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Engineered promoters enable constant gene expression at any copy number in bacteria

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The internal environment of growing cells is variable and dynamic, making it difficult to introduce reliable parts, such as promoters, for genetic engineering. Here, we applied control-theoretic ideas to design promoters that maintained constant levels of expression at any copy number. Theory predicts that independence to copy number can be achieved by using an incoherent feedforward loop (iFFL) if the negative regulation is perfectly non-cooperative. We engineered iFFLs into *Escherichia coli* promoters using transcription-activator-like effectors (TALEs). These promoters had near-identical expression in different genome locations and plasmids, even when their copy number was perturbed by genomic mutations or changes in growth medium composition. We applied the stabilized promoters to show that a three-gene metabolic pathway to produce deoxychromoviridans could retain function without re-tuning when the stabilized-promoter-driven genes were moved from a plasmid into the genome.

Genetic engineering projects often require balancing of gene expression; however, achieving and maintaining this balance is difficult[1]. For example, in metabolic engineering the expression of enzymes may need to be regulated to optimize flux to the product, and variable expression could result in diversion of carbon to other pathways or in the accumulation of toxic intermediates[2,3]. Similarly, the construction of intracellular molecular machines requires the correct ratios of components[4], which could be confounded by gene expression variability.

Balancing expression can be achieved by using a variety of genetic parts, such as different promoters or ribosome-binding sites (RBSs), to control each gene. Directed evolution also can be used to make random changes, by using mutagenesis and screening thousands of variants for improvements[5,6]. A body of research has been built in efforts to improve measurement[7] and reliability[8] of genetic parts, including the development of computational methods that use this information to guide a search[9]. It is frustrating that after going through such effort to fine-tune a system any changes to the host cells can disrupt gene expression, with 're-tuning' required to fix the problem[10,11]. Changes in host cells can arise due to differences in the growth medium, the growth phase or the environment (such as use of a bioreactor, or of soil or gut microbiota), changing the genetic location of a pathway, adding more genetic systems or through mutation[12]. In metabolic engineering, once a pathway is optimized for expression in one plasmid backbone, it often requires re-tuning when it is moved to a different plasmid or to the bacterial genome[13].

DNA copy number is a huge source of uncertainty when designing genetic systems. Although it is often treated as a constant, the copy number of plasmids can vary widely. Within a clonal population, plasmid copy number varies both over time and between cells due to stochastic fluctuations[14]. Many changes to the cell or the environment—including the host strain used[15,16], composition of the medium[16,17], growth temperature[18] and growth rate[17]—can alter plasmid copy number. Changing the size of a plasmid or the genes being transcribed also can affect plasmid copy number[19–21]. Even the insertion of a system into the genome does not protect it from copy-number effects. Fast-dividing bacteria initiate genome replication more than once per cell division and contain partially replicated genomes, which leads to an enrichment of genes that are located closer to the origin of replication[22]. As a consequence, the average copy number of different locations on the genome can span up to eightfold, depending on the rate of cell division[23]. The stabilization of a genetic system with respect to copy number is hypothesized to improve robustness and enable it to be modified or transferred between genetic locations with less chance of disruption.

The total level of gene expression produced by constitutive promoters closely reflects the copy number at which they are present in the cell[22,24]. Minimal constitutive (unregulated, and thus always on) bacterial promoters consist of DNA sequences, which are recognized by the σ factor of the RNA polymerase holoenzyme complex, and a downstream transcriptional start site (**Fig. 1a**). The performance of minimal promoters can be perturbed by changing the surrounding genetic sequence, and they are often flanked by insulators to counteract this[8] (**Fig. 1a**).

Stabilized promoters incorporate additional elements to decouple gene expression from copy number. Stabilization of a promoter requires the introduction of a regulatory mechanism that detects changes in copy number and compensates promoter activity accordingly. This can be achieved by autoregulatory feedback, integral feedback or iFFLs[25,26]. In comparing approaches, one has to consider design feasibility and the ability to achieve perfect adaptation (i.e., convergence to a constant level of expression regardless of copy number). Although

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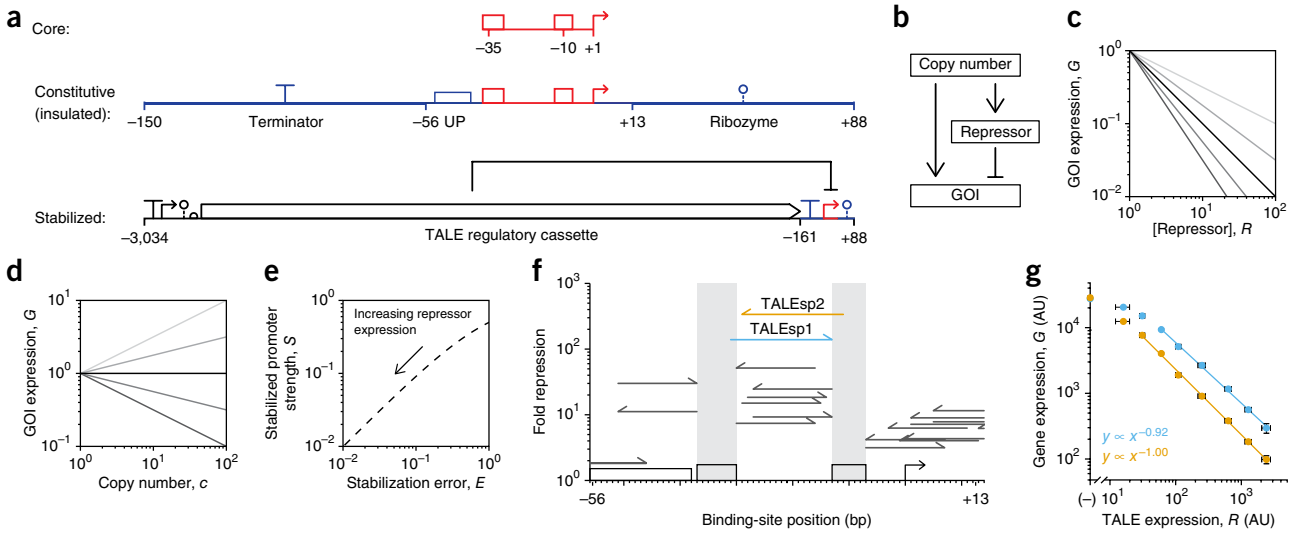

