were induced (right) with 0.3 mM 3mBz at* OD_{600} *0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12 h. A control in non-inducing conditions shows the low basal expression levels (left). Datapoints and error bars represent the mean* OD_{600} *and 5(6)-FAM/*OD_{600} *values and standard error of four biological replicates.*

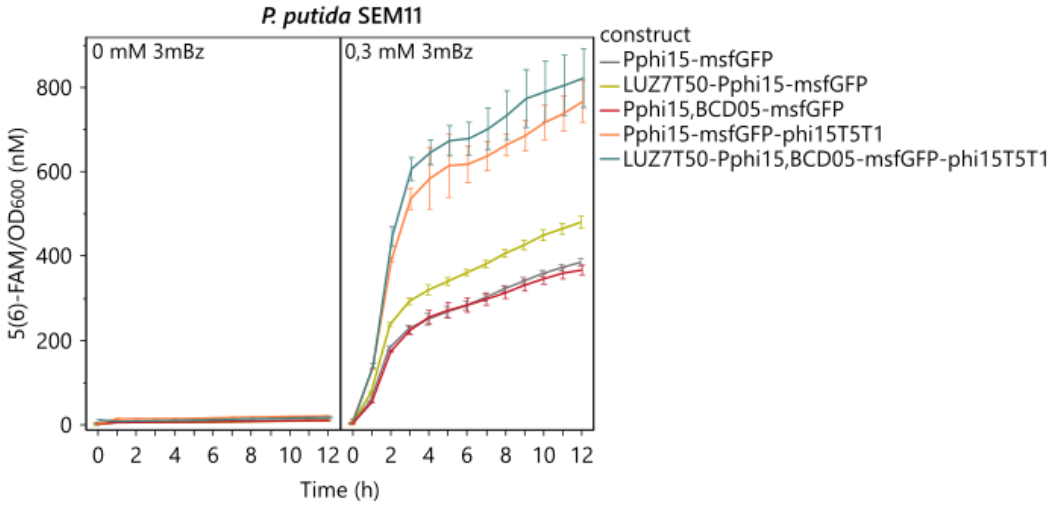


Figure S4: Optimized expression vector pPUT delivers high expression levels of the gene-of-interest. P. putida *P15 strains with pBGDes.P_{phi15}-msfgfp, pBGDes.LUZ7T50-P_{phi15}-msfgfp, pBGDes.$P_{phi15-BCD05}$-msfgfp, pBGDes.P_{phi15}-msfgfp-phi15T5-phi15T1 and pPUT.msfGFP (top to bottom) were induced with 0.3 mM 3mBz at OD_{600} 0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12h. Datapoints and error bars represent the mean and standard error of 5(6)-FAM/OD_{600} values of four biological replicates.*

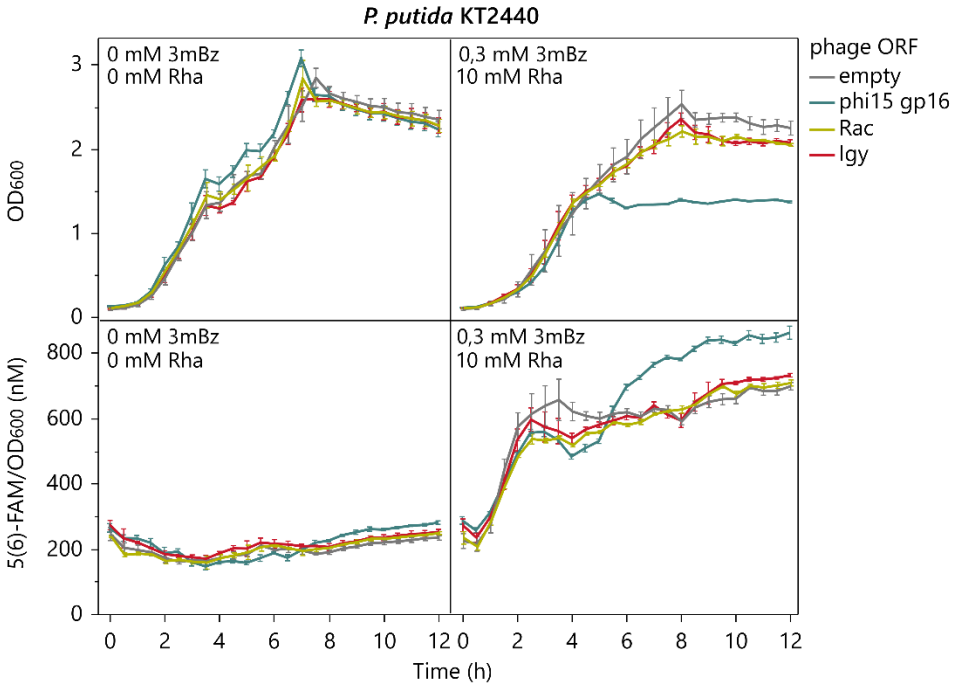


Figure S5: Growth-decoupling with phage ORFs. P. putida *KT2440 with pBGDesXa·phi15RNAP, pBGDes.P_{phi15}-msfgfp and pSTDesR with different phage ORFs (phi15 gp16, Igy, Rac) were induced with 0.3 mM 3mBz and 10 mM Rha at OD_{600} 0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12h. Datapoints and error bars represent the mean and standard error of 5(6)-FAM/OD_{600} values of four biological replicates.*

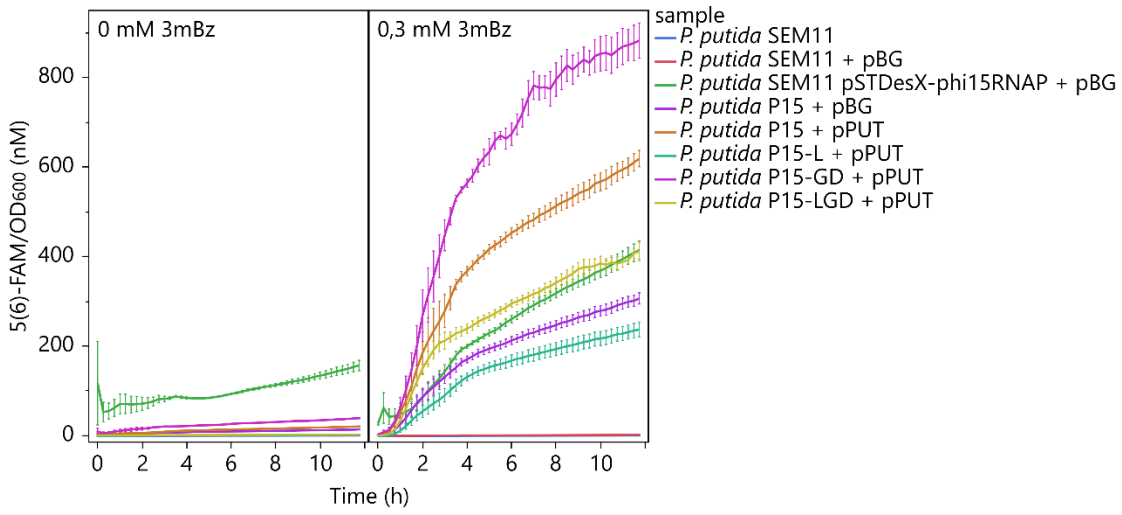


Figure S6: All intermediate steps of the optimization of the phi15 expression system. All optimization steps to create vector pPUT. All P. putida *SEM11, P15, P15-L, P15-GD and P15-LGD strains with pBGDes.P_{phi15}-msfgfp or pPUT.msfGFP induced with 0.3 mM 3mBz at OD_{600} 0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12h. Datapoints and error bars represent the mean and standard error of 5(6)-FAM/OD_{600} values of four biological replicates.*

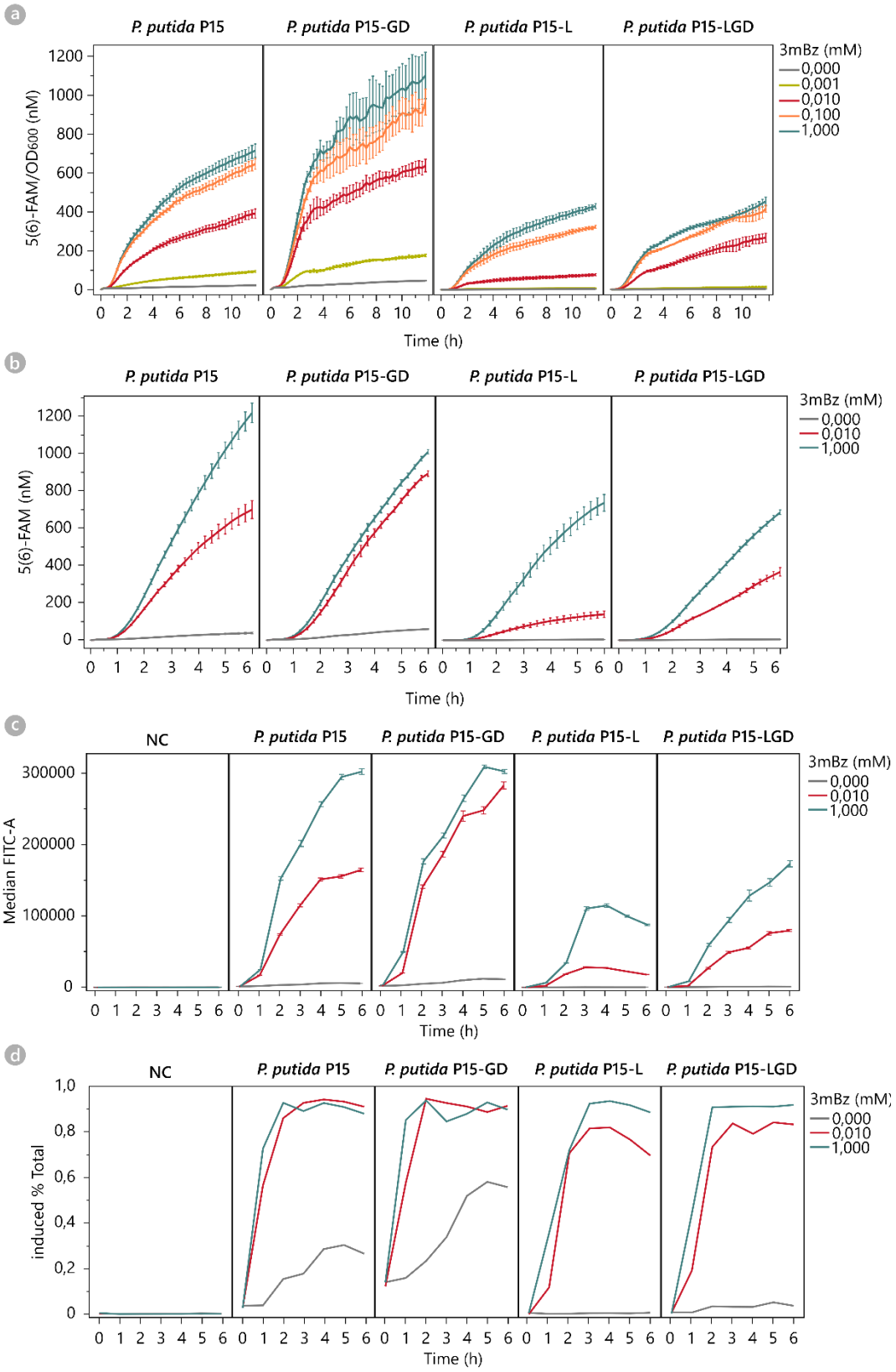


Figure S7: Characterization of P. putida *P15, P15-L, P15-GD and P15-LGD. a)* P. putida *P15, P15-L, P15-GD and P15-LGD were induced with 0-1 mM 3mBz at* OD_{600} *0.1 (and 10 mM Rha for strains P15-GD and P15-LGD), after which the fluorescence intensity and cell growth is monitored every half hour for 12h. Datapoints and error bars represent the mean 5(6)-FAM/*OD_{600} *values and standard error of four biological replicates. b) Identical assay to a), datapoints and error bars represent the mean 5(6)-FAM values and standard error of four biological replicates over 6h, to make a comparison with the flow cytometric data presented in c). c and d) Flow cytometric analysis of* P. putida *P15, P15-L, P15-GD and P15-LGD, which were induced with 0-1*

mM 3mBz at OD_{600} 0.1 (and 10 mM Rha for strains P15-GD and P15-LGD). 5000 cells were analyzed every hour P.I. for a total of 6h. Datapoints and error bars represent the mean msfGFP-FITC-A value (c) or percentage induced cells (msfGFP-FITC-A above 10´) (d) and standard error of two biological replicates.

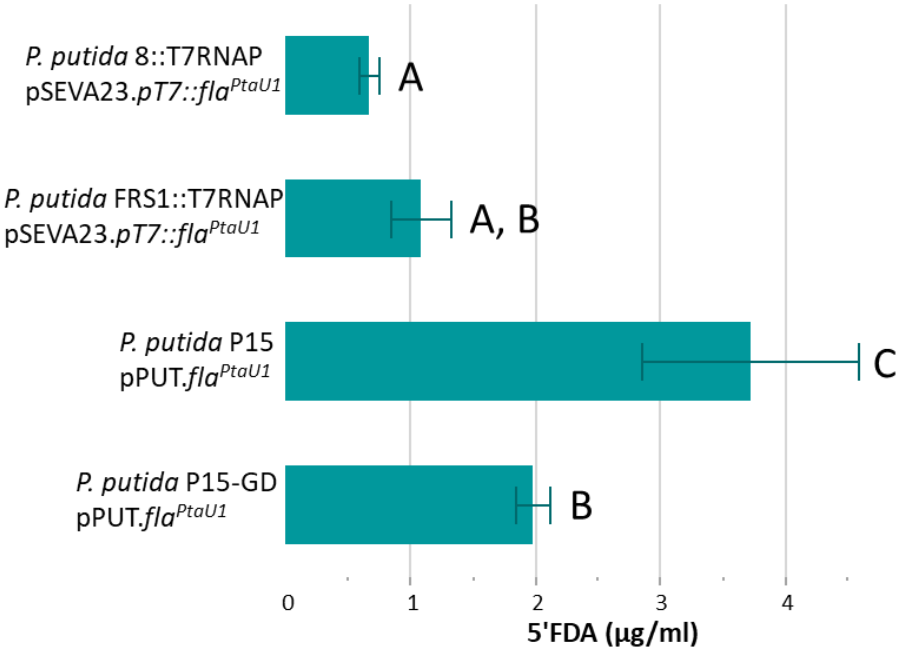


Figure S8: Mean 5'- FDA yield of the fluorinase reaction for each tested strain (bars), per ml reaction (µg/ml). Four different strains were tested, representing a Xyls/Pm-based system (P. putida 8::T7RNAP pSEVA23.pT7::fla^{ftaU1}), FRS1-based system ((P. putida FRS1::T7RNAP pSEVA23.pT7::fla^{ftaU1}), the phi15-based system ((P. putida P15 pPUT.fla^{ftaU1}) and phi15-based system with growth decoupler (P. putida P15-GD pPUT.fla^{ftaU1}). Error bars represent the standard error of three biological replicates, samples not connected by same letters are significantly different (Tukey HSD, α=0.05).