Figure 1 Stabilized promoter design. (**a**) The stabilized promoter is shown along with nested promoters. The minimal, core promoter is shown in red; insulators flanking the minimal promoter are shown in blue; and regulatory elements in the stabilized promoter are shown in black. (**b**) The schematic of the iFFL is shown. Copy number influences both repressor and GOI expression. The repressor counteracts the effect on GOI expression. (**c**) Predictions from the mathematical model for gene expression as a result of repressor concentration (**Supplementary Note 1**). The theoretical power-law response functions for the repression of the promoter are shaded by the cooperativity (n = 1 is black, and from top to bottom, n = 0.5, 0.75, 1.25 and 1.5). (**d**) The predicted stabilization by the response functions from **c**. (**e**) The predicted trade-off between stabilization error (E) and stabilized promoter strength (S) when varying the level of repressor expression (according to equation 19 in **Supplementary Note 1**). (**f**) The ability of TALEs to repress transcription from bacterial promoters, based on the position of their operator. Lines indicate the location of the TALE binding site, and arrows indicate its orientation. Gray lines indicate TALE1 binding sites; the blue line indicates the TALEsp1 binding site; and the orange line indicates the TALEsp2 binding site. (**g**) The response function for the repression of the P_{UPsp1} and P_{UPsp2} promoters by TALEsp1 (blue) and TALEsp2 (orange), respectively. The TALEs were expressed on a second plasmid from the IPTG-inducible P_{Tac} promoter (IPTG concentrations used were (from left to right) 0, 5, 10, 16, 25, 40, 63, and 100 μM) that was characterized separately in parallel using a fluorescent reporter to generate the x axis values (**Supplementary Fig. 4**). On the x axis, the symbol "(–)" indicates a control with no TALE (**Supplementary Fig. 1**). Lines indicate power function fits ($R^2 > 0.99$). AU, arbitrary units. For all data, the mean ± s.d. of three replicates from different days are shown.

autoregulation is straightforward to implement, it cannot achieve perfect adaptation without infinitely cooperative repression, and it has the potential to cause oscillations. In contrast, integral feedback can achieve perfect adaptation but is complicated to implement[27].

We chose to base our approach on an iFFL because it is simple, is predicted to achieve perfect adaptation[25,26] and has been demonstrated to function in a wide range of contexts. Feedforward loops are commonly found in natural transcription networks[28,29] and have been used in a number of engineered systems. In studies most relevant to this work, Benenson and co-workers used transcriptional and posttranscriptional iFFLs to partially decouple gene expression from the amount of DNA transfected into mammalian cells[26]. Similar designs have been shown to reduce changes in gene expression caused by growth differences[30].

A feedforward loop is 'incoherent' when an input signal is split, and it both positively and negatively controls the output[29]. In this case the input signal is copy number, which affects gene expression from all of the promoters in the construct equally. Our design centers on a stabilized promoter, the core of which is the same as the insulated promoter in **Figure 1a**. On its own, expression from this promoter is expected to be positively correlated with copy number, which functions as the input signal. Negative regulation is introduced by making the promoter responsive to a protein repressor, which itself is controlled by an insulated promoter (**Fig. 1b**). Thus, increased copy number leads to increased expression of the repressor, which interacts with the stabilized promoter to cancel out the change in expression of the gene(s) of interest (GOI) caused by copy number.

Transcriptional repression in bacteria at steady-state generally follows the Hill equation. If the dynamic range of repression is high, then this equation approaches a simple power law as the repressor concentration increases above its dissociation constant (**Supplementary Note 1**). Under these conditions, the GOI expression level, G can be modeled as

$$G \propto \frac{c}{R^n} \quad (1)$$

where c is the copy number, R is the concentration of repressor, and n is the cooperativity of repression (**Fig. 1c**). Because repressor expression is controlled with an unregulated promoter, R scales with the copy number and

$$G \propto c^{1-n} \quad (2)$$

Hence, when n = 1 (repression is non-cooperative), GOI expression is predicted to be independent of copy number (**Fig. 1d**). However, there is a tradeoff between the GOI expression level and adaptation. Increasing expression of the repressor lowers the stabilization error (the relative change in GOI expression as copy number is increased) but also decreases GOI expression (**Fig. 1e**). The key parameter is the expression level of the repressor at the lowest copy number; expression must be sufficient to ensure good adaptation but not so high as to render the output too weak to be useful (**Supplementary Note 2**).

We chose TALE proteins to build stabilized promoters because they can be programmed to tightly bind arbitrary DNA sequences[31]

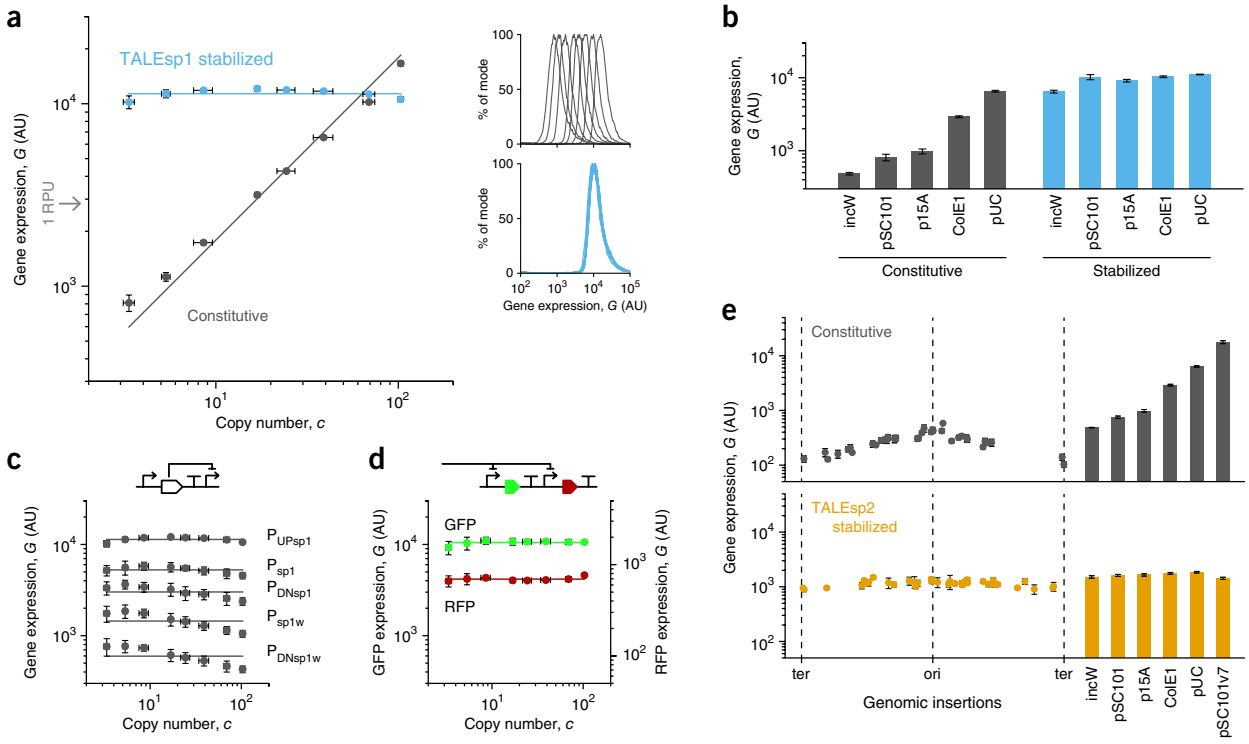

Figure 2 Stabilized promoters compensate for changes in copy number. (**a**) Performance of a constitutive (gray) and TALEsp1 stabilized (blue) promoter on a set of pSC101 plasmid variants with different copy numbers. The *x* axis shows the copy number of each pSC101 variant backbone, as measured using qPCR. Cytometry distributions that correspond to these data are shown to the right. The gray line is a linear fit of the constitutive promoter data, and the blue line shows the geometric mean of the stabilized promoter data. The gray arrow shows the approximate AU value that corresponds to 1 relative promoter unit (RPU) (**Supplementary Fig. 8**). (**b**) Performance of the constitutive or the TALEsp1 stabilized promoter across different plasmid backbones. (**c**) The expression levels achieved by variants of stabilized promoters on the pSC101 plasmid variants are shown. The TALEsp1 expression cassette remained constant, and the promoter names to the right indicate the sequence of the TALE-repressible promoter (**Supplementary Fig. 14**). Each line is the geometric mean of the corresponding data. (**d**) The expression levels of two operons stabilized by TALEsp1 on the pSC101 plasmid variants are shown. The green symbols indicate GFP expression levels driven by the P_{UPsp1} promoter, and the red symbols indicate RFP expression levels driven by the P_{DNsp1} promoter. (**e**) Comparison of the performance of the constitutive promoter (gray) and TALEsp2 stabilized promoter (orange) when integrated into the genome and on different plasmid backbones. ori, genomic origin of replication; ter, genome terminus. For all data, the mean ± s.d. of three replicates from different days are shown.

and because they have previously been shown to achieve ~100-fold repression in *E. coli*[32]. Notably, TALEs bind as monomers to a single operator, which is consistent with non-cooperative repression. To identify the optimal site for the operator, a previously characterized TALE[33] was used, and the location and orientation of the operator was varied in the backbone of a strong constitutive promoter[7] (P_{T7A1}) (**Fig. 1f** and **Supplementary Figs. 1** and **2**). Then, TALEs were designed that bound 18-bp operators and were predicted to not bind to the *E. coli* genome[31]. The TALE-encoding genes were recoded to avoid predicted evolutionary instability[34] (**Supplementary Fig. 3**). Two TALE–promoter pairs (TALEsp1 and TALEsp2) were identified that were orthogonal to each other and generated at least 90- and 230-fold repression, respectively (**Fig. 1g** and **Supplementary Figs. 4** and **5**). Notably, the response functions approached the predicted power-law approximation of Eq. 1 at higher repressor expression levels, with $n = 0.92 \pm 0.03$ and 1.00 ± 0.02 for TALEsp1 and TALEsp2, respectively (**Fig. 1g**).

We characterized the effect of copy number on an insulated constitutive promoter (P_{T7A1w1}; **Supplementary Fig. 6a**) by moving it onto a set of pSC101 plasmid backbones with a range of different copy numbers[35] (**Supplementary Fig. 7**). These plasmids are nearly identical but contain up to four mutations in the pSC101 origin of replication that alter copy numbers regulation. During exponential growth, the copy numbers of these plasmid backbones spanned a range from 3 to 100 copies per cell, and expression from the constitutive promoter varied by 20-fold when present on these backbones (**Fig. 2a**).