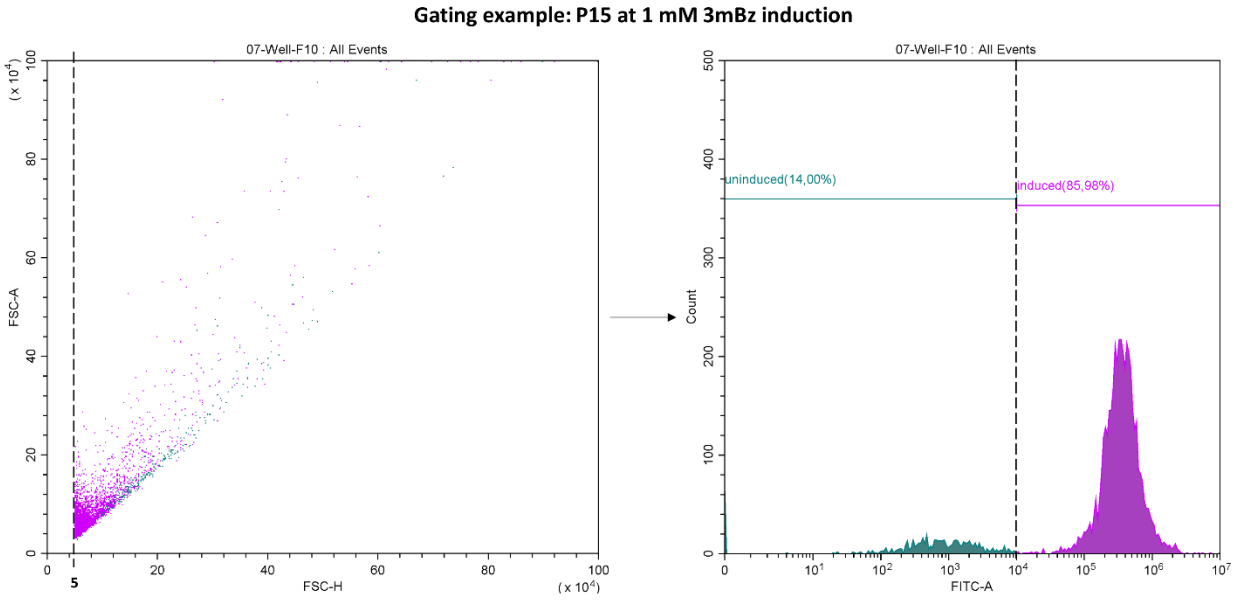


Figure S9: Gating strategy of flow cytometry experiments. Gating was applied for all samples and an example of P15 at 1 mM 3mbZ induction is given here (left) events with FSC-H values above 50,000 are retained for analysis. (right) events with a FITC-A (msfGFP) value above 10^4 are considered to be induced and below to be uninduced.

Supplementary Experiments

1. Vector backbone of optimized expression vector

In order to obtain high yields of the desired protein with minimal cell burden, it is important to integrate the genetic expression construct in the optimal genetic background. Multiple factors can influence the expression of the construct, such as the plasmid copy number and proper insulation from upstream and downstream sequences. So far, the msfGFP reporter constructs have been genomically integrated in the host as a single copy. To determine the impact of the copy number on msfGFP production, the reporter construct $P_{phi15,MCP}$-*msfgfp* is integrated in identical vectors with different origins of replication: pSEVA621 (RK2 – low copy number), pSEVA631 (pBBR1 – medium copy number), pSEVA641 (pR01600/ColE1 – high copy number) and pSEVA651 (RSF1010 – high copy number)(1). Not to our surprise, when introducing the vectors in *P. putida* carrying pSTDesXa·phi15RNAP none of the transformants contained the desired vector. This result is in line with the work of other research groups, who did not succeed to support both a T7-like phage RNAP and the corresponding promoter on DNA vectors (2). Therefore, we attempted to introduce the vectors in *P. putida* where *phi15rnap* is genomically integrated in PP0013, PP5322 and PP5042, respectively. All of the strains were able to tolerate and replicate pSEVA62·$P_{phi15,MCP}$-msfGFP, while no transformants were obtained for the pSEVA641 backbone. Furthermore, only *P. putida* PP5042::*phi15rnap* was electroporated successfully with pSEVA63·$P_{phi15,MCP}$-msfGFP and pSEVA65·$P_{phi15,MCP}$-msfGFP.

The OD_{600} and fluorescence intensity of all obtained transformants were measured for 12 h after induction with 0.3 mM 3mBz and displayed in Figure S9. For all integration sites, the cell cultures have a significantly lower OD_{600} after 12h of growth when the reporter construct vector-borne instead of integrated in the host's Tn7 landing site (Tukey HSD, $P<0.0001$). Moreover, for integration site PP0013, almost no cell growth occurred after 12 h of incubation in the presence of 0.3 mM 3mBz (Figure S9). Due to this very stunted cell growth, it would be incorrect to compare the performance of the different strains based on their normalized msfGFP levels. Therefore, the absolute msfGFP levels were examined instead (Figure S9), clearly indicating that a single copy reporter construct is highly favorable over multicopy variants, no matter in which locus *phi15rnap* is integrated. For *P. putida* strains with *phi15rnap* integrated in PP0013, PP5322 or PP5042, the single copy construct shows minimal impact on cell growth,

while enabling very high absolute and normalized msfGFP expression levels. Based on these results, the pBGDes vector will be used as a backbone to create our final expression construct.

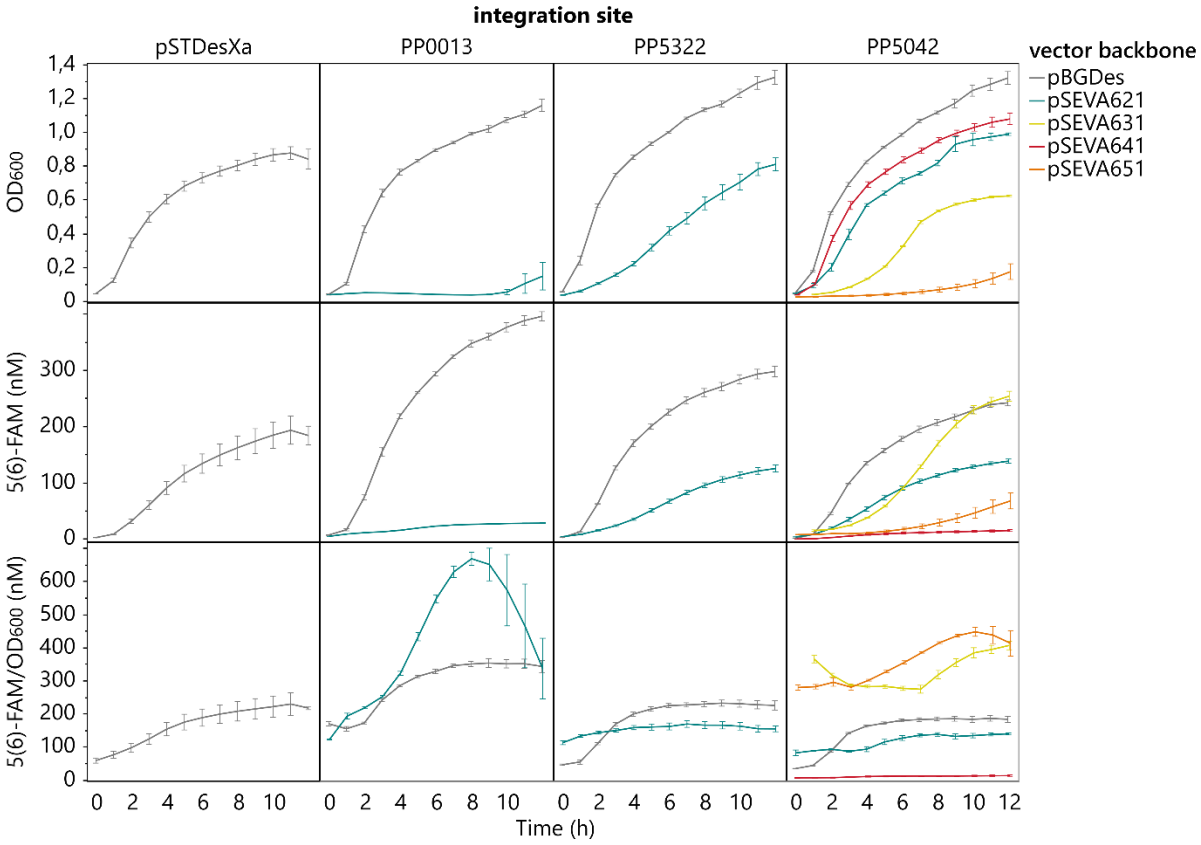


Figure S10: The copy number of the reporter construct has a large impact on cell growth and fluorescence intensity. Fluorescence intensity assay to analyze the effect of plasmid copy number of $P_{phi15,MCP}$-msfGFP on cell growth and fluorescence intensity in P. putida. All P. putida *strains were induced with 0.3 mM 3mBz at OD_{600} 0.1, after which the fluorescence intensity and cell growth is monitored every half hour for 12 h. Datapoints represent the mean OD_{600} values (top), mean 5(6)-FAM values (middle) and mean 5(6)-FAM/OD_{600} values (bottom) of four biological replicates. Error bars represent the standard error.*

2. Upstream terminator

Nine strong, validated terminators of phages LUZ7 and LUZ100 were screened as potential upstream insulators of the expression construct (3). All terminators were individually placed directly upstream of the phi15 promoter driving msfGFP expression. Upon integration of the reporter constructs in the host's Tn7attB site, the basal fluorescence level of the resulting strains was measured in absence of the phi15 RNAP, to assess potential read-through of neighboring sequences. As a negative control, a reporter construct without upstream terminator and the *P. putida* KT2440 wildtype strain were included in the assay. The constructs with terminators LUZ7 T7 and LUZ7 T60 showed a msfGFP output that was significantly higher than the wildtype strain (Tukey HSD, $P<0.05$) (Figure S11a). It is unclear whether this increased

msfGFP level is caused by the terminator sequence itself or another factor, but nevertheless these two terminators will not be considered as upstream insulators.

Apart from the basal fluorescence level, we confirmed that the tested terminators did not impact the *msfgfp* expression levels upon induction of the phi15 RNAP (Figure S11b). After introduction of *phi15rnap* on pSTDesX, the msfGFP levels of all strains were monitored in induced and uninduced conditions. None of the terminators showed a significant impact on msfGFP output in comparison to the terminatorless control, except for LUZ100 T6 (Tukey HSD, $P<0,05$). Based on these results, terminator LUZ7 T50 is selected as an upstream insulator, as it does not influence the performance of the expression system and has been characterized as strong, bidirectional terminator (3).

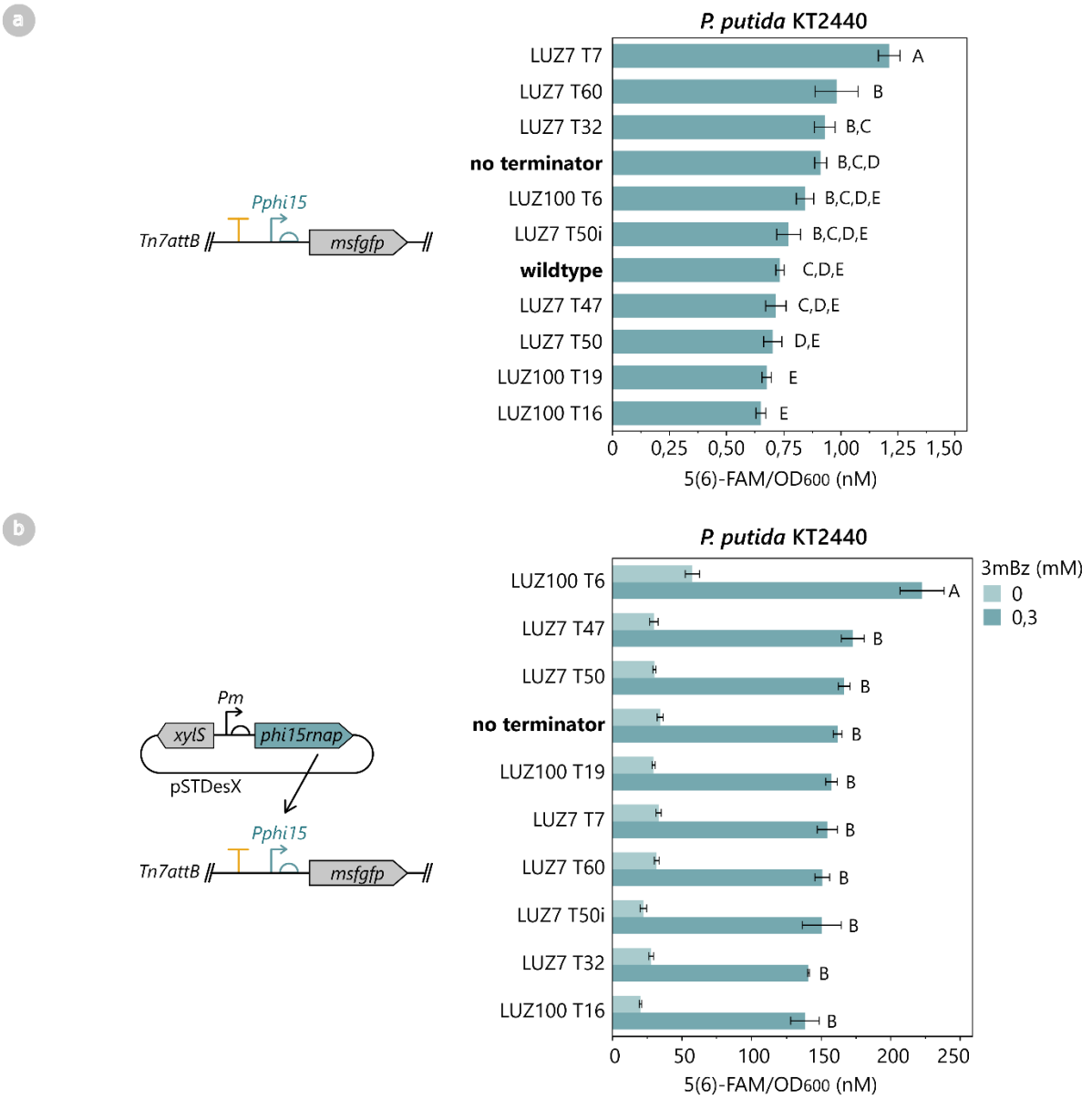


Figure S11: a) *Fluorescence intensity assay to assess the basal expression level of reporter constructs with an upstream terminator in* P. putida. P. putida *KT2440 strains with a* P_{phi15}-msfgfp *reporter construct with upstream terminator LUZ7 T7, LUZ7 T32, LUZ7 T47, LUZ7 T50, LUZ7 T50i, LUZ7 T60, LUZ100 T6, LUZ100 T16 or LUZ100 T19 were monitored for fluorescence intensity and cell growth for 12h. Bars and error bars represent the mean msfGFP/*OD_{600} *values and standard error of four biological replicates after 12h. Samples not connected by the same letter are significantly different (Tukey HSD,* α*=0.05) b) Fluorescence intensity assay to assess the potential influence of an upstream terminator on expression of the reporter construct in* P. putida. P. putida *KT2440 strains with pSTDesX.phi15rnap and a* P_{phi15}-msfgfp *reporter construct with upstream terminator LUZ7 T7, LUZ7 T32, LUZ7 T47, LUZ7 T50, LUZ7 T50i, LUZ7 T60, LUZ100 T6, LUZ100 T16 or LUZ100 T19 were induced with 0.3 mM 3mBz at* OD_{600} *0.1, after which the fluorescence intensity and cell growth is measured for 12h of induction. Bars and error bars represent the mean msfGFP/*OD_{600} *values and standard error of four biological replicates after 12h. Samples not connected by the same letter are significantly different (Tukey HSD,* α*=0.05).*

3. Downstream terminator

Efficient transcription termination is known to play a crucial role in circuit performance (7, 8). Therefore, eleven phage terminators were screened for efficient transcription termination of the phi15 RNAP transcript using the SEVAtile terminator trap (Figure S12) (3, 9). In this trap, a terminator is placed in between msfGFP and mCherry reporters, such that low mCherry levels are indicative for transcriptional termination, while the msfGFP output can be related to increased or decreased mRNA stability by the terminator sequence. The terminator efficiency of all tested terminators was significantly higher than the terminatorless control construct (Tukey HSD, $P<0.05$) (Figure S12). Moreover, terminators phi15 T1 and phi15 T5 outperformed all other terminators with an efficiency of 96.1% and 94.5%, respectively. Interestingly, not only was the normalized mCherry level of the phi15 T1 sample 15-fold lower than the control, the msfGFP levels were also twice as high compared to the control (Figure S12).

To increase the termination efficiency even more, terminators phi15 T1 and phi15 T5 were placed in tandem in both possible orders. For the phi15 T5-T1 construct, a terminator efficiency of 99.5% was observed, which was 2% higher than the phi15 T1-T5 combination. As such, the terminator pair phi15 T5-T1 is selected for placement downstream of the reporter construct, to efficiently terminate the phi15 RNAP transcript.