We built a stabilized promoter using TALEsp1 (**Supplementary Fig. 6b**) and characterized it with the pSC101 backbones. The promoter and RBS controlling TALE expression were chosen to express the repressor to a level sufficient for good adaptation at copy numbers equal to or greater than the copy number of pSC101 (~3/cell) while maintaining high GOI expression (**Supplementary Figs. 8** and **9**). This stabilized promoter successfully buffered against most of the effects of copy number and showed near-identical expression levels from the various pSC101 backbones (**Fig. 2a**). Notably, the population distributions, as measured by flow cytometry, collapsed into a single distribution (**Fig. 2a**, histograms).

The stabilized promoter was then tested in different plasmid backbones. Five commonly used origins of replication were chosen: incW, pSC101, p15A, ColE1 and pUC. Similar to the pSC101 set, they vary widely in copy number (from ~2 to 38 copies/cell during exponential growth; **Supplementary Fig. 10**), but they also vary in size, the genes expressed and, possibly, in cellular localization[36]. When the constitutive promoter was used in each backbone, expression varied 16-fold

(**Fig. 2b**). In contrast, the stabilized promoter was able to eliminate most of the variability (**Fig. 2b**). Similar results were seen in other strains of *E. coli* and when multiple plasmids were maintained in one cell (**Supplementary Figs. 11** and **12**).

Stabilized promoters have to be tunable so that the expression level can be set depending on the optimal expression level required for a gene. Additionally, it is undesirable for the promoter controlling each gene in a genetic system to have its own corresponding TALE, because this would increase the construct's DNA size and the number of characterized repressors required for regulation. Thus, we built a series of stabilized promoters that generated varied expression levels and were controlled by the same TALE (**Supplementary Fig. 13a**). We made mutations to the TALEsp1-repressible promoter sequence (P_{UPsp1}) to generate four promoters with a range of strengths that were effectively repressed by the TALEsp1 repressor (**Supplementary Fig. 14**). The promoters were combined with the TALEsp1 expression cassette to create a set of four variant stabilized promoters that represented a range of strengths. All of the promoters were able to maintain consistent expression levels across a wide range of copy numbers (**Fig. 2c**). In addition, the RBS controlling expression of the GOI could be changed without considerably affecting stabilization (**Supplementary Fig. 15**).

With these promoters, one TALE can be used to stabilize multiple operons at different ratios, a feat required for more complex genetic systems, such as metabolic pathways and molecular machines[6]. The P_{UPsp1} and P_{DNsp1} promoters were used with the TALEsp1 expression cassette in a single cell to maintain expression of green fluorescent protein (GFP) and red fluorescent protein (RFP) at high and low levels, respectively (**Supplementary Fig. 13b**). This multi-operon stabilized system maintained a nearly constant ratio of gene expression across the pSC101 plasmid set (**Fig. 2d**).

To use stabilized promoters at copy numbers below the copy number of pSC101, we built a second stabilized promoter using the TALEsp2 repressor. For this promoter, TALE expression was set to a higher level so that good stabilization could be achieved at copy numbers down to ~1/cell (**Supplementary Figs. 6c** and **9**). The higher level of TALE expression led to a lower level of expression from the stabilized promoter (**Supplementary Note 1**). The expression of TALEs had only a slight effect on the growth rate when carried on the highest-copy plasmids (**Supplementary Figs. 16** and **17**).

The TALEsp2 stabilized promoter was tested in the genome for its ability to buffer against copy-number differences caused by rapid cell division. The stabilized promoter driving GFP expression was flanked by insulators and inserted randomly in the genome by using a Tn5 transposon system[37] (**Supplementary Fig. 18** and Online Methods). The positions of single-insertion events were determined using arbitrary PCR. This yielded strains with 35 insertions of the stabilized promoter distributed across the genome. This process was repeated with the insulated constitutive promoter to create 35 additional strains with distributed insertions (**Supplementary Table 1**). As expected[22,23], the library of constitutive promoter insertions showed a clear trend of maximal expression near the genomic origin of replication (**Fig. 2e** and **Supplementary Fig. 19**). In contrast, the library of stabilized promoter insertions showed almost no position-dependent differences in gene expression (**Fig. 2e** and **Supplementary Fig. 19**). Furthermore, the levels of expression from the stabilized promoter matched those obtained when the promoters were carried on plasmids (**Fig. 2e**, bars). This demonstrates that a stabilized promoter can allow a system to be moved from plasmids to arbitrary locations in the genome without greatly impacting the level of expression.

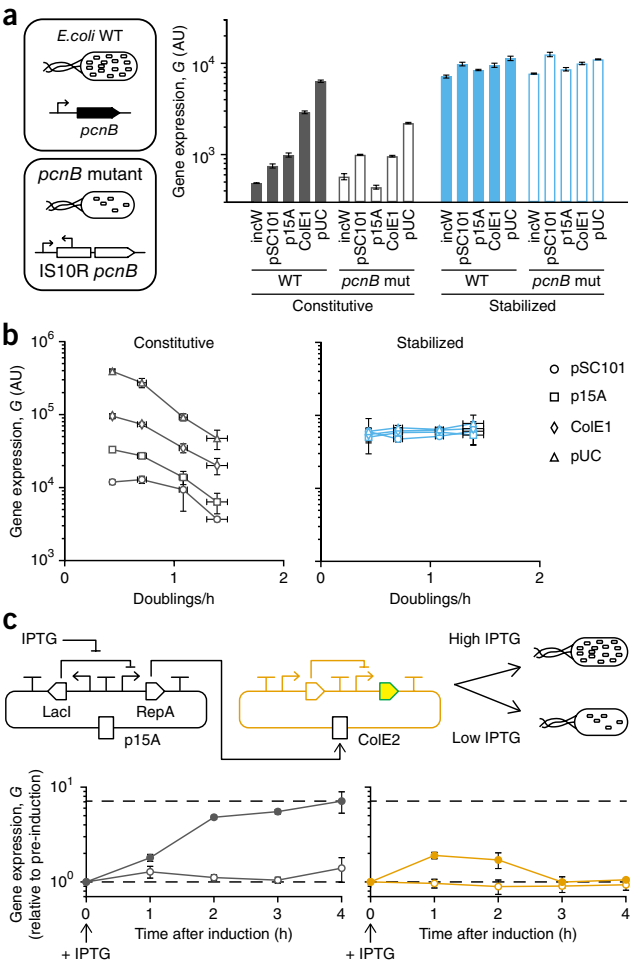

Figure 3 Stabilized promoters reduce the effect of perturbations that affect copy number. (**a**) Performance of a constitutive promoter (gray) and the TALEsp1 stabilized promoter (blue) across plasmid backbones in wild-type *E. coli* DH10B and in a mutant strain with an insertion element (IS10R) that disrupts *pcnB*. (**b**) Performance of the constitutive and the TALEsp1 stabilized promoter during exponential growth when *E. coli* DH10B was grown in different media. Each line is a different plasmid backbone. From left to right, the medium used was: (i) M9 with 0.4% glycerol and 0.5 mM leucine, (ii) M9 with 0.4% glucose and 0.5 mM leucine, (iii) M9 with 0.4% glycerol and 0.2% casamino acids, and (iv) M9 with 0.4% glucose and 0.2% casamino acids. The average doubling time of *E. coli* in each medium was determined (Online Methods). (**c**) The transient response of a constitutive promoter (gray) and the TALEsp2 stabilized promoter (orange) to a change in copy number, as measured using a plasmid whose copy number was controlled by a LacI-repressed, IPTG-inducible *trans*-factor. After cultures reached steady-state with 10 μM (low) IPTG, the concentration was increased to 40 μM (high) IPTG (solid circles), which led to an increase in copy number when compared to cultures that were treated with only 10 μM IPTG (hollow circles). Gene expression is shown normalized to pre-induction levels. The dashed lines indicate the maximum and minimum values from the constitutive system. For all data, the mean ± s.d. of three replicates from different days is shown.

The stabilized promoters were designed to buffer against changes in DNA copy number. However, many perturbations can affect gene expression through DNA copy number as an intermediate. It is expected that stabilized promoters would additionally buffer against these changes. For example, mutations to the host genome can affect