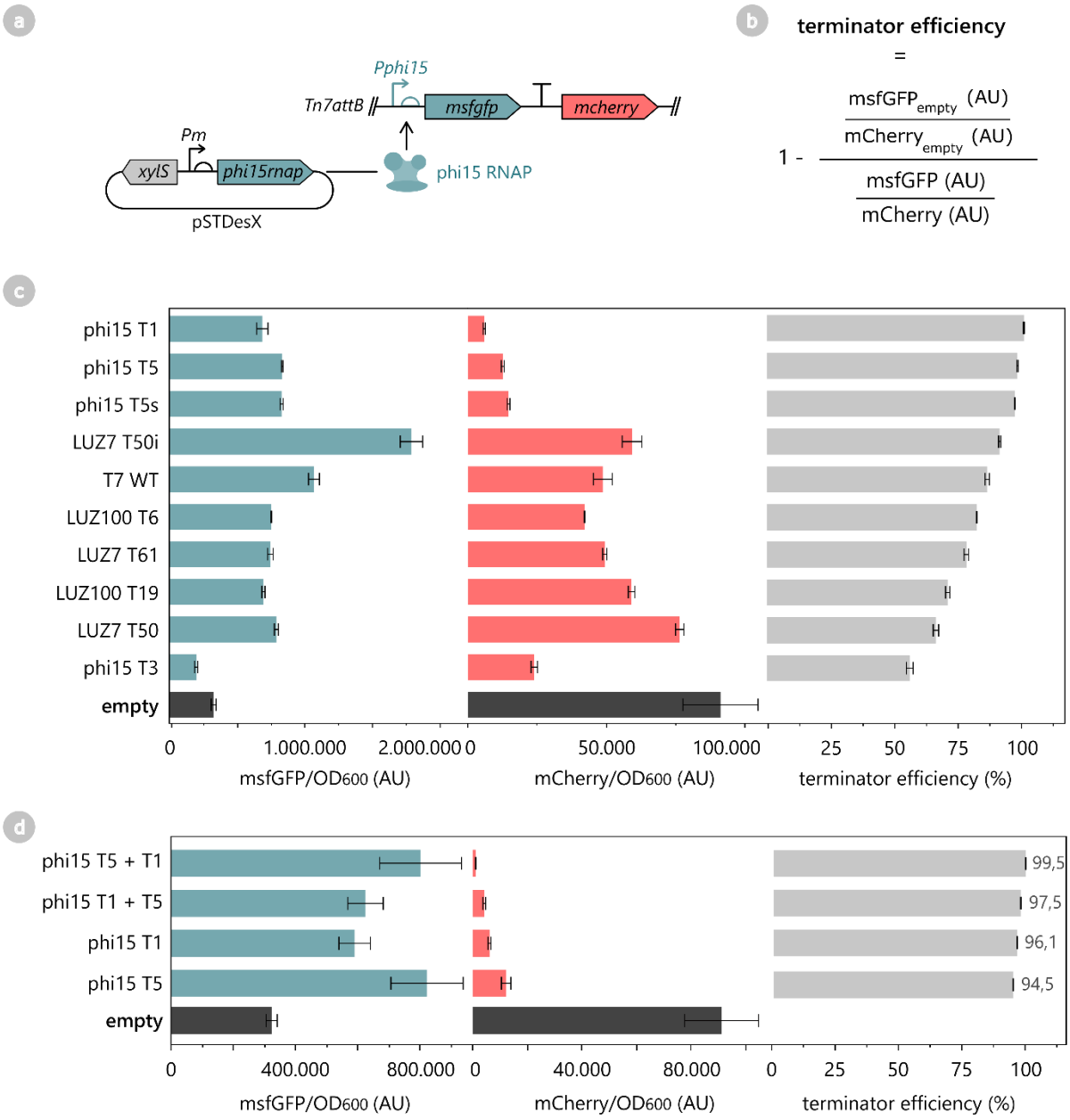


Figure S12: a) *General lay-out of the terminator trap to determine the terminator efficiency of phage terminators for the phi15 RNAP.* b) *Calculation of the terminator efficiency. Empty refers to a terminator trap without terminator.* c) *Fluorescence intensity assay to assess the terminator efficiency of phage terminators for phi15 RNAP in* P. putida. P. putida *KT2440 strains with pSTDesXa·phi15rnap and a* P_{phi15}-msfgfp-terminator-BCD1-mcherry *reporter construct with terminator phi15 T1, phi15 T3, phi15 T5, phi15 T5s, LUZ7 T50, LUZ7 T50i, LUZ7 T61, LUZ100 T6, LUZ100 T19 or T7 T7 were induced with 0.3 mM 3mBz at* OD_{600} *0.1, after which the fluorescence intensity and cell growth is measured after 16h of induction. Bars represent the mean msfGFP/*OD_{600} *values (left), mean mCherry/*OD_{600} *values (middle) and mean terminator efficiency values (right) of four biological replicates. Error bars represent the standard error. d) Assay identical to c, with terminator phi15 T1, phi15 T5 or terminator pair phi15 T1 + T5 or phi15 T5 + T1.*

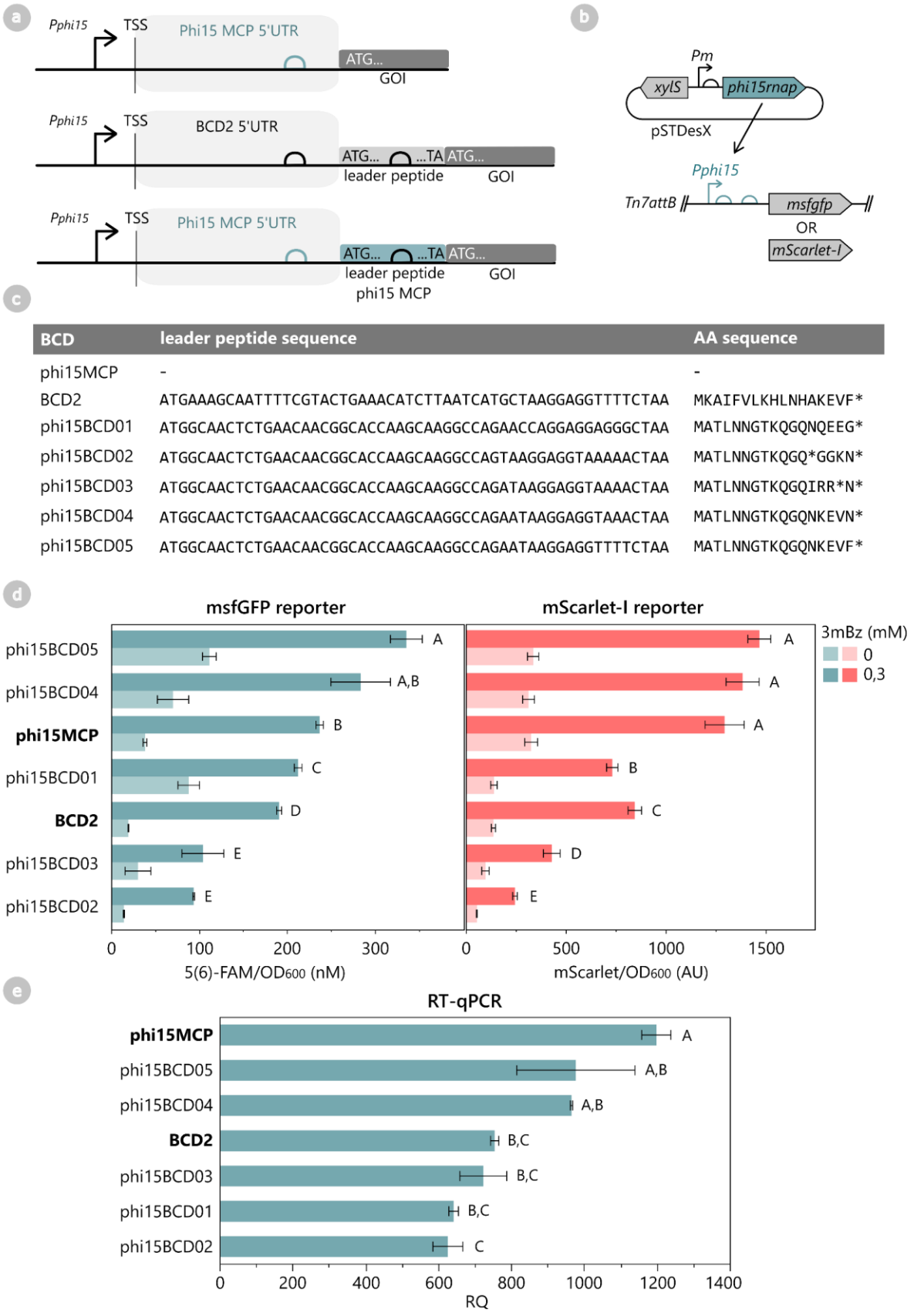
4. BCD design

We attempted to combine the translational strength of the phage MCP 5'UTR with the standardized performance of BCD2 by creating novel BCDs based on the phages' MCP 5'UTR sequence. More specifically, we created five different BCDs for the phi15 system by ligating the phi15 promoter, phi15 MCP 5'UTR and the first 17 codons of the phi15 MCP gene, in which we introduced the second RBS and linker to the start codon in five different ways (Figure S11a-c). In phi15BCD01, the MCP sequence was maximally maintained, while in phi15BCD02, phi15BCD03 and phi15BCD04 three RBS-startcodon spacers with different lengths were introduced that are reported to yield high expression levels in *P. putida* (4). Lastly, in phi15BCD05 we introduced the BCD2 spacer in the phi15 MCP. All five phi15BCDs were ligated to an msfGFP of mScarlet-I reporter and introduced together with the phi15 RNAP in *P. putida* (Figure S11d) and their performance was compared to the original phi15 MCP 5'UTR and the phi15 GC-GCAG-BCD2 UTR.

As displayed in Figure S11d, the five phi15-based BCDs generate broadly varying msfGFP expression levels, ranging from 93,4 nM 5(6)-FAM/OD_{600} (phi15BCD02) to 335 nM 5(6)-FAM/OD_{600} (phi15BCD05). Interestingly, phi15BCD05, phi15BCD04 and phi15BCD01 show the highest expression levels in the same range as the original phi15 MCP 5'UTR and all have a 7 nt spacer. This is in contrast to phi15BCD02 and phi15BCD03, which display much lower msfGFP expression levels and have a 9 nt and 8 nt spacer, respectively. This result was unexpected, as Aparicio *et al.* reported higher expression levels for the 9 nt spacer than the shorter 8 nt spacer (4). However, this result could be explained by the early stop codons in the leader peptides of phi15BCD02 and phi15BCD03 (Figure S11c), which often lead to lower expression levels in BCDs (5). The most important observation in this assay is that the msfGFP output of phi15BCD05 is significantly higher than the original phi15 MCP 5'UTR, showing a 41% increase (Tukey HSD, $P<0,05$). Therefore, this phi15-based BCD can be used as a highly potent 5'UTR in the *Pseudomonas*-optimized pET system.

To verify that the previous results are independent of the sequence of the gene-of-interest, two additional assays were performed. First, a similar fluorescence assay was performed in which the msfGFP reporter was replaced with mScarlet-I, which have 43,9% overall sequence similarity on the DNA level (EMBOSS Needle (6)). As shown in Figure S11d, the expression levels of the phi15-based BCDs show the same order for both the msfGFP and mScarlet-I

reporters, supporting the idea that BCD designs perform more independently from the gene's sequence than traditional monocistronic designs. Furthermore, phi15BCD05 shows a 13% increase of mScarlet-I expression levels, although this difference is not significant (Tukey HSD, P>0,05). Second, a RT-qPCR experiment was performed on the strains with the msfGFP reporter constructs. It is expected that the different BCDs do not impact transcription of the reporter constructs, but solely influence the translation efficiency. The RT-qPCR experiment revealed that only the phi15 MCP and BCD02 samples had a relative mRNA quantity that was significantly different from other samples (Tukey HSD, P<0,05), supporting our hypothesis. Based on these results, we carefully conclude that phi15BCD05 is a reliable 5'UTR for use in genetic circuitry.



BCD	leader peptide sequence	AA sequence
phi15MCP	-	-
BCD2	ATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCTAAGGAGGTTTTCTAA	MKAIFVLKHLNHAKEVF*
phi15BCD01	ATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGAACCAGGAGGAGGGCTAA	MATLNNGTKQGQNQEEG*
phi15BCD02	ATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGTAAGGAGGTAAAAACTAA	MATLNNGTKQGQ*GGKN*
phi15BCD03	ATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGATAAGGAGGTAAAACTAA	MATLNNGTKQGQIRR*N*
phi15BCD04	ATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGAATAAGGAGGTAAACTAA	MATLNNGTKQGQNKEVN*
phi15BCD05	ATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGAATAAGGAGGTTTTCTAA	MATLNNGTKQGQNKEVF*

Figure S13: a) Conceptual comparison of phi15 MCP 5'UTR (top), BCD2 (middle) and phi15-based BCDs (bottom). b) Experimental set-up to assess the performance of phi15-based BCDs. c) Leader peptide sequence and amino acid (AA) sequence of the different phi15-based BCDs. 3) Fluorescence assay to assess the translational strength of different phi15-based BCDs. For the msfGFP reporter (left), P. putida strains carrying pSTDesXa·phi15RNAP and a reporter construct with

$P_{phi15,MCP}$ or $P_{phi15BCDXX}$ were induced with 0.3 mM 3mBz at OD_{600} 0.3, after which the fluorescence intensity and cell growth is monitored every half hour for 12h. A similar assay was performed for the mScarlet-I reporter (right).Bars represent the mean 5(6)-FAM/OD_{600} value of four biological replicates at 12h post induction. Error bars represent the standard error. Samples not connected by same letters are significantly different (Tukey HSD, α=0.05). e) RT-qPCR experiment to assess the relative quantity (RQ) of transcripts of the msfGFP reporter construct in P. putida *upon induction with 0.3 mM 3mBz.*

Supplementary Tables

Table S1: Flow cytometry data of different P. putida *strains induced with 0; 0.1 or 1 mM 3mBz (mM) and 10 mM Rha for strains P15-GD and P15-LGD. The median msfGFP-FITC-A level of the entire population is listed from 0-6 h post-induction (P.I.).*

Strain	**3mBz (mM)**	**Median msfGFP-FITC-A of entire population**						
		0h P.I.	**1h P.I.**	**2h P.I.**	**3h P.I.**	**4h P.I.**	**5h P.I.**	**6h P.I.**
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.00	217.10	198.50	224.00	220.10	234.40	254.40	287.30
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.00	164.60	215.90	243.00	233.30	218.40	218.30	241.10
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.01	194.20	231.30	247.80	235.90	222.00	211.30	234.10
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.01	185.60	218.60	226.10	226.70	222.90	224.10	247.40
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	1.00	183.70	211.60	222.50	222.10	242.30	253.10	269.80
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	1.00	202.20	186.20	215.10	230.50	250.80	252.40	283.00
P. putida P15 + pPUT·*msfgfp*	0.00	1383.60	2026.40	3376.20	4480.10	6132.30	5984.20	5310.50
P. putida P15 + pPUT·*msfgfp*	0.00	1307.50	1936.40	3412.10	3514.80	5338.50	6448.80	5965.00
P. putida P15 + pPUT·*msfgfp*	0.01	1290.00	30192.10	69408.40	119139.30	156850.20	169501.90	172919.40
P. putida P15 + pPUT·*msfgfp*	0.01	1464.10	3881.70	79109.80	110931.70	145736.60	141588.80	156112.70
P. putida P15 + pPUT·*msfgfp*	1.00	1490.90	33874.40	158638.50	195517.20	255012.50	292400.40	305185.50
P. putida P15 + pPUT·*msfgfp*	1.00	1549.90	16404.40	146987.50	207768.50	258315.70	297034.90	299263.70
P. putida P15-GD + pPUT·*msfgfp*	0.00	1851.20	3127.30	5003.70	6197.80	10849.80	14086.80	9863.20
P. putida P15-GD + pPUT·*msfgfp*	0.00	2518.60	2734.20	5169.80	6754.70	9517.60	10196.00	13254.30
P. putida P15-GD + pPUT·*msfgfp*	0.01	3196.60	2080.50	132856.70	184598.30	246982.50	275918.80	274485.80
P. putida P15-GD + pPUT·*msfgfp*	0.01	1506.50	38338.80	149408.10	188256.00	233088.80	220201.30	291358.40
P. putida P15-GD + pPUT·*msfgfp*	1.00	3546.40	44423.40	179854.70	194682.70	278458.10	306904.50	294407.80
P. putida P15-GD + pPUT·*msfgfp*	1.00	3170.20	55329.00	173186.20	231037.50	251627.40	311121.10	310506.90
P. putida P15-L + pPUT·*msfgfp*	0.00	175.10	217.10	272.60	448.20	384.70	288.50	334.30
P. putida P15-L + pPUT·*msfgfp*	0.00	180.40	231.60	336.50	353.50	371.00	405.90	353.50
P. putida P15-L + pPUT·*msfgfp*	0.01	205.40	706.50	17267.30	32162.60	30313.80	23836.30	20419.00
P. putida P15-L + pPUT·*msfgfp*	0.01	180.10	3407.80	19963.90	24322.50	24386.50	21375.40	15672.50