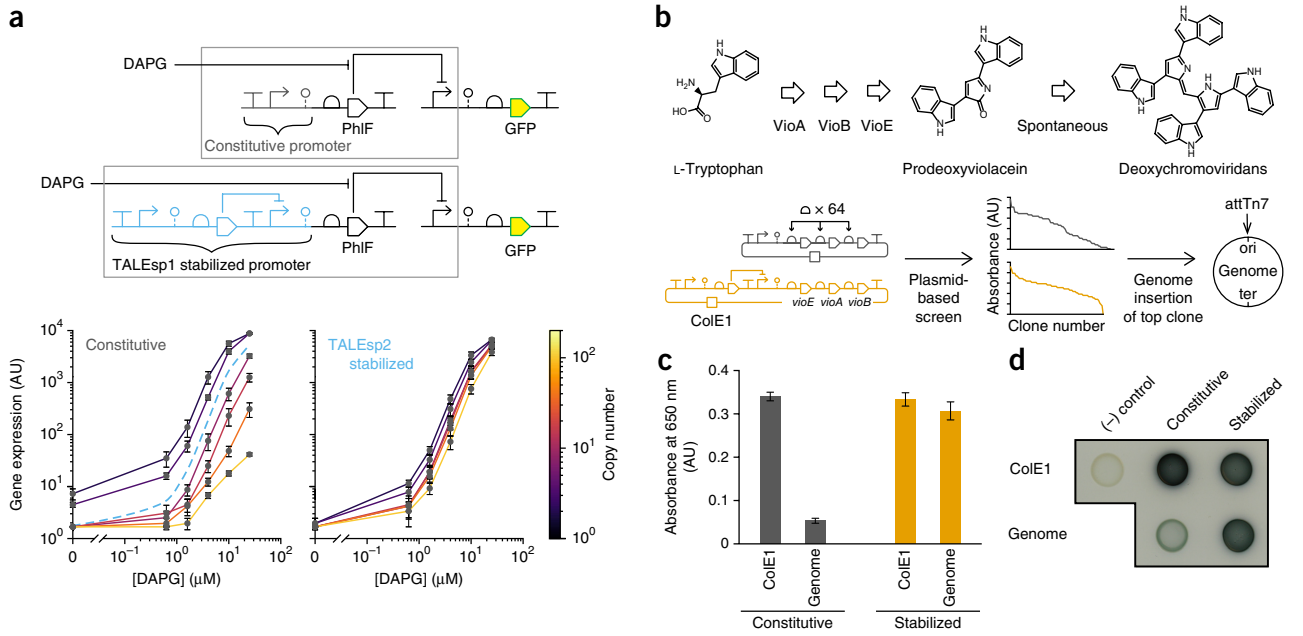

Figure 4 Copy-number stabilization of a small-molecule sensor and metabolic pathway. (**a**) The PhlF repressor was expressed from different plasmid backbones (low to high copy: incW, pSC101, pSC101v2, ColE1, pUC and pSC101v7), using either the constitutive promoter or the TALEsp1 stabilized promoter. PhlF represses a P_{PhlF} promoter on a p15A backbone, which can be induced by titrating DAPG. The dashed blue line indicates the geometric mean of the TALEsp2 stabilized induction curves. The mean ± s.d. of three replicates from different days is shown. (**b**) RBS libraries of the three-gene (*vioA*, *vioB* and *vioE*) deoxychromoviridans pathway driven by either a constitutive promoter (gray) or TALEsp2 stabilized promoter (orange) were constructed and screened on a ColE1 plasmid. The titer was measured as an absorbance, and the rank orders of 94 (constitutive) and 84 (TALEsp2) screened clones are shown. (**c**) Comparison of deoxychromoviridans production after 12 h of growth in liquid LB medium from tuned pathways driven by the constitutive (gray) or TALEsp2 stabilized (orange) promoter. The pathways were characterized on a ColE1 plasmid or when integrated into the attTn7 site in the genome. The mean ± s.d. of three replicates from different days is shown. (**d**) Representative image ($n = 3$) of deoxychromoviridans production from the strains shown in **c** after 24 h of growth on a LB agar plate. "(–)" indicates cells that did not contain the deoxychromoviridans pathway.

plasmid replication systems[15,16]. In previous work, we serendipitously observed this effect when the growth effect of a toxic genetic circuit carried on a plasmid was reduced by the host strain's acquisition of a genomic mutation that reduced the copy number[38]. This occurred due to the insertion of a mobile genetic element upstream of *pcnB*, which encodes a protein that affects the copy number of plasmids that rely on RNA regulation (e.g., p15A, ColE1 and pUC)[15]. The stabilized promoter was able to ameliorate the effect of this mutation and achieve similar levels of expression across both strains for all of the plasmid backbones (**Fig. 3a**). This stabilization has the potential to improve evolutionary robustness, as the use of a stabilized promoter eliminates some mutational paths that could disrupt function. It is analogous to increasing the stability of a genetic construct by eliminating repeats that provoke homologous recombination or by removing transposons from a strain[34,39].

Changes in medium and growth conditions also can change copy number and break genetic circuits and metabolic pathways[40,41]. We tested whether a stabilized promoter could reduce the variation in gene expression caused by different growth media (**Fig. 3b**). Four variants of M9 medium were made with different carbon sources (glucose or glycerol) or amino acids (casamino acids or leucine) (Online Methods). These changes in composition led to large differences in the growth rate, with average doubling times ranging between 40 and 140 min (**Fig. 3b**). Furthermore, medium composition is known to affect plasmid copy number differentially depending on the plasmid's origin of replication[16,17]. When the performance of the constitutive promoter was compared between all of the different media and the origins of replication, there was a 90-fold spread in expression levels (**Fig. 3b**). The TALEsp1 stabilized promoter eliminated most of this effect (**Fig. 3b**).

The copy number of plasmids and of the genome can change dynamically. For example, as cells shift from exponential to stationary phase, the copy number of plasmids can go up by fourfold to fivefold[19,24]. Similarly, the relative copy number of the genome can change as a function of the cell division rate[22,23]. iFFLs are predicted to respond transiently to perturbations, returning to their set point without overcorrection, a property called 'disturbance rejection' in control theory[42]. Experiments were designed to measure the temporal response of stabilized promoters to a change in copy number. Copy number can be controlled by using a ColE2 plasmid whose *trans*-acting RepA protein is under isopropyl-β-D-thiogalactoside (IPTG) control from a second plasmid[24] (**Fig. 3c** and **Supplementary Fig. 20**). When the concentration of IPTG was raised from 10 μM to 40 μM, the expression of GFP from a constitutive promoter increased by sixfold (**Fig. 3c**). The TALEsp2 stabilized promoter showed a transient increase of twofold and returned to the set point after 3 h (**Fig. 3c**).

The stabilized promoters were then evaluated for their ability to reduce the copy-number dependence of synthetic regulation. Genetic sensors respond to an environmental stimulus by changing the activity of an output promoter[43]. How the output changes as a function of the stimulus at steady-state is referred to as the 'response function'. When a sensor is based on a regulatory protein, the response function will be sensitive to genetic context due to changes in expression. Here we evaluated how stabilized promoters could be applied to maintain a

constant response function irrespective of the genetic location of the regulatory protein. This was applied to a 2,4-diacetylphloroglucinol (DAPG) sensor, which is based on the PhlF repressor[44] (**Fig. 4a** and **Supplementary Fig. 21**). When a constitutive promoter controlled PhlF, the response function varied substantially depending on the plasmid backbone (**Fig. 4a**). In contrast, when PhlF was controlled with a TALEsp1 stabilized promoter, the response functions collapsed onto a single curve (**Fig. 4a**).

There is a trade-off in metabolic engineering between plasmids, which are easy to manipulate, and genomic insertion, which is required for genetic stability of cells grown in a bioreactor. It is much easier to build libraries of pathways on plasmids, for example by using part variants[6,9] or directed evolution[5]. However, library constructs may perform differently when inserted into the genome. Stabilized promoters offer a solution; once a variant is identified it should be possible to move it into the genome without negatively affecting activity. We tested whether this was the case by using a three-gene operon that encoded a pathway to synthesize deoxychromoviridans. For product to be synthesized most effectively, specific expression levels of these three genes are required[3]. To this end, we constructed libraries by simultaneously varying all three RBSs and screening for deoxychromoviridans production titer (**Fig. 4b**, **Supplementary Fig. 22** and Online Methods). When a tuned pathway was controlled by a constitutive promoter, the activity declined considerably after insertion into the genome (**Fig. 4c**), which could be seen clearly by eye (**Fig. 4d**). However, when a tuned pathway was controlled by the TALEsp2 stabilized promoter, the titer was preserved after genomic insertion (**Fig. 4c**,**d**). It is noteworthy that the titers achieved by RBS libraries that were made by using the constitutive and stabilized promoters were nearly identical. This result demonstrates that the use of stabilized promoters enables a variant discovered by directed evolution of a plasmid-borne library to be integrated into the genome with no need for re-tuning.

This project started with a simple question. Could we design a promoter that produces the same protein concentration no matter where it is placed? Based on a simple model, we were able to design a class of stabilized promoter that maintained the same level of gene expression irrespective of the plasmid backbone or its location in the genome. This was achieved by harnessing the feedforward loop, a common motif in natural regulatory networks that is responsible for maintaining homeostasis between proteins, implementing dynamic ordering and producing a pulse of gene expression[29,45–47]. Although our stabilized promoter was designed to buffer gene expression against the effects of changing DNA copy number, our results demonstrated broad robustness of the promoter design to genome mutations and medium composition. Collectively, robustness to these conditions eliminates much of the context dependence that plagues precision genetic engineering.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

T.H.S.-S., E.D.S. and C.A.V. conceived the study and designed the experiments; T.H.S.-S. performed the experiments and analyzed the data; and T.H.S.-S., E.D.S. and C.A.V. wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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Supplementary Information for:

Constant gene expression at any copy number using feedforward stabilized promoters
Thomas H. Segall-Shapiro, Eduardo D. Sontag, Christopher A. Voigt

Supplementary Notes

Supplementary Figures

Supplementary Tables

Supplementary Note 1: Guidelines for building stabilized promoters

Two stabilized promoters - TALEsp1 and TALEsp2 – are described in this manuscript. In this note, we detail the steps to build new stabilized promoters, tuned to achieve copy number independence. These calculations assume that the repressors used in the new stabilized promoters have a response function that follows a noncooperative Hill function with no leak (Equation 14).

1. *Determine the desired expression level of the repressor per copy number.* Three parameters guide tuning the repressor expression level in a stabilized promoter: the lowest copy number at which the promoter will function, acceptable stabilization error, and desired promoter strength. The most important parameter is the lowest copy number, since this influences both the maximum promoter activity and how the expression level of repressor affects stabilization error. Based on the lowest copy number c_{min}, the required expression level of repressor per copy β relative to its dissociation constant for binding the promoter K_d can be calculated as (following from Equation 15):

$$\frac{\beta}{K_d} = \frac{1}{\mathrm{E}c_{min}} \quad , \tag{18}$$

where E is the acceptable stabilization error. From this, it follows that lower errors require higher repressor expression. However, there is also a tradeoff where higher repressor expression leads to lower promoter strength S (following from Equation 16),

$$S = \frac{1}{\frac{\beta c_{min}}{K_d}+1} \quad . \tag{19}$$

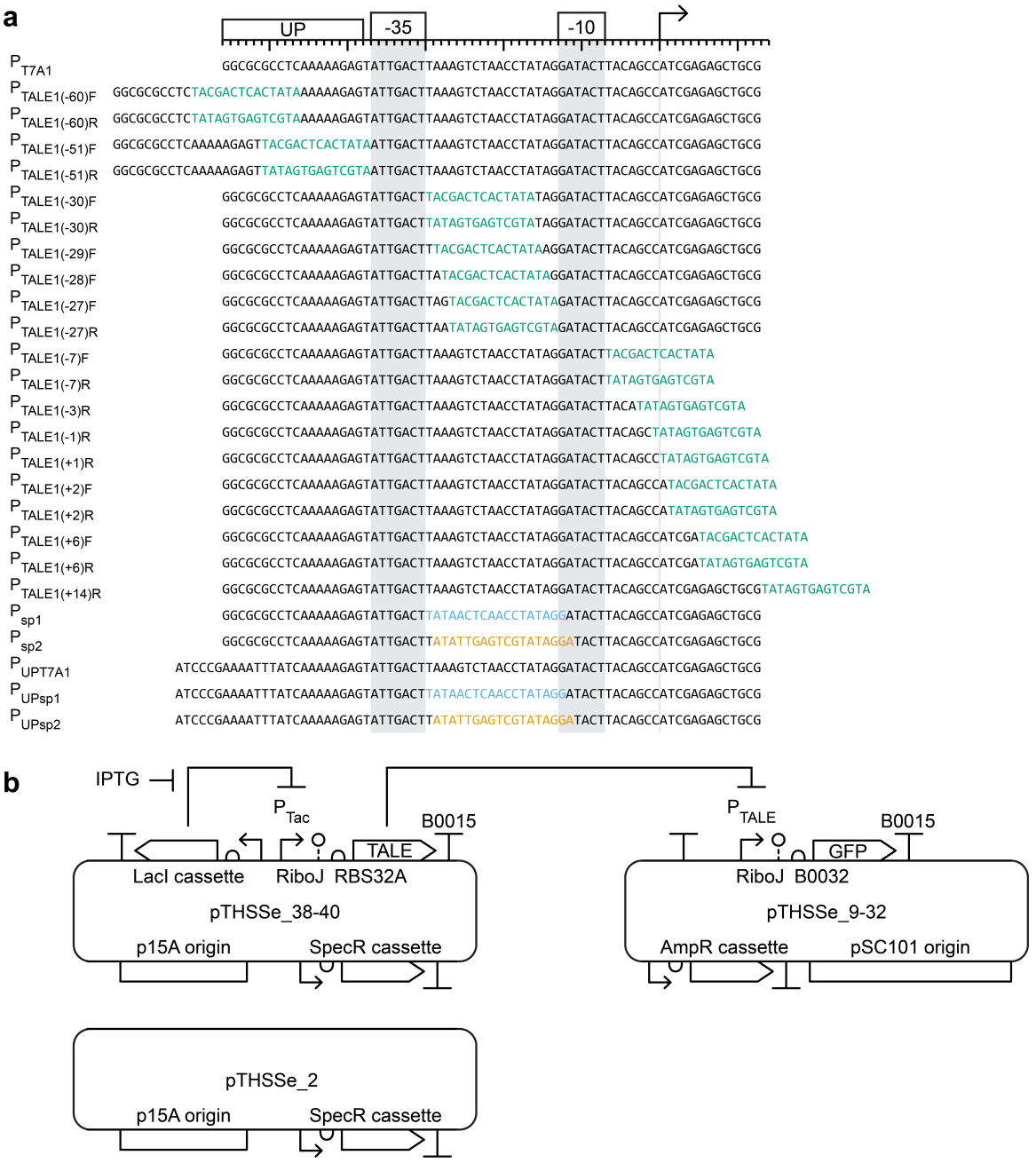
Balancing these effects is dependent on the expression of the repressor relative to its K_d. In practice, we have found that this balance is achieved by expressing the repressor to 2-5x the K_d at the lowest copy number, depending on whether strength or accuracy is prioritized. Supplementary Figure 9 shows this tuning for TALEsp1 and TALEsp2.