P. putida P15-L + pPUT·*msfgfp*	1.00	203.90	8658.50	35622.80	103339.00	119739.30	99912.00	100503.80
P. putida P15-L + pPUT·*msfgfp*	1.00	177.10	4501.40	33063.80	117631.00	109829.30	99707.90	75172.70
P. putida P15-LGD + pPUT·*msfgfp*	0.00	630.80	597.50	765.50	938.50	1073.60	1316.90	1196.30
P. putida P15-LGD + pPUT·*msfgfp*	0.00	670.20	703.30	771.30	1084.00	1008.10	1244.80	1158.70
P. putida P15-LGD + pPUT·*msfgfp*	0.01	589.10	1676.10	21039.30	35608.10	33051.90	46947.20	50246.90
P. putida P15-LGD + pPUT·*msfgfp*	0.01	712.80	2694.60	32942.10	62657.90	77885.30	104977.70	108923.30
P. putida P15-LGD + pPUT·*msfgfp*	1.00	582.70	11447.60	66642.40	101087.40	135144.80	161681.70	165156.90
P. putida P15-LGD + pPUT·*msfgfp*	1.00	694.30	5848.70	52457.40	87954.80	121944.10	132496.70	181203.50

Table S2: Flow cytometry data of different P. putida strains induced with 0; 0.1 or 1 mM 3mBz (mM) and 10 mM Rha for strains P15-GD and P15-LGD. The percentage of the entire population that is induced (events with a msfGFP-FITC-A value above 10^4) are listed for 0-6h post-induction (P.I.).

Strain	**3mBz (mM)**	**Induced population (msfGFP-FITC-A > 10^4)**						
		0h P.I.	**1h P.I.**	**2h P.I.**	**3h P.I.**	**4h P.I.**	**5h P.I.**	**6h P.I.**
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.00	0.24%	0.08%	0.08%	0.10%	0.24%	0.36%	0.24%
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.00	0.20%	0.05%	0.08%	0.10%	0.10%	0.24%	0.20%
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.01	0.38%	0.25%	0.24%	0.17%	0.26%	0.12%	0.28%
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	0.01	0.00%	0.05%	0.18%	0.27%	0.16%	0.08%	0.18%
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	1.00	0.88%	0.04%	0.10%	0.06%	0.10%	0.18%	0.30%
P. putida SEM11 + pBG-P_{phi15}·*msfgfp*	1.00	0.00%	0.06%	0.08%	0.12%	0.08%	0.16%	0.10%
P. putida P15 + pPUT·*msfgfp*	0.00	4.19%	5.94%	16.16%	21.80%	31.22%	27.88%	23.96%
P. putida P15 + pPUT·*msfgfp*	0.00	3.24%	1.76%	14.66%	13.76%	25.90%	32.82%	29.08%
P. putida P15 + pPUT·*msfgfp*	0.01	3.32%	76.68%	80.86%	94.88%	94.04%	93.56%	89.14%
P. putida P15 + pPUT·*msfgfp*	0.01	2.25%	36.43%	91.24%	90.61%	94.42%	93.00%	92.94%
P. putida P15 + pPUT·*msfgfp*	1.00	2.44%	86.20%	94.54%	88.79%	93.28%	90.82%	89.96%
P. putida P15 + pPUT·*msfgfp*	1.00	3.10%	59.17%	90.98%	89.51%	92.08%	91.00%	85.98%
P. putida P15-GD + pPUT·*msfgfp*	0.00	14.07%	21.29%	23.30%	32.52%	54.24%	64.58%	51.02%
P. putida P15-GD + pPUT·*msfgfp*	0.00	13.93%	10.47%	23.42%	35.10%	49.56%	51.56%	60.44%
P. putida P15-GD + pPUT·*msfgfp*	0.01	17.07%	37.53%	94.06%	93.00%	94.26%	95.17%	88.42%

P. putida P15-GD + pPUT·*msfgfp*	0.01	7.31%	77.71%	95.04%	92.36%	88.01%	82.26%	94.50%
P. putida P15-GD + pPUT·*msfgfp*	1.00	12.67%	74.93%	93.26%	82.17%	91.86%	92.96%	87.14%
P. putida P15-GD + pPUT·*msfgfp*	1.00	15.06%	95.44%	94.36%	87.02%	84.02%	92.92%	92.38%
P. putida P15-L + pPUT·*msfgfp*	0.00	0.42%	0.08%	0.06%	0.16%	0.34%	0.38%	0.60%
P. putida P15-L + pPUT·*msfgfp*	0.00	0.63%	0.24%	0.36%	0.66%	0.52%	0.32%	0.68%
P. putida P15-L + pPUT·*msfgfp*	0.01	0.00%	8.27%	69.28%	86.06%	82.30%	77.14%	73.70%
P. putida P15-L + pPUT·*msfgfp*	0.01	0.00%	15.18%	72.00%	77.00%	81.60%	76.10%	65.54%
P. putida P15-L + pPUT·*msfgfp*	1.00	0.70%	43.82%	73.06%	91.28%	93.72%	91.72%	89.66%
P. putida P15-L + pPUT·*msfgfp*	1.00	0.26%	27.42%	70.92%	93.52%	93.36%	91.70%	87.44%
P. putida P15-LGD + pPUT·*msfgfp*	0.00	1.40%	0.52%	3.70%	2.98%	3.96%	4.94%	3.90%
P. putida P15-LGD + pPUT·*msfgfp*	0.00	0.26%	0.96%	3.14%	3.46%	2.42%	5.42%	3.42%
P. putida P15-LGD + pPUT·*msfgfp*	0.01	0.08%	16.06%	70.24%	80.16%	72.04%	80.28%	80.46%
P. putida P15-LGD + pPUT·*msfgfp*	0.01	1.08%	22.28%	76.52%	87.28%	86.32%	88.06%	86.08%
P. putida P15-LGD + pPUT·*msfgfp*	1.00	0.10%	55.54%	92.20%	92.02%	91.74%	92.28%	91.16%
P. putida P15-LGD + pPUT·*msfgfp*	1.00	0.48%	33.02%	89.40%	90.10%	90.66%	89.90%	92.58%

Table S3: Strains used and created in this work

Name	Description	Reference
E. coli TOP10	Intermediate host for vector cloning (Invitrogen™); F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ *lac*X74 *rec*A1 *ara*D139 Δ(*araleu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	-
P. putida KT2440	Derivative of *P. putida* mt-2 lacking the TOL plasmid	(10)
P. putida FRS1::T7RNAP	Derivative of *P. putida* KT2440 with the fluoride responsive FRS1 5'UTR and *t7 rnap* gene integrated in the *attTn7* site	(11)
P. putida 8::T7RNAP	Derivative of *P. putida* KT2440 with XylS/*Pm:: t7rnap* gene integrated in the *attTn7* site; created with pTn7-8T7RNAP	This work
P. putida SEM11	Genome-reduced derivative of *P. putida* KT2440 with deletion of prophages, β-lactamases, *benABCD* and *pvdD*	(12)
P. putida P15	Derivative of *P. putida* SEM11 with a XylS/*Pm::phi15rnap(RBS-D)* integration in the PP0013 locus	This work
P. putida P15-L	Derivative of *P. putida* SEM11 with a XylS/*Pm::phi15rnap(RBS-D)* integration in the PP0013 locus and a p14c-BCD22-phi15lys(G3RQ) integration in the PP5388 locus	This work
P. putida P15-GD	Derivative of *P. putida* SEM11 with a XylS/*Pm::phi15rnap(RBS-D)* integration in the PP0013 locus and vector pSTDesR·*phi15gp16*	This work
P. putida P15-LGD	Derivative of *P. putida* SEM11 with a XylS/*Pm::phi15rnap(RBS-D)* integration in the PP0013 locus, a p14c-BCD22-phi15lys(G3RQ) integration in the PP5388 locus and vector pSTDesR·*phi15gp16*	This work

Table S4: All vectors used and created in this work

Name	Relevant features	Reference
pSTDesXa	Destination vector; *oriT*; *oriV(pRO1600/ColE1)*; *xylS/Pm* → PT3-*SacB*-PT4; Km^R	(9)
pSTDesXa·phi15RNAP	pSTDesXa in which the *SacB* cassette is substituted with PT3-*phi15rnap*-PT4	(13)
pPS36	ProUSER vector; *oriT*; *oriV(pRO1600/ColE1)*; *xylS/Pm* → USER cassette; Sp^R/Sm^R	(14)
pPS37	ProUSER vector; *oriT*; *oriV(pRO1600/ColE1)*; *araC/ParaBAD*→ USER cassette; Sp^R/Sm^R	(14)
pPS39	ProUSER vector; *oriT*; *oriV(pRO1600/ColE1)*; *rhaRS/PrhaBAD*→ USER cassette; Sp^R/Sm^R	(14)
pPS44	ProUSER vector; *oriT*; *oriV(pRO1600/ColE1)*; *lacI/PlacUV5*→ USER cassette; Sp^R/Sm^R	(14)
pPS36·*msfGFP*	pPS36 derivative with *msfGFP* insertion	This work
pPS37·*msfGFP*	pPS37 derivative with *msfGFP* insertion	This work
pPS39·*msfGFP*	pPS39 derivative with *msfGFP* insertion	This work
pPS44·*msfGFP*	pPS44 derivative with *msfGFP* insertion	This work
pPS-F^--riboswitch·*msfGFP*	pPS36 backbone with F^--riboswitch-*msfGFP* insertion	This work
pTn7frsv1_T7RNAP	*oriT*; *oriV(R6K)*; *Tn7L* and *Tn7R* extremes; Km^R/Gm^R; F^--riboswitch-*t7rnap*	(11)
pSNW2	Integration vector; *oriT*; *oriV(R6K)*; P_{14g}-*BCD2-msfGFP* selection marker; MCS, Km^R	(15)
pSNW2·PP0013	pSNW2 with PP0013(HA1)-XylS/*Pm::phi15rnap*-PP0013(HA2)	This work
pSNW2·PP0013-C	pSNW2 with PP0013(HA1)-XylS/*Pm::phi15rnap(RBS-C)*-PP0013(HA2)	This work
pSNW2·PP0013-D	pSNW2 with PP0013(HA1)-XylS/*Pm::phi15rnap(RBS-D)*-PP0013(HA2)	This work
pSNW2·PP5322	pSNW2 with PP5322(HA1)-XylS/*Pm::phi15rnap*-PP5322(HA2)	This work
pSNW2·PP5042	pSNW2 with PP5042(HA1)-XylS/*Pm::phi15rnap*-PP5042(HA2)	This work
pSEVA6231s	Constitutive expression of I-SceI for genome engineering in Pseudomonas; *oriT*; *oriV(RK2)*; Gm^R	(16)
pQURE6	Inducible expression of I-SceI with 3-mBz for genome engineering in Pseudomonas; *riT*; *oriV(RK2,inducible)*; Gm^R	(15)

pSNW2·PP4305	pSNW2 with PP4305(HA1)-*P14c-RBS22::phi15lysG3RQ*-PP4305(HA2)	This work
pSNW2·PP5388	pSNW2 with PP5388(HA1)-*P14c-RBS22::phi15lysG3RQ*-PP5388(HA2)	This work
pBG	Destination vector; *oriT*; *oriV(R6K)*; *Tn7L* and *Tn7R* extremes; Km^R/Gm^R	(17)
pBG42	pBG derivative with P14g-BCD2-msfGFP	(17)
pBGDes·*BCD2-msfgfp*	pBG derivative with *BCD2-msfgfp* insertion	(9)
pBGDes·$P_{phi15,MCP}$-*msfgfp*	pBG derivative with $P_{phi15,MCP}$-*msfgfp* insertion	(13)
pBGDes·*LUZ100T6-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ100T6-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ100T16-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ100T16-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ100T19-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ100T19-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ7T7-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ7T7-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ7T32-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ7T32-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ7T47-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ7T47-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ7T50-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ7T50-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ7T50i-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ7T50i-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·*LUZ7T60-*$P_{phi15,MCP}$-*msfgfp*	pBG derivative with *LUZ7T60-*$P_{phi15,MCP}$-*msfgfp* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-phi15T1-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-phi15T1-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-phi15T3-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-phi15T3-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-phi15T5-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-phi15T5-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-phi15T5s-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-phi15T5s-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-phi15T1T5-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-phi15T1T5-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-phi15T5T1-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-phi15T5T1-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-LUZ100T6-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-LUZ100T6-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-LUZ100T19-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-LUZ100T19-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-LUZ7T7-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-LUZ7T7-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-LUZ7T50-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-LUZ7T50-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-LUZ7T50i-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-LUZ7T50i-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-LUZ7T61-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-LUZ7T61-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15,MCP}$-*msfgfp-T7T7wt-BCD1-mcherry*	pBG derivative with $P_{phi15,MCP}$-*msfgfp-T7T7wt-BCD1-mcherry* insertion	This work
pBGDes·$P_{phi15BCD01}$-*msfgfp*	pBG derivative with $P_{phi15BCD01}$-*msfgfp* insertion	This work
pBGDes·$P_{phi15BCD02}$-*msfgfp*	pBG derivative with $P_{phi15BCD02}$-*msfgfp* insertion	This work
pBGDes·$P_{phi15BCD03}$-*msfgfp*	pBG derivative with $P_{phi15BCD03}$-*msfgfp* insertion	This work
pBGDes·$P_{phi15BCD04}$-*msfgfp*	pBG derivative with $P_{phi15BCD04}$-*msfgfp* insertion	This work
pBGDes·$P_{phi15BCD05}$-*msfgfp*	pBG derivative with $P_{phi15BCD05}$-*msfgfp* insertion	This work
pBGDes·P_{phi15}-*BCD2-msfgfp*	pBG derivative with P_{phi15}-*BCD2-msfgfp* insertion	This work
pBGDes·*BCD2-msfgfp*	pBG derivative with *BCD2-msfgfp* insertion	This work
pBGDes·$P_{phi15BCD01}$-*mscarlet-I*	pBG derivative with $P_{phi15BCD01}$-*mscarlet-I* insertion	This work
pBGDes·$P_{phi15BCD02}$-*mscarlet-I*	pBG derivative with $P_{phi15BCD02}$-*mscarlet-I* insertion	This work
pBGDes·$P_{phi15BCD03}$-*mscarlet-I*	pBG derivative with $P_{phi15BCD03}$-*mscarlet-I* insertion	This work
pBGDes·$P_{phi15BCD04}$-*mscarlet-I*	pBG derivative with $P_{phi15BCD04}$-*mscarlet-I* insertion	This work
pBGDes·$P_{phi15BCD05}$-*mscarlet-I*	pBG derivative with $P_{phi15BCD05}$-*mscarlet-I* insertion	This work

pBGDes·P_{phi15}-*BCD2-mscarlet-I*	pBG derivative with P_{phi15}-*BCD2-mscarlet-I* insertion	This work
pSEVA621	SEVA vector; *oriT*; *oriV(RK2)*; MCS; Gm^R	(18)
pSEVA631	SEVA vector; *oriT*; *oriV(pBBR1)*; MCS; Gm^R	(18)
pSEVA641	SEVA vector; *oriT*; *oriV(pRO1600/ColE1)*; MCS; Gm^R	(18)
pSEVA651	SEVA vector; *oriT*; *oriV(RFS1010)*; MCS; Gm^R	(18)
pSEVA62·$P_{phi15,MCP}$-*msfgfp*	pSEVA621 in which the MCS is substituted for $P_{phi15,MCP}$-*msfgfp*	This work
pSEVA63·$P_{phi15,MCP}$-*msfgfp*	pSEVA621 in which the MCS is substituted for $P_{phi15,MCP}$-*msfgfp*	This work
pSEVA64·$P_{phi15,MCP}$-*msfgfp*	pSEVA621 in which the MCS is substituted for $P_{phi15,MCP}$-*msfgfp*	This work
pSEVA65·$P_{phi15,MCP}$-*msfgfp*	pSEVA621 in which the MCS is substituted for $P_{phi15,MCP}$-*msfgfp*	This work
pPUT-ST	pBG derivative with *LUZ7T50-*$P_{phi15BCD05}$*-AATG-*P_{14g}*-BCD2-msfGFP-TAAC-phi15T5T1* insertion; *AATG-*P_{14g}*-BCD2-msfGFP-TAAC* is flanked with BsaI recognition sites and can be substituted for a desired insert	This work
pPUT-GS	pBG derivative with *LUZ7T50-*$P_{phi15BCD05}$*-AATG-*P_{14g}*-BCD2-msfGFP-GCTT-phi15T5T1* insertion; *AATG-*P_{14g}*-BCD2-msfGFP-GCTT* is flanked with BsaI recognition sites and can be substituted for a desired insert	This work
pPUT-GSN	pBG derivative with *LUZ7T50-*$P_{phi15BCD05}$*-CCAT-*P_{14g}*-BCD2-msfGFP-GCTT-phi15T5T1* insertion; *CCAT-*P_{14g}*-BCD2-msfGFP-GCTT* is flanked with BsaI recognition sites and can be substituted for a desired insert	This work
pPUT-GS2C	pBG derivative with *LUZ7T50-*$P_{phi15BCD05}$*-AATG-*P_{14g}*-BCD2-msfGFP-GGTA-phi15T5T1* insertion; *AATG-*P_{14g}*-BCD2-msfGFP-GGTA* is flanked with BsaI recognition sites and can be substituted for a desired insert	This work
pPUT-GS2N2C	pBG derivative with *LUZ7T50-*$P_{phi15BCD05}$*-CCAT-*P_{14g}*-BCD2-msfGFP-GGTA-phi15T5T1* insertion; *CCAT-*P_{14g}*-BCD2-msfGFP-GGTA* is flanked with BsaI recognition sites and can be substituted for a desired insert	This work
pPUT-ST·*msfgfp*	pPUT-ST derivative with *msfgfp* insertion	This work
pPUT-ST·fla^{PtaU1}	pPUT-ST derivative with fla^{PtU1} insertion	This work
pTNS2	Helper plasmid; *oriV(R6K)*; *tnsABCD*; Amp^R	(19)
pSTDesR	Destination vector; *oriT*; *oriV(RK2)*; *RhaRS/*p_{RhaBAD}→MCS*;* Sm^R/Sp^R	(9)
pSTDesR·*phi15lys*(*G3RQ*)	pSTDesR in which the MCS is substituted with *phi15lysozyme(G3RQ)*	(13)
pSTDesR·*phi15gp16*	pSTDesR in which the MCS is substituted with *phi15gp16*	This work
pSTDesR·*rac*	pSTDesR in which the MCS is substituted with *rac*	This work
pSTDesR·*igy*	pSTDesR in which the MCS is substituted with *igy*	This work
pSEVA421	*oriT*; *oriV(RK2)*; *MCS;* Sm^R/Sp^R	
pSEVA42·*phi15lys(G3RQ)(BCD2)*	pSEVA421 derivative in which the MCS is substituted with *p14c-BCD2-phi15lys(G3RQ)*	This work
pSEVA42·*phi15lys(G3RQ)(BCD13)*	pSEVA421 derivative in which the MCS is substituted with *p14c-BCD13-phi15lys(G3RQ)*	This work
pSEVA42·*phi15lys(G3RQ)(BCD22)*	pSEVA421 derivative in which the MCS is substituted with *p14c-BCD22-phi15lys(G3RQ)*	This work
pSEVA23·$pT7::fla^{PtaU1}$	pSEVA231 derivative in which the MCS is substituted with *pT7-*fla^{PtaU1}	(20)
pTn7-8T7RNAP	pTn7·xylS vector with *t7rnap* gene integrated in the MCS under *Pm* promoter control	This work

Table S5: DNA sequences of all genetic parts used in this work

Name	**Sequence**
msfGFP	ATGATCATGGGAATTCATAAAGGTGAAGAACTGTTCACCGGTGTTGTTCCGATCCTGGTTGAACTGGATGGTGATG TTAACGGCCACAAATTCTCTGTTCGTGGTGAAGGTGAAGGTGATGCAACCAACGGTAAACTGACCCTGAAATTCAT CTGCACTACCGGTAAACTGCCGGTTCCATGGCCGACTCTGGTGACTACCCTGACCTATGGTGTTCAGTGTTTTTCTC GTTACCCGGATCACATGAAGCAGCATGATTTCTTCAAATCTGCAATGCCGGAAGGTTATGTACAGGAGCGCACCAT TTCTTTCAAAGACGATGGCACCTACAAAACCCGTGCAGAGGTTAAATTTGAAGGTGATACTCTGGTGAACCGTATT GAACTGAAAGGCATTGATTTCAAAGAGGACGGCAACATCCTGGGCCACAAACTGGAATATAACTTCAACTCCCATA

	ACGTTTACATCACCGCAGACAAACAGAAGAACGGTATCAAAGCTAACTTCAAAATTCGCCATAACGTTGAAGACGG TAGCGTACAGCTGGCGGACCACTACCAGCAGAACACTCCGATCGGTGATGGTCCGGTTCTGCTGCCGGATAACCA CTACCTGTCCACCCAGTCTAAACTGTCCAAAGACCCGAACGAAAAGCGCGACCACATGGTGCTGCTGGAGTTCGTT ACTGCAGCAGGTATCACGCACGGCATGGATGAACTCTACAAATAA
mCherry	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGG CTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCA AGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAA GGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGC GTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAAGACGGCGAGTTCATCTAC AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGC CTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACG GCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACG TCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCC GCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAA
mScarlet-I	ATGGTGAGCAAGGGCGAGGCAGTGATCAAGGAGTTCATGCGGTTCAAGGTGCACATGGAGGGCTCCATGAACGG CCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTG ACCAAGGGTGGCCCCCTGCCCTTCTCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAGGGCCTTCATCAA GCACCCCGCCGACATCCCCGACTACTATAAGCAGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTC GAGGACGGCGGCGCCGTGACCGTGACCCAGGACACCTCCCTGGAGGACGGCACCCTGATCTACAAGGTGAAGCT CCGCGGCACCAACTTCCCTCCTGACGGCCCCGTAATGCAGAAGAAGACAATGGGCTGGGAAGCGTCCACCGAGCG GTTGTACCCCGAGGACGGCGTGCTGAAGGGCGACATTAAGATGGCCCTGCGCCTGAAGGACGGCGGCCGCTACC TGGCGGACTTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGATGCCCGGCGCCTACAACGTCGACCGCAAGT TGGACATCACCTCCCACAACGAGGACTACACCGTGGTGGAACAGTACGAACGCTCCGAGGGCCGCCACTCCACCG GCGGCATGGACGAGCTGTACAAG
6xHis-Fla^{PtaU1}	ATGCATCATCACCACCACCATTTGCAAAATACCAAAAATGAGTATAAGCCGCGCGATGGCGGGGGGCACCGCCCT ATCATCGGCTTCATGTCCGACCTCGGTACGGAGGATGATAGCGTGGGCATCTGCAAGGGTGTCATGTTGGGGCTG TGTCCTGACGCCGTGATCGTGGATATTAGCCACTCCATGACGCCCTGGGACATTGATCAAGGGTCGCGCTTGATTG TGGATCTCCCAAAGTTTTTCCCCAACTGGACGACGTTTGCGACCACGTCCTACCGCGAAACCGGCACCAGCGCACG TTCGGTCGCTATCAAGCTCCCAAGCGGGCATGTCTACGTGGCGCCCAATAACGGTTTGCTGACCCGTGTGATCGAG GATCATGGGTATGTGGAGGCATACGAAGTGACCACCGTCGGCGCCATCCCAGCTGAACCGGAGCCAACGTGGTTT AGCCGTGATATGGTGGCTTACCCTGCTGCgGCTATTGCAGCGGGCTTTCCGTTGGAGAATGTGGGCCGTCCCCTGA ATGATTCGGAGATTGTCCGCGCGGACCTGCCTCGGTATACGCAGGCTGAAGATGGTATCATCCAGGGTATCGTGA CCACCATTGATCGTCCTTTCGGTAACATTTGGACGAATATCCCTCGGCGTATTATCGAGAACGAACTGCGGATCGC ATACGGCACCAACATTCGCGTGGTCTTGGATAATTTGTTGGTGCTCGAAGTGCCTTTTATGCGGACGTTTGGCGAT GTCGGCCTGAAGAAACCGATGTGTTATATTAATAGCCGCGGCTACTTTTCCTTGGCCTACTATGGGGGCAATTTGG CCGACCCTTACAATATTCGTCGCGGTATGCCGGTCAAAATCGAATCCATCACGCGTTAG
$P_{phi15,\ MCP}$	TAAAAACCCACACAATAGACAGAGGGAGCCGTTCCGTGCTCCTTCTGGACCCAATTTCTGACTCAAGGAGAACTAC ATATG
$P_{phi15BCD01}$	TAAAAACCCACACAATAGACAGAGGGAGCCGTTCCGTGCTCCTTCTGGACCCAATTTCTGACTCAAGGAGAACTAC ATATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGAACCAGGAGGAGGGCTAATG
$P_{phi15BCD02}$	TAAAAACCCACACAATAGACAGAGGGAGCCGTTCCGTGCTCCTTCTGGACCCAATTTCTGACTCAAGGAGAACTAC ATATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGTAAGGAGGTAAAAACTAATG

$P_{phi15BCD03}$	TAAAAACCCACACAATAGACAGAGGGAGCCGTTCCGTGCTCCTTCTGGACCCAATTTCTGACTCAAGGAGAACTAC ATATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGATAAGGAGGTAAAACTAATG
$P_{phi15BCD04}$	TAAAAACCCACACAATAGACAGAGGGAGCCGTTCCGTGCTCCTTCTGGACCCAATTTCTGACTCAAGGAGAACTAC ATATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGAATAAGGAGGTAAACTAATG
$P_{phi15BCD05}$	TAAAAACCCACACAATAGACAGAGGGAGCCGTTCCGTGCTCCTTCTGGACCCAATTTCTGACTCAAGGAGAACTAC ATATGGCAACTCTGAACAACGGCACCAAGCAAGGCCAGAATAAGGAGGTTTTCTAATG
P14c	GTGAATTGACATGTCAATTTTTATGTTGTATAATATAACTA
PEM7	TTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACC
BCD1	GGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCACAGGAG ACTTTCTAATG
BCD2	GGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCTAAGGAG GTTTTCTAATG
BCD13	GGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCAATGGAG GCTTTCTAATG
BCD22	GGCCCAAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCCTAGGAA GTTTTCTAATG
RBS-C	CAACGCGGTCCGATGTG
RBS-D	CAACGGGGTCCGAGGTG
Phi15 RNAP	GTGATTGAAGTAGCAAAGAACGATTTCTCCGATGTCAAGACTGACTGGGCGTTTCGTGTACTGAGTGAGCTGTAC GGTGAAGAACTGGCAGCCGCTCAACTGGCTCTGGAACACGAGTCGCACGAGATCGGTGAGGCAAAGTTCAAGAA AGCCCTTGATCGCCAGATGAAACGTGGCGAGACCTCTGAGACCTCCGTGGCAAAGCCGCTGGTCGCAATGCTGGT CCCTAAGTTTGTCGAGAAGATGGACGCATGGGTAGAGCACCAGATGAAGAACGTGCGCCGCAAGTCCGTGGCCCT GAAGTTCATCCAGATGGTCGCCACCGAGCGCGTTGCGGTCATCACCATCAAGACCGTAATTAACGCGATGTCGCAA GGAGACGTAGTGCTTCAGGCAATCGCCGGTCGCATCGGTCGCGGCATCGAGGAGGAGGCTCGGTTCGGTCGCAT CCGTGACCAAGAGGCGAAGCACTTCAAGAAGTACATCCGTGAGGCCCTCAACAAGCGCAACGGTCACACCTACAA GCGGGCCTACATGCACGCCGTAGAGGATCGGATGCTGGAGGCGGGGGAACTCAACGGTGCATGGTCGGACTGG GACAACGAAGACCCAACGATCATCGCCCACATCGGTCTCCGCTGCATCGAGGCGCTCATTGAGTCCTCTGGGCTGG TCCGCATTACTCGCCGCAGCGCTGGCAACGTCAAGGAGGACTGCAATGTCCTCGAACTGGAGCCGCAATGGGTCG AGATGTTGAACCAACGGGCCTTTACGCTGGCCGGGGTGAACACCTATCACCAGCCTTGCGTTGTCCCTCCGCGCCC TTGGACCCGCCCTGTGGGTGGAGGATACTGGGGCAAGGGTCGTCGCCCGACTCGATTCATCCGGGTCCACAACAA GAAGGCCCTTGAGCGATACCGCGACGTTGACATGGAAGCGGTCTACAAGGCCGTGAACATCGCGCAGAACACCG CGTGGTCGATCAACAAGCGGATCCTTGAGGTGGCCGAAGCGCTCGCCTCATGGACCAACGTGCCTATCAGCAAGT GGCCCAAAGCGGAAACCCAAGAGCTGCCCGTTAAGCCTCACGATATTGAGACCAACGAGGAAGCACGGAACGCA TGGAAGAAACAGGCGTCCGGCGTGTACCGCAGTGAGTCCTCTCGGGTCTCCCGCAGGATGTCGCTTGAGACCACT CTGGAGACCGCACGGAAGTTTGCAGACTTCGAGGCGATCTACTTCCCTCACAACCTCGACTGGCGTGGCCGTGTGT ACGCCCTGCCGGTATTCAACCCTCAACGTGACGACCTGACCAAGGGTCTCCTACAGGCCTCCAAAGGTGAGCCGGT AGGGGAAGACGGCATCAAGTGGCTTATGATCCACGGTGCGAACACGGCAGGCGTCGATAAGGTACCTTTCGATG AACGACAACAATGGGTTCGAGACAATGAACGAACCATCCTACAGTGCGCTGAAGACCCACTTACACACACTGAGT GGATGTCAATGGATAGTCCCTTCTGCTTCCTTGCCTTCTGTTTTGAATGGGCTGGAGTCGTTAAAGATGGTCCGAAC CACGTATCTGCTCTCCCCATTGCTTTTGATGGGAGTTGCTCGGGCATTCAGCACTTTTCTGCAATGCTCCGAGACGA AACAGGCGGTCGGGCAGTCAATCTGCTCCCTTCCGAGCGTGTGCAAGATATCTACCGACTGGTCTCCGATGGTGTT

	AACGCTGCGCTTAGAGATGACGCTGTTCACGGAACGGATGACTCCACAGACGTACACGTTGACGAGAAAACTGGA GAGATTACAGAGCGGCGAGTCCTCGGAACTCGAACCCTCGCTGCTGCATGGCTTGCTCACGGGGTTGATCGCAGC GTCACCAAGCGCTCAGTAATGACCCTCGCCTACGGCTCCAAGGAGTTCGGCTTCACGGACCAAGTGCGGGACGAC ATCATCACCCCAGCGGTGGACGCAGGGTCCCTTAACTTCCCTCAGCCCCAGCAAGCGGCCCGCTACATGGCGCACT TGATCTGGGTCTCCGTAGGGAAGACCGTAGTGGCTGCCGTAGAGGCCATGGAGTGGCTCCAGAAGTCCGCCAAG CTCCTCGCGGCCATCGTCAAGGAGAAGAAGGGCCCCGAGAAGGGCAAGATCCTCAAGCCAGCCATGCCGGTCTAC TGGGTGACCCCTGACGGCTTCCCGGTATGGCAAGAGTACCGCGTACAGCAAGCCAAGCGGATCGACATGATCCTC ATGGGCGACGTAAGGCTGACCGCCACGGTGCTCCATCAGCAGGACCAGATTGACGCCCGCAAGCAGGAGTCAGG GATCTCCCCTAACTTCGTGCACAGCATGGACGGCAACCACCTGCGACAGACCGTGGTCCACGCTCACGATGCGTAC GACATCACGTTCTTCGCCCTGATCCACGATTCATTCGGTACCATCCCGGCCAAGGCTGGGCAGCTCTTCAAGGCCG TCCGTGAAACCATGGTCACCGCCTATGAGCACAACGATGTCCTCGCTGACTTCCGTGAGCAGTTCATCGACCAGCT ACACGAGACCCAGATGGATAAGATGCCTGAGCTGCCCAAGAAGGGCACCTTGGACATCCGTGAGATCCTCAAGTC TCAATTCGCTTTCGCTTAA
Phi15 lysozyme (G3RQ)	ATGGCTCGTCAAGTCAAATTTAAGGAGCGCTTGAGTACCAAGATGATCGTTGTGCACTGTTCGGCCACCAAGGCCA GCATGGACATCGGTCGTAAAGAGATCCAAATGTGGCACGTTCAGCAAGGCTGGCTGGCCATTGGGTACCACCTCG TGATTCGTCGCGACGGTACCATCGAGCAAGGCCGACCACACAAGGCCATCGGGTCCCACGTTAAGGGTCACAACA GTGACTCCATCGGGATCTGCCTCGTGGGCGGTATCGACGACTCGGGGAAACCTGAGGACAACTTCACGGACCAGC AAAAGGCTGCGCTGAGCGGCCTGCTGTGGGACATGACGCAATCTGGCGTGACCTATGGGGACACCTATAAGGAG CTGCCAGTGGTTGGTCACCGTGATCTCGATAGTGGTAAAGCCTGCCCGAGCTTCGATGTGAAGGCATGGTGGGCC GCTCAGATCAATTAA
Phi15 gp16	ATGAATGTCGAAAAGCAAAAGATGAAACGCTTCGAGGCCGAACTGGTCGGCAAATTCACCAGCTCGTGGGTCCCT GTATGGGCTCGCAATGTCGATGAGGCCCTTGAGGCTGCCGAGATGGAATATGGCGAGGACAACGTGGGCCGTGT GCGTCCCAAGGAGGTCGAATAA
Igy	ATGAAATCCACTGAACCCCAAGTCTATTGGTTGGCCAGTCCCGATGGTGAATTCCACCTGAAGGTCATCAACTTCG GCCAGTTCATCACCGAAACCCTTTACGAACTCGGCATTCCGGTGGACCACAAGGTTCATCGGGTCCACTGA
Rac	ATGAACAAGTCCATCTGGCGAGTCCACGCAAAGGCCGGCACTCCCTCGGAACTCCAGGGCCTGTGCTGGCTGGCG ATACAGGAGTTGGAGGAGTTCACCCTCTTCCGCTCGAAAGACGACGCCCTGAATACGATGCTGGATAGTATCGAG GGCAATGATCGAACCGAGCTGCTGGTATTCCGCGACGGGCAATTGGCCGGCGGTGCCTGCATCGTGTTCGAGGAC GACCCCCACGTCGGCCCGTGCGTCACAGCACAGTGGCAGTACGTCCTACCACGCTACCGCAATTCAGGTGTGGTTC GGGAGTTCATCCGCGAACTCCACCGTCAGGCCGGCTGGGGTCAAATCCCCCTCGTGTGCTGGAGCCATCGTGAAA GCGATAGCCGGTACACGATCCACTACCGGAGAGCCAAGCCTTATGGGCAAGAAAGTAAAGAAGGTGTTGGGCAA GACCATCATCGGCAAACTCGCTGA
T7 WT	TAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGT
Phi15 T1	GCGTGGCCATCTTCGGGAGTTAAGTCCACTGAAGGGCATCCTCATGGGTGCCTTTTGGGGAACAAATACCCACACT ATCGGGAATATCGAAATGAAATAC
Phi15 T3	CAATTGGGTCAACCAGTAGAGTAAGGTCACGAGCCTCATCGACTTCGGTCGGTGGGGCTTTTTGCGTTTAACTTCC AAGGAGACTTGACATGACACCGAA
Phi15 T5	GTGGTTACCGTTCGATAAGCTGAAACCAAACCCCTTGGGTCCCATAACGGGGCTTGAGGGGTTTTTATTTAGAACA GGAGGAGCTATGGCTCTACAAGATG
Phi15 T5s	GCTGAAACCAAACCCCTTGGGTCCCATAACGGGGCTTGAGGGGTTTTTATTT

LUZ7 T7	AGAGCGCGCGCTCGACCTGTAACACCATGCCCAACCTCCTCTTGGGGGTTGGGCGTGTTCTTTGTGCTATCCGTCA GTCCGTAAGGAGTTCCAACATGAA
LUZ7 T32	TCCGTGGGGTCCGCTCCTTCCCCATAGGTTTCATCCAACCTCAACCCCCTACAGGCATGTCGCTACCTGTAGGGGGT TTTTTTATACCTGGTCGAAGGGA
LUZ7 T47	GTCTCCTAGCCTCTGAGTTCACCCACCCTTTCTAGATAGCCAGCTTTATGCTGGCTTTCTTATGTCTATGTCTCGTAG ACATAAGGGAGTGCTCTGGCCC
LUZ7 T50	TTCTGGGAGATCCTGGCAAACGCATTCAAAAAGAAAGGGGAGCATTAGCTCCCCTTCTTCTTTACAGGTTCATCAA ACCTTTCACCATCGGCAGTTCACT
LUZ7 T50i	AGTGAACTGCCGATGGTGAAAGGTTTGATGAACCTGTAAAGAAGAAGGGGAGCTAATGCTCCCCTTTCTTTTTGAA TGCGTTTGCCAGGATCTCCCAGAA
LUZ7 T61	GACGGGGAGGCTGGGCGTAAGGGTGAAGGGGAACCAGGACGGTTCCCCTTTTTCTTTTAGGCTTTGCCGGTACTA CCGAACCCACCTTCACCTCGACTGG
LUZ100 T6	CCAATTACCACACAGGTATGGAAGACCGCCCCCGGTATGCCCAGCGCTGCCGGGGGTTTCTGTTTCAAGACCCACG ACAACTGTGAGGAGAACCCCATGA
LUZ100 T16	CTGTTGCGTAAGCAACTATCCACCATCGCATAGGAGGGCCAGAAATGGCTCTCCTATTTTTTTCTTATGGGGGTTCC ATGATTTCTCCAACCACTGAGCT
LUZ100 T19	TTCTGGGATCAGTAACAACCATTCGTTCAACGCACAGGGGCGGCCTTCGGGTCGCCCTTTTTCTTTGTCTGGAGGA AAAGCATGGCTCTTGCACGAGTCA
PP0013 HA up	TCATCGAAAGTAACGCGCTGTCGGTGTCGAACCTGGACTTCTGATCAGGTGTGTAGGTTCACAGAAAAGCTGCAC GCTTTCACAAATAGATGACCGGCCGGGCTTCAACCATGGCCCCTGTCGGTCACCATCAAGCCCGCCTTCGCGGGCT TTTTTTTGCCTGGAATTCCTGCCGGGGCGCCCTACTCCGCTCATTGGAAAATGATGCAGATGGGGGGATTTGAGGA AGTGATTTGATGGGGCGCTGGAAGTGCTGAGAGGGTCGGCGGGCGGATCTGAGCAGCAGAGAGATTGAGAGGA CTTTAAAACTAATATTTGAACACCTTAATAGTTAATTAAGCGTACGTGTTAAGTATTAATTTGAACAATTTAGTATGG AAATGGAATTTGGTGGAAGTTGCCATTTTGGATTTTTTGTAGATTTCGAAGGGATGTTGGAATAAGATCTATGACC AACTTTTCCGCTTTGCACTGAAGATTTTCAGCCAAATCAGCTGTTTGAAGCGAGAGCTTCAGACCTGATC
PP0013 HA dn	CAAATTGCTCCAGCGATCACGAAGTGGCCGTTTAGAAATAAAGAGAGCAAGCCGATTGCTTTAGATTCTGATCAGT CATGAGAGTCATGACCAATGAGAGAAAAGGAGACCTGAAAATGTAGAAAAACGCGATCAATCCCGAGAGGACTG ACCGCGCTTAGCGAAGCAGACTTGCGTCCTCAGAACCCGCAATGTCTGCTTTTTCGAAGCGACTGTCAATCCTCTCC CATTTTTTCGACCTTAGCGAGGTTACTCGACGGCACAGTGTTGCCTGTCGAAAGGCCTCACTTCGCCCGCCCTGCGC CTTGATTTTTTGGCGCAGCGTCGGCGCTCGTTTCGAGATTTTCGGAGGTGTTTCATGACAGTACGAAAGGTGGTCA CGCGGCGGTCCAACCATTACCGCGGCTACTTCCCATCGCTCAAAAATAAAAAGCCTGTGCCCTGGGAGTCCCAGCT CGAAGGCGCTCTTTTTCGGCTTCTTGAGCTGTCTCCTGCGGTCATCGGCTACGTCCCGCAGCCCAGTGAGGAACGT GTTC
PP5322 HA up	GTACGACGCCGAGCGCGAAGAATTCACCGGCCTGCTGCACATCAAGGACCTGCTGCTGGAGCTGGCTGAGCTGGA GCACTTGCCAGAGACCATCGACCTGGAGGACCTTGCCCGGCCACTCGAACGCGTGTCGCGGCACATGCCGCTGGC ACAGTTGCTGGAGCAGTTCCGTAAGGGCGGCGCGCACTTCGTGCTGGTCGAGGAAGCCGATGGCAAGGTGATCG GCTACCTGACCATGGAAGACGTGCTGGAAGTGCTGGTAGGCGACATCCAGGACGAGCACCGCAAGACCGAGCGT GGCATTCTTGCCTACCAGCCGGGCAAGCTGCTGGTGCGTGGCGACACCCCACTGTTCAAGGTCGAGCGCCTGCTG GGTATCGACCTTGACCACATCGAGGCCGAAACCCTGGCCGGGTTGATCTACGAGACGCTCAAGCGCGTGCCTGAA GAAGAGGAAGTGCTGGAGGTCGAAGGGCTGCGCATCATCATCAAGAAGATGAAAGGCCCCAAGATCGTACTGGC CAAGGTGCTGAAGCTGGATTGATGGCCC

PP5322 HA dn	AATCGGTCAGGGGTTGCCGGCAGTGAAATCCGGCAACCCGCTGATCGGCCTGTCGAAGCGGTAAGGTATCGACTC CAGCGCCAACCCCACATTGCGCTGCACCACAAAATGCAGGTGCGGCCCAGTGCTGTTGCCGGTATTGCCCGACTTG GCCAGCATCTGGCCCTGCCGTACCCGTTGCCCCTCCGCCACCACCACCGAACCACGCATCAGGTGAAGGTATACGC CCATGGTGCCATCCGGGTGCAAAATCCTGACAAAGTTACCCGAGGGGTTGGGGCCGCGCCCGCTCTGGCTGTTCT GCGTCTTCACCACCACCCCTTCCCGCGCTGCGATGATGGGCGTGCCTTCGGGCATGGCGATATCCATGGCGTAGCG CCCTTTGGGTCCAAAATGGCTGACGCGGCCATTGGGGCCCTGAGTGACGCGGAACGGCCCGCCAATCCACGGGAA CGCATAGCGGAAGCCCTGGGGCCGTTTGCCCGGGTCACCCATGGCCTGCAGCAGGGTCGAACGGTAGTTCAGCA GCAGCCCGGGCCGCCCCCTGAGC
PP5042 HA up	TCTGCGATTCCGGCAAAAGGTTGTTCATAAATTGAACAATTCCGGTATCATCGCGCGCCCCAAGACCGTGATCCAC CCTATAAAGATGAAAACGACCCTGATCGCCGCGGCCGAAGTCGACCGCCTGGAGACCTGGCAGCGCTACACCAGC AACATGTGCCATGGCTGCCATTCGACCTGCTGCACCCTGCCGGTGGAGGTGAAGATCAAGGATCTGATCCGTATC GGCGTGGTCGACGAGTTCGAAAAAGACGAACCACCCAAGAACGTGGCCAAGCGCCTGCAAAAAGAAGGCATCAT CGAGCGCTTCAACCAGAAGTCCGGGATCTTCACCCTGACCCGGATGAGCAACGACGACTGCATGTACCTGGATCG TAAAAGCCGGCTGTGCACCATTTATGACAAGCGCCCGGATACCTGCCGCAACCACCCCAAGGTCGGGCCACGGCC GGGGTATTGCGCCTACAAGCCCAAGGTGGTTGGGCGTTGAGTCTTGCGCCGGGGCATAAATCGAGCGCCGCGCG GGCGGCGCTCGATTCTGTGGCGATGC
PP5042 HA dn	CGAGCTGGGCATGAAGCACTGAACCCACCGCTCCCATTTCAGCCAGCGGGGAAGCTCCGGCAAGCACCCCCCTGA TCCGCTGGTGGATGCTCAGGATAAGTCTGCGGCAAAACGCTGACCGGGCTGGTGACAACGAAACCATCAGCAAAC CGCGCCTCTACGAACCGCGCCTGCCAGCCTTGCGCCGGCGGCGCCCAGTCAAGGCTGAACGCCTCACTGGGGATG ATCGTCTGCGATGTGTAGCGTACCCCGCAGGCATGGCGGAAGTCCCGATCCAAGGGGTTAGCCGCAGTCCATACC TTCACTGCTACAGGCATTTCCGAAAGCCGTACCAGCACATGCGCAGCTCCTTCGCCCGGAGCCACTTGCACGCGAG GCATCGGCAGGCTGGCCTGGATACGCTGAACCATCGGCACGAGGGTAGTGACAACATGCGCACGCACGCCCCCAT GGTCCGAGTTCGGCAGCACCCTGAGGCTGGTCTGGCCAGGCAGGCGCTGCAGGTAGTCCGTGGCGTGATCAGGG GCGAAGAAATCATCCCCGCTGGCGG
PP4305 HA up	GGTAATGGCCGCTATACCTACCTGTCGGCCTGGGGCTACGTACTCAAGCAAGGCGGCGATGAAAGCAAAGCCCGC GACTTCGTCGGCAAGCTGTTCAAGCAGGCACCCGTACTGGACACCGGTGGCCGTGCCGCCACCACCACGTTCATG ACCAACCAGATCGGTGACGTGCTGGTCACCTTCGAGAACGAAGCCGAGATGATCGCCCGCGAGTTTGGCCGCGAC CAGTTCGAAGTCGTTTACCCAAGCGTATCGGCCGAAGCCGAGCCACCGGTGACGGTGGTCGACAAGGTAGTGGCC CGGAAAGGCACCCAGGCCGTGGCCGAGGAATACCTGAAGTACCTGTGGTCACCCGCAGCCCAGGAGATCGCCGC CAACAACTACCTGCGCCCGCGTGATGCAACGGTACTGGCCAAGTACACCGACCGCTTCCCGAAAGTGGACTTCCTG TCGGTGGAGAAAACCTTCGGTGACTGGCGTACCGTGCAGAAGACCCACTTCAATGATGGTGGGGTGTTTGACCAG ATCTATACCGGTCAGTAACGCACAA
PP4305 HA dn	GCAACATCAACCCCGGCGGAACACCAAGGCCTTCAGCCCCCCCGCCGGGTCAACGTCCGGAAACTCTGGCGGGTT TTCCAGGCGCTCGACATAAGCCAACGAAGGCGCCTGCTCTGCCATGCCTTCGATCAGAAACTCAGGCCCGATCCCC GGGTCGTTGACACACGCCAGCACCGTCCCGCCTTCGCTCAACAACTCCGGCAAACGCCGCAGGATCTTTGCGTAAT CCTGGGTCAGCACAAAGCTGCCACGCTGGAAGGTCGGCGGGTCGATGATGATCAGGTCATACGGCCCGTACTTGC GCACCTTGCCCCACGACTTGAACAGTTCATGCCCCAGGTACGCCACCCGTGATGCATCGTGGCCATTGAGGCGATG GTTGTCCCGGCCCCGCGACAGCGCCGACTTGGCCATGTCCAGGTTCACCACCTGCTCAGCGCCACCGGCAATCGCT GCCACAGAAAAGCCGCAGGTATAGGCGAACAGGTTCAGCACGCGCTTGCCCGCCGCCTGCTCGCGCACCCAGCGG CGGCCATAGCGCATGTCGAGG
PP5388 HA up	TGACCGCACCGTTGCTCTCTCTCTTCGTGATTCCCGCAGCGTATTGGCTGGTCCGACGCCGCGATCTTGTAGTACCT CATAATTCCACACCAGGAGACATCCGATGAAAAAGCTCTACCTCAGCATTGCACTGCTCTTTGCCTTCGCATCAGGC

	GCGCAAGCCCAAGACTCCATGGCCGGGATGAGCATGGATGGAATGGATATGAAGGAAACCCAATCAGCACCTTCC GCTCATGCAGAAGGGACGGTAAAGGCAATTGACGCCCAAGGCGGCACAGTGACCCTGATGCATGGACCGGTTGC TGCGTTGAAATGGCCGGCCATGACCATGGCCTTCAAAGCCTCTGCGCAACAGCTCGATGGATTGAAGGTGGGAGA CAACGTAGAGTTTGATTTCCGGATGGATGGCAGCACGGCAACGATTGTTGATATTCGCAAACAGTAATTCGCCTAA TTATCCTGTCTGCCAACGCTTGATTCGCCCATGACCTCCTGACGCCTCCTAGCAACTGGAAAAGCTTGGAAGCATCT AGGAAGTCAGGGCGATTCA
PP5388 HA dn	GATCATGGTAACCCCGGCCGCTGGAGCCATTTCTGAAGTACTGACACGGCGCTGAAGCGATGTGGCAGTTCCGCC TAAGGCGGCGAGCAGCAGCGAGAAAAGATCGTCACCCCAAGTTTTCTTCTTGCAGGCGACTGAATAGAGGAGAA ATCGAGATAACAGCTCGCAAGGCATCCTGATTGGCGCCCGACCATGCCTGATCCTGTACATCATTGAGCACGTGCT GTGTCGGAATTGGCCGAAACGCTTGCGTCGCGGGGTCGTAGACGTAAACGTGAAGACCTATTTCAATCGCTCGCG AGGCCTGACGGTGCCAGTTAAAGTTGCCACCATTCGTCATCGTGTCCGCAGCTACGAGGATGTCGTAGTCATTAAC GATGTAGCCGAAAAGTACATGCTGCGAGATATCGCGGAGAGCTAGAGAATGACGCCTGCTCGCCGATCGCCAGA CTAGGTTGCGTGCAAAGTGTCGCCTACTGACCCTTGGGTCATCCAAAAGCGTGACCTTGTCGTAATAGGCTACTGA CAAATCAATATCGTCGACCAGGGC
T1-P14c-BCD22-phi15lys(G3RQ)-T0 cassette	ATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTT CGTTTTATTTGATGCCTTTAATTAATCTAGTGAATTGACATGTCAATTTTTATGTTGTATAATATAACTAGCAGGCCC AAGTTCACTTAAAAAGGAGATCAACAATGAAAGCAATTTTCGTACTGAAACATCTTAATCATGCCTAGGAAGTTTTC TAATGGCTCGTCAAGTCAAATTTAAGGAGCGCTTGAGTACCAAGATGATCGTTGTGCACTGTTCGGCCACCAAGGC CAGCATGGACATCGGTCGTAAAGAGATCCAAATGTGGCACGTTCAGCAAGGCTGGCTGGCCATTGGGTACCACCT CGTGATTCGTCGCGACGGTACCATCGAGCAAGGCCGACCACACAAGGCCATCGGGTCCCACGTTAAGGGTCACAA CAGTGACTCCATCGGGATCTGCCTCGTGGGCGGTATCGACGACTCGGGGAAACCTGAGGACAACTTCACGGACCA GCAAAAGGCTGCGCTGAGCGGCCTGCTGTGGGACATGACGCAATCTGGCGTGACCTATGGGGACACCTATAAGG AGCTGCCAGTGGTTGGTCACCGTGATCTCGATAGTGGTAAAGCCTGCCCGAGCTTCGATGTGAAGGCATGGTGGG CCGCTCAGATCAATTAACACTAGTCTTGGACTCCTGTTGATAGATCCAGTAATGACCTCAGAACTCCATCTGGATTT GTTCAGAACGCTCGGTTGCCGCCGGGCGTTTTTTATTGGTGAGAAT
T1-XylS/Pm::phi15rnap-T17 integration cassette	ATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTT CGTTTTATTTGATGCCTTTAATTAAACGTTCGTAATCAAGCCACTTCCTTTTTGCATTGACGCAGGGTGTCGGAAGG CAACTCGCCGAACGCGCTCCTATAGTTTTCAGCGAAGCGTCCCAAATGTAAGAAGCCGTAGTCTAGGGCTATCTCA GTTATACTACGCACATTGGCACTGGGATCGTTCAAGCAGGCGCGGATGCTTTCGAGCTTGCGGTTGCGGATGTAG TTCTTCGGCGTGGTGCCGGCGTGCTTCTCGAACAAATTGTAGAGCGAGCGTGGACTCATCATCGCCAGCTCCGCTA ACCGCTCAAGGCTGATATTCCGTTTGAGATTCTCCTCAATGAATTGAACGACTCGCTCGAAAGACGGGTTACCTTTG CTGAAAATTTCACGGCTGACATTGCTGCCCAGCATTTCGAGCAGCTTGGAAGCGATGATCCCCGCATAGTGCTCTT GGACCCGAGGCATCGACTTTGTATGTTCCGCTTCGTCACAAACTAACCCGAGTAGATTGATAAAGCCATCGAGTTG CTGGAGATTGTGTCGCGCGGCGAAACGGATACCCTCCCTCGGCTTGTGCCAATTGTTGTCACTGCACGCCCGATCA AGGACCACTGAGGGCAATTTAACGATAAATTTCTCGCAATCTTCTGAATAGGTCAGGTCGGCTTGGTCATCCGGAT TGAGCAGCAATAGTTCGCCCGGCGCAAAATAGTGCTCCTGGCCATGGCCACGCCACAGGCAATGGCCTTTGAGTA TTATTTGCAGATGATAACACGTCTCTAATCCAGGCGAGATTACCCTCACGCTACCGCCGTAGCTGATTCGACACAGA TCGAGGCATCCGAAGATTCTGTGGTGCAGCCTGCCTGCCGGGCGCCCGCCCTTGGGCAGGCGAATAGAGTGCGTA CCGACATACTGGTTAACATAATCGGAGACTGCATAGGGCTCGGCGTGGACGAAGATCTGACTTTTCTCGTTCAATA AGCAAAAATCCATAGTTCACGGTTCTCTTATTTTAATGTGGGCTGCTTGGTGTGATGTAGAAAGGCGCCAAGTCGA TGAAAATGCATCTCGACGTGATGCGTATACGGGTTACCCCCATTGCCACGTTGCGCCATCCTTTTTGCAATCAGTGA CCACTTTTCCAAGCAAAAATAACGCCAAGCAGAACGAAGACGTTCTTTTTAAGAAGCGAGAACACCAGAAGTTCGT

	GCTGTCGGGGCATGGGGCGACGAATTGGCGGATAAAGGGGATCTGCTGGATATTACGGCCTTTTTAAAGACCGTA AAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCATCCGTAATTACG TATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACT GAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGC GTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGG TGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTCACCATGCATGGGAAT TAGCTTGATCTGACCAACGACCGGTAGCGGAGCTATCCAACGGCGGTATACCAGGAAAACACACAGCAGGTACAT CAGAACAGTACCATGACTGAAGAACAAATAGTTTTTTCCTGATCCATAAAGCAGAACGGCCTGCTCCATGACAAAT CTGGCTCCCCAACTAATGCCCCATGCAGCCAGCATAACCAGCATAAAGTGCAGTGTCCGGTTTGATAGGGATAAGT CCAGCCTTGCAAGAAGCGGATACAGGAGTGCAAAAAATGGCTATCTCTAGTAAGGCCTACCCCTTAGGCTTTATGC AACAGAAACAATAATAATGGAGTCATGACCATGAGGAGGAAAAACAAATGATTGAAGTAGCAAAGAACGATTTCT CCGATGTCAAGACTGACTGGGCGTTTCGTGTACTGAGTGAGCTGTACGGTGAAGAACTGGCAGCCGCTCAACTGG CTCTGGAACACGAGTCGCACGAGATCGGTGAGGCAAAGTTCAAGAAAGCCCTTGATCGCCAGATGAAACGTGGC GAGACCTCTGAGACCTCCGTGGCAAAGCCGCTGGTCGCAATGCTGGTCCCTAAGTTTGTCGAGAAGATGGACGCA TGGGTAGAGCACCAGATGAAGAACGTGCGCCGCAAGTCCGTGGCCCTGAAGTTCATCCAGATGGTCGCCACCGAG CGCGTTGCGGTCATCACCATCAAGACCGTAATTAACGCGATGTCGCAAGGAGACGTAGTGCTTCAGGCAATCGCC GGTCGCATCGGTCGCGGCATCGAGGAGGAGGCTCGGTTCGGTCGCATCCGTGACCAAGAGGCGAAGCACTTCAA GAAGTACATCCGTGAGGCCCTCAACAAGCGCAACGGTCACACCTACAAGCGGGCCTACATGCACGCCGTAGAGGA TCGGATGCTGGAGGCGGGGGAACTCAACGGTGCATGGTCGGACTGGGACAACGAAGACCCAACGATCATCGCCC ACATCGGTCTCCGCTGCATCGAGGCGCTCATTGAGTCCTCTGGGCTGGTCCGCATTACTCGCCGCAGCGCTGGCAA CGTCAAGGAGGACTGCAATGTCCTCGAACTGGAGCCGCAATGGGTCGAGATGTTGAACCAACGGGCCTTTACGCT GGCCGGGGTGAACACCTATCACCAGCCTTGCGTTGTCCCTCCGCGCCCTTGGACCCGCCCTGTGGGTGGAGGATA CTGGGGCAAGGGTCGTCGCCCGACTCGATTCATCCGGGTCCACAACAAGAAGGCCCTTGAGCGATACCGCGACGT TGACATGGAAGCGGTCTACAAGGCCGTGAACATCGCGCAGAACACCGCGTGGTCGATCAACAAGCGGATCCTTGA GGTGGCCGAAGCGCTCGCCTCATGGACCAACGTGCCTATCAGCAAGTGGCCCAAAGCGGAAACCCAAGAGCTGCC CGTTAAGCCTCACGATATTGAGACCAACGAGGAAGCACGGAACGCATGGAAGAAACAGGCGTCCGGCGTGTACC GCAGTGAGTCCTCTCGGGTCTCCCGCAGGATGTCGCTTGAGACCACTCTGGAGACCGCACGGAAGTTTGCAGACT TCGAGGCGATCTACTTCCCTCACAACCTCGACTGGCGTGGCCGTGTGTACGCCCTGCCGGTATTCAACCCTCAACGT GACGACCTGACCAAGGGTCTCCTACAGGCCTCCAAAGGTGAGCCGGTAGGGGAAGACGGCATCAAGTGGCTTAT GATCCACGGTGCGAACACGGCAGGCGTCGATAAGGTACCTTTCGATGAACGACAACAATGGGTTCGAGACAATGA ACGAACCATCCTACAGTGCGCTGAAGACCCACTTACACACACTGAGTGGATGTCAATGGATAGTCCCTTCTGCTTCC TTGCCTTCTGTTTTGAATGGGCTGGAGTCGTTAAAGATGGTCCGAACCACGTATCTGCTCTCCCCATTGCTTTTGAT GGGAGTTGCTCGGGCATTCAGCACTTTTCTGCAATGCTCCGAGACGAAACAGGCGGTCGGGCAGTCAATCTGCTC CCTTCCGAGCGTGTGCAAGATATCTACCGACTGGTCTCCGATGGTGTTAACGCTGCGCTTAGAGATGACGCTGTTC ACGGAACGGATGACTCCACAGACGTACACGTTGACGAGAAAACTGGAGAGATTACAGAGCGGCGAGTCCTCGGA ACTCGAACCCTCGCTGCTGCATGGCTTGCTCACGGGGTTGATCGCAGCGTCACCAAGCGCTCAGTAATGACCCTCG CCTACGGCTCCAAGGAGTTCGGCTTCACGGACCAAGTGCGGGACGACATCATCACCCCAGCGGTGGACGCAGGGT CCCTTAACTTCCCTCAGCCCCAGCAAGCGGCCCGCTACATGGCGCACTTGATCTGGGTCTCCGTAGGGAAGACCGT AGTGGCTGCCGTAGAGGCCATGGAGTGGCTCCAGAAGTCCGCCAAGCTCCTCGCGGCCATCGTCAAGGAGAAGA AGGGCCCCGAGAAGGGCAAGATCCTCAAGCCAGCCATGCCGGTCTACTGGGTGACCCCTGACGGCTTCCCGGTAT GGCAAGAGTACCGCGTACAGCAAGCCAAGCGGATCGACATGATCCTCATGGGCGACGTAAGGCTGACCGCCACG GTGCTCCATCAGCAGGACCAGATTGACGCCCGCAAGCAGGAGTCAGGGATCTCCCCTAACTTCGTGCACAGCATG

	GACGGCAACCACCTGCGACAGACCGTGGTCCACGCTCACGATGCGTACGACATCACGTTCTTCGCCCTGATCCACG ATTCATTCGGTACCATCCCGGCCAAGGCTGGGCAGCTCTTCAAGGCCGTCCGTGAAACCATGGTCACCGCCTATGA GCACAACGATGTCCTCGCTGACTTCCGTGAGCAGTTCATCGACCAGCTACACGAGACCCAGATGGATAAGATGCCT GAGCTGCCCAAGAAGGGCACCTTGGACATCCGTGAGATCCTCAAGTCTCAATTCGCTTTCGCTTAATTCTGGGAGA TCCTGGCAAACGCATTCAAAAAGAAAGGGGAGCATTAGCTCCCCTTCTTCTTTACAGGTTCATCAAACCTTTCACCA TCGGCAGTTCACT

Table S6: All primers used in this work. Yellow: BsaI recognition site; grey: BsaI restriction site; blue: SapI recognition site; green: SapI restriction site; red: uridine nucleotide for USER cloning;

Name	**Sequence**
General primers	
SEVA_PS1	AGGGCGGCGGATTTGTCC
SEVA_PS2	GCGGCAACCGAGCGTTC
insertpBG_F	GCGTTCGGTCAAGGTTCTGGAC
insertDesR_F	CGGCGAAATAGTAATCACGAGGTCAG
Optimal induction system	
pPS_BsaI_F	GACGGTCTCTTAACatcgcacctcagcgtcgtgactgg
pPS36_RBS_BsaI_R	GTCGGTCTCCCATTTATATTCCTCCTatcgcctcagcattattattg
pPS37_RBS_BsaI_R	GTCGGTCTCCCATTTATATTCCTCCTatcgcctcagcatccaaaaaaacggg
pPS39_RBS_BsaI_R	GTCGGTCTCCCATTTATATTCCTCCTatcgcctcagcgaatttc
pPS44_RBS_BsaI_R	GTCGGTCTCCCATTTATATTCCTCCTatcgcctcagcgtgtgaaattg
pPSempty_BsaI_R	TATGGTCTCCTCTAacgcgatcggcctcctgtgtgaaattg
NaFswitch_F	TATGCTCTTCTAGAGGTCTCTTCTATCCTCTtttggccctctttcgtaag
NaFswitch_R	TATGCTCTTCAAGAGGTCTCTCATTCGTCGAAATTTAGACATTTGGCC
msfGFP_F	GACGCTCTTCCAGAGGTCTCGAATGATCATGGGAATTCATAAAGGTG
msfGFP_R	TAGGCTCTTCTCTTGGTCTCAGTTATTTGTAGAGTTCATCCATGCCG
Constitutive expression of phi15 lys G3RQ	
pSTDesR_BsaI_F	GACGGTCTCCTAACAACTAGTCTTGGACTCCTGTTG
pS6x1_BsaI_R	GTCGGTCTCCTAGAttaattaaaggcatcaaataaaacg
p14c_F	TAAGCTCTTCTAGAGGTCTCTTCTAGTGAATTGACATGTCAATTTTTATGTTG
p14c_R	TAAGCTCTTCTCTTGGTCTCTCTGCTAGTTATATTATACAACATAAAAATTGAC
BCDX_F	GGCGCTCTTCCAGAGGTCTCTGCAGGCCCAAGTTCACTTAAAAAGG
BCD2_R	GCCGCTCTTCTCTTGGTCTCGAATGACCTCCTTAGCATGATTAAG
BCD13_R	TAAGCTCTTCTCTTGGTCTCTCATTAGAAAGCCTCCATTGCATG
BCD22_R	TAAGCTCTTCTCTTGGTCTCTCATTAGAAAACTTCCTAGGCATG
phi15lysG3RQ_F	AGAGGTCTCGAATGGCTCGTCAAGTCAAATTTAAGGAGCGC
phi15lys_R	TAGGCTCTTCTCTTGGTCTCAGTTAATTGATCTGAGCGGCCC
Genomic integration of phi15rnap and phi15 lys G3RQ	
pSNW_seq_F	TGTAAAACGACGGCCAGT
pSNW_seq_R	CTTTACACTTTATGCTTCCGG

userphi15_R	AACAAGACUAGTTGTTAAGCGAAAGCGAAT
userL7T17_F	AGTCTTGTUCTGGGAGATCCTGGCAAA
PP0013_HAup_F	AGATCCUTCATCGAAAGTAACGCGC
PP0013_HAup_R	AAATGAUCAGGTCTGAAGCTCTCGCTTCA
userphi15_F2	ATCATTUGTCCTACTCAGGAGAGCGTTCA
userL7T17_R2	ATTTGAGUGAACTGCCGATGGTGAAA
PP0013_HAdn_F	ACTCAAAUTGCTCCAGCGATCACGAA
PP0013_HAdn_R	AGGTCGACUGAACACGTTCCTCACTGGGC
PP5322_HAup_F	AGATCCUGTACGACGCCGAGCGCGAAGA
PP5322_HAup_R	ATGGGCCAUCAATCCAGCTTCAGCACCTTGGCC
userphi15_F3	ATGGCCCAUTTGTCCTACTCAGGAGAGC
userL7T17_R3	ACCGATTAGUGAACTGCCGATGGTGAAA
PP5322_HAdn_F	ACTAATCGGUCAGGGGTTGCCGGCAGTGAAATCC
PP5322_HAdn_R	AGGTCGACUGCTCAGGGGGCGGCCCGG
PP5042_HAup_F	AGATCCUTCTGCGATTCCGGCAAAAGG
PP5042_HAup_R	AAATGCAUCGCCACAGAATCGAGCGC
userphi15_F3	ATGCATTUGTCCTACTCAGGAGAGCGTTCA
userL7T17_R3	AGCTCGAGUGAACTGCCGATGGTGAAA
pSNW2_F	ATAGGTCTCAAGTCGACCTGCAGGCATGCAAGC
pSNW2_R	ATAGGTCTCACTAGAGGATCCCCGGGTACCGAGC
PP5388_HAup_F	ATAGGTCTCACTAGTGACCGCACCGTTGCTCTCTCTCTTCG
PP5388_HAup_R	ATAGGTCTCATAGATGAATCGCCCTGACTTC
PP5388_HAdn_F	ATTGGTCTCAGGCTGATCATGGTAACCCCGGCCGC
PP5388_HAdn_R	ATAGGTCTCAGACTGCCCTGGTCGACGATATTG
Trrnb_F	ATAGGTCTCATCTAATTTGTCCTACTCAGGAGAGCG
T0term_R	CTTGGTCTCTAGCCCTGGATTCTCACCAATAAAAAAC
Verification of genomic integrations	
PP0013_up	TGGGTGATGCGGTCGAGC
PP0013_dn	ATTTGAAGTAGCCCTGGAGCG
PP5322_up	CCTCTGAAGCAGATCCTGGC
PP5322_dn	GCGCACCACGCAGATGCTC
PP5042_up	TACGGTTCGCAGTTTTCATGCC
PP5042_dn	GGGTGCTCGCCTGAAGATAC
PP4305_up	CAAGGACGGTGTGCAGGTG
PP4305_dn	CCTGCCAGCAACGCGAAG
PP5388_up	ATTCTTTGGAGCAACGGAAC
PP5388_dn	CAGCTGCAAGTGGAGATC
RBS-C and RBS-D introduction in pSNW2.PPXXXX vectors	
RBSC_F	AGCTTTATCTATTTAACAACGCGGTCCGATGTGattgaagtagcaaagaacg
RBSD_F	AGCTTTATCTATTTAACAACGGGGTCCGAGGTGattgaagtagcaaagaacg
RBSX_R	TTATTATTGTTTCTGTTGCATAAAGCCTAAGGGGTAGGC

Expression vector optimization - backbone	
pS6x1_BsaI_F	GACGGTCTCCTAACactagtcttggactcctg
pS6x1_BsaI_R	GTCGGTCTCCTAGAttaattaaaggcatcaaataaaacg
Phi15prom_F	GACGCTCTTCCAGAGGTCTCGTCTATAAAAACCCACACAATAG
msfGFPend_R	TAGGCTCTTCTCTTGGTCTCATCTTATTTGTAGAGTTCATCCATGCCG
Expression vector optimization – upstream terminator	
LUZ100T6_F	TATGCTCTTCAAGAGGTCTCTTAACccaattaccacacaggtatg
LUZ100T6_R	TATGCTCTTCACTTGGTCTCTAGCCTCATGGGGTTCTCCTCACAG
LUZ100T16_F	TATGCTCTTCAAGAGGTCTCTTAACctgttgcgtaagcaactatc
LUZ100T16_R	TATGCTCTTCACTTGGTCTCTAGCCAGCTCAGTGGTTGGAGAAATC
LUZ100T19_F	TATGCTCTTCAAGAGGTCTCTTAACttctgggatcagtaacaacc
LUZ100T19_R	TATGCTCTTCACTTGGTCTCTAGCCTGACTCGTGCAAGAGCCATGC
LUZ7T7_F	TATGCTCTTCAAGAGGTCTCTTAACagagcgcgcgctcgacc
LUZ7T7_R	TATGCTCTTCACTTGGTCTCTAGCCTTCATGTTGGAACTCCTTAC
LUZ7T47_F	TATGCTCTTCAAGAGGTCTCTTAACgtctcctagcctctgagttc
LUZ7T47_R	TATGCTCTTCACTTGGTCTCTAGCCGGGCCAGAGCACTCCCTTATG
LUZ7T50_F	TATGCTCTTCAAGAGGTCTCTTAACttctgggagatcctggcaaac
LUZ7T50_R	TATGCTCTTCACTTGGTCTCTAGCCAGTGAACTGCCGATGGTG
LUZ7T50i_F	TATGCTCTTCAAGAGGTCTCTTAACagtgaactgccgatggtg
LUZ7T50i_R	TATGCTCTTCACTTGGTCTCTAGCCTTCTGGGAGATCCTGGCAAAC
LUZ7T32i_F	TATGCTCTTCAAGAGGTCTCTTAACtccgtggggtccgctccttc
LUZ7T32i_R	TATGCTCTTCACTTGGTCTCTAGCCTCCCTTCGACCAGGTATAAAAAAAC
LUZ7T60i_F	TATGCTCTTCAAGAGGTCTCTTAACccagtcgaggtgaaggtggg
LUZ7T60i_R	TATGCTCTTCACTTGGTCTCTAGCCGACGGGGAGGCTGGGCGTAAGGG
phi15prom_PM5_F	GAAGCTCTTCTAGAGGTCTCTGGCAtaaaaacccacacaatag
pBG_BsaI_PM4_R	GTCGGTCTCCGTTATTAATTAAGACGTCTTGAC
Expression vector optimization – downstream terminator	
phi15T1_F	TATGCTCTTCAAGAGGTCTCTTAACgcgtggccatcttcgggag
phi15T1_R	TATGCTCTTCACTTGGTCTCTAGCCGTATTTCATTTCGATATTCCC
phi15T3_F	TATGCTCTTCAAGAGGTCTCTTAACcaattgggtcaaccagtag
phi15T3_R	TATGCTCTTCACTTGGTCTCTAGCCTTCGGTGTCATGTCAAGTCTCC
phi15T5_F	TATGCTCTTCAAGAGGTCTCTTAACgtggttaccgttcgataag
phi15T5_R	TATGCTCTTCACTTGGTCTCTAGCCCATCTTGTAGAGCCATAGC
phi15T5short_F	TATGCTCTTCAAGAGGTCTCTTAACgctgaaaccaaacc
phi15T5short_R	TATGCTCTTCACTTGGTCTCTAGCCAAATAAAAACCCCTC
LUZ100T6_F	TATGCTCTTCAAGAGGTCTCTTAACccaattaccacacaggtatg
LUZ100T6_R	TATGCTCTTCACTTGGTCTCTAGCCTCATGGGGTTCTCCTCACAG
LUZ100T19_F	TATGCTCTTCAAGAGGTCTCTTAACttctgggatcagtaacaacc
LUZ100T19_R	TATGCTCTTCACTTGGTCTCTAGCCTGACTCGTGCAAGAGCCATGC
LUZ7T50_F	TATGCTCTTCAAGAGGTCTCTTAACttctgggagatcctggcaaac
LUZ7T50_R	TATGCTCTTCACTTGGTCTCTAGCCAGTGAACTGCCGATGGTG

LUZ7T50i_F	TATGCTCTTCAAGAGGTCTCTTAACagtgaactgccgatggtg
LUZ7T50i_R	TATGCTCTTCACTTGGTCTCTAGCCTTCTGGGAGATCCTGGCAAAC
LUZ7T61_F	TATGCTCTTCAAGAGGTCTCTTAACgacggggaggctgggcg
LUZ7T61_R	TATGCTCTTCACTTGGTCTCTAGCCCCAGTCGAGGTGAAGGTGGG
T7wt_F	TATGCTCTTCAAGAGGTCTCTTAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTC
T7wt_R	TATGCTCTTCACTTGGTCTCTAGCCACAAAAAACCCCTCAAGACCCGTTTAGAGG
mCherend_F	TATGCTCTTCAAGAGGTCTCTTAATGGTGAGCAAGGGCGAGG
mCheCYFPend_R	TAGGCTCTTCTCTTGGTCTCATCTTACTTGTACAGCTCGTCCATG
BCD1_PM5_F	TAAGCTCTTCCAGAGGTCTCTGGCTGCCCAAGTTCACTTAAAAAGG
BCD1_PM4_F	TAAGCTCTTCCAGAGGTCTCTTAACGCCCAAGTTCACTTAAAAAGG
BCD1_PM6_R	GCCGCTCTTCTCTTGGTCTCCATTAGAAAGTCTCCTGTGCATGATTAAGATGTTTC
pBG_BsaI_F	GACGGTCTCTAAGAGAATTCGAGCTCGGTAC
pBG_BsaI_R	GTCGGTCTCCTAGATTAATTAAGACGTCTTGAC
phi15T1PM4_R	TATGCTCTTCACTTGGTCTCTGTTAGTATTTCATTTCGATATTCCC
phi15T5PM4_R	TATGCTCTTCACTTGGTCTCTGTTACATCTTGTAGAGCCATAGC
Expression vector optimization – bicistronic design and RT-qPCR	
Phi15prom_F	GACGCTCTTCCAGAGGTCTCGTCTATAAAAACCCACACAATAG
phi15BCD01_R	CTTGGTCTCTCATTAGCCCTCCTCCTGGTTCTGGCCTTGCTTGGTGC
phi15BCD02_R	CTTGGTCTCTCATTAGTTTTTACCTCCTTACTGGCCTTGCTTGGTGC
phi15BCD03_R	CTTGGTCTCTCATTAGTTTTACCTCCTTATCTGGCCTTGCTTGGTGC
phi15BCD04_R	CTTGGTCTCTCATTAGTTTACCTCCTTATTCTGGCCTTGCTTGGTGC
phi15BCD05_R	CTTGGTCTCTCATTAGAAAACCTCCTTATTCTGGCCTTGCTTGGTGC
msfGFP_F	GACGCTCTTCCAGAGGTCTCGAATGATCATGGGAATTCATAAAGGTG
msfGFPend_R	TAGGCTCTTCTCTTGGTCTCATCTTATTTGTAGAGTTCATCCATGCCG
EL_ST_mScarlet_F	AATGCTCTTCTAGAGGTCTCTAATGGTGAGCAAGGGCGAGGCAG
EL_ST_mScarletend_R	TATGCTCTTCTCTTGGTCTCATCTTACTTGTACAGCTCGTCCATG
pBG_BsaI_F	GACGGTCTCTAAGAGAATTCGAGCTCGGTAC
pBG_BsaI_R	GTCGGTCTCCTAGATTAATTAAGACGTCTTGAC
qPCR_msfGFP_F	CACCGCAGACAAACAGAAGA
qPCR_msfGFP_R	ACTGGGTGGACAGGTAGTGG
qPCR_rpoD_F	TCGCCAAGAAGTACACCAAC
qPCR_rpoD_R	TTTCATCAGACCGATGTTGC
qPCR_algQ_F	GAGATCAACCCACGGATCAACGACA
qPCR_algQ_F	TCATGCAACAACCCGCCCAAT
Expression vector optimization – pPUT series	
pPUT_pBG_F	TAGGCTCTTCTTCGGAATTCGAGCTCGGTAC
pPUT_BCD05_R	TAGGCTCTTCTAGAAAACCTCCTTATTCTGGCCTTGCTTGGTGC
pPUT_GFP_F1	TAGGCTCTTCTTCTAATGAGAGACCgcccattgacaaggctctc
pPUT_GFP_F2	TAGGCTCTTCTTCTAATGGCCATAGAGACCgcccattgacaaggctctc
pPUT_GFP_R1	TAGGCTCTTCTCACGTTAAGAGACCttatttgtagagttcatcc
pPUT_GFP_R2	TAGGCTCTTCTCACAAGCAGAGACCttatttgtagagttcatcc

pPUT_GFP_R3	TAGGCTCTTCTCACTACCAGAGACCttatttgtagagttcatcc
pPUT_T5_F	TAGGCTCTTCTGTGGTTACCGTTCGATAAG
pPUT_T1_R	TAGGCTCTTCTCGAGTATTTCATTTCGATATTCCC
pPUT_L7T50_R	TAGGCTCTTCATGCCAGTGAACTGCCGATGGTG
pPUT_BCD05_F	TAGGCTCTTCTGCATAAAAACCCACACAATAGACAGAGG
pPUT_flaPtaU1_F	GGTCTCCGTTACTAACGCGTGATGGATTCGATT
pPUT_flaPtaU1_R	GGTCTCCAATGCATCATCACCACCACCATTTGCA
Growth decoupling module	
phi15gp16_F	GACGCTCTTCCAGAGGTCTCGAATGAATGTCGAAAAGCAAAAG
phi15gp16_R	TAAGCTCTTCTCTTGGTCTCAGTTATTCGACCTCCTTGGGACGCAC
igy_F	GACGCTCTTCCAGAGGTCTCGAATGAAATCCACTGAACCCCAAG
igy_R	TAAGCTCTTCTCTTGGTCTCAGTTAGTGGACCCGATGAACCTTG
rac_F	GACGCTCTTCCAGAGGTCTCGAATGAACAAGTCCATCTGGCGAG
rac_R	TAAGCTCTTCTCTTGGTCTCAGTTAGCGAGTTTGCCGATGATG
pSTDesR_BsaI_F	GACGGTCTCCTAACAACTAGTCTTGGACTCCTGTTG
pSTDesR_RBS_BsaI_R	GTCGGTCTCCCATTGTCGACCACCTCCTGAATTTCATTACGACCAGTC

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