2. *Determine the amount of gene expression to achieve the desired repressor expression level.* It is difficult to directly measure the repressor concentration or its dissociation constant in cells. However, β / K_d can be inferred from the gene expression of a stabilized promoter G divided by the gene expression from an unrepressed promoter at the same copy number $G|_{\beta=0}$ using Equation 14. This relationship can be used to guide the construction of tuned stabilized promoters. The repressible promoter should therefore be characterized when completely unrepressed at a specific copy number (ideally c_{min}). With this value, a gene expression level can be determined that corresponds to the desired amount of repressor expression.

$$G = \frac{G|_{\beta=0}}{1+\frac{\beta c}{K_d}} \quad . \tag{20}$$

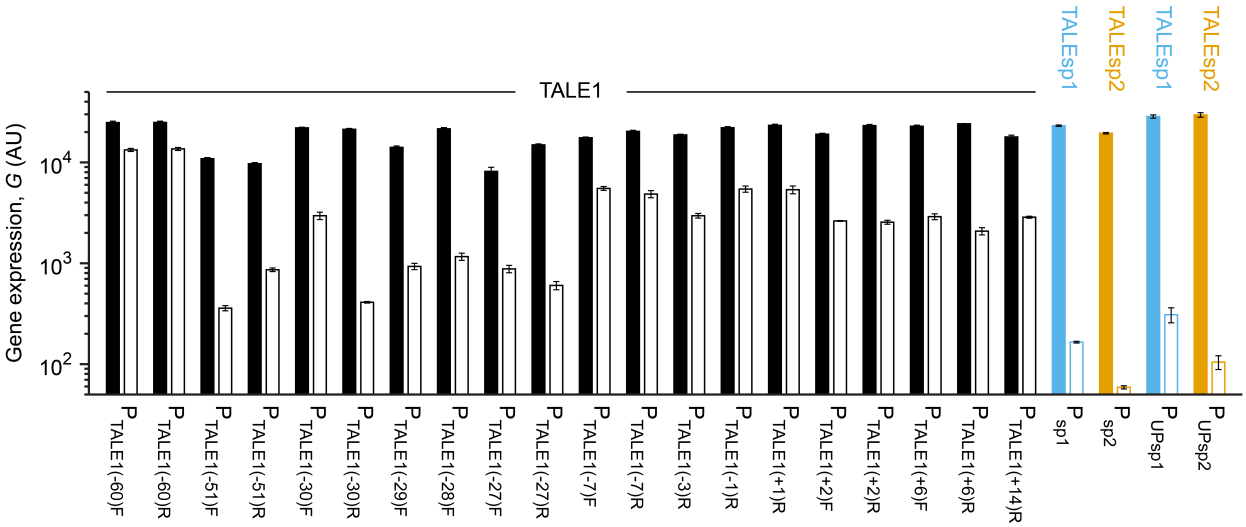
3. *Build a stabilized promoter to the specifications determined from steps 1-2.* In practice, we have found that the simplest approach is to construct a library of stabilized promoters with different promoters and RBSs driving repressor expression in order to find a variant with the correctly tuned parameters. This library should be constructed at the same copy number as the unrepressed promoter in step 2. By comparing gene expression from the library members with that of the unrepressed promoter using Equation 20, library members can be identified that are tuned to the correct value of β.

Addressing the potential growth impact of stabilized promoters. Very high levels of repressor expression – corresponding to low acceptable *E*, low *S*, and high copy numbers – can lead to toxicity (Supplementary Figure 18). This typically occurs as a threshold, beyond which toxicity is observed. Depending on the repressor, this may lead to an additional constraint that needs to be incorporated into the promoter design. To determine if this is an issue before stabilized promoter construction, a response function relating repressor concentration in arbitrary units *R* to fold-repression should be measured (similar to Fig. 1g) along with a measurement of the growth impact of expressing the repressor from the same system (similar to Supplementary Fig. 18). Assuming that repression follows a perfect non-cooperative Hill function, the transfer function should follow Equation 20 with $\beta c \propto R$. Using the desired β / K_d value with the highest copy number that the stabilized promoter is intended to work on, a repression value can be calculated, mapped onto the response function, and the equivalent amount of repressor expression checked for a growth impact.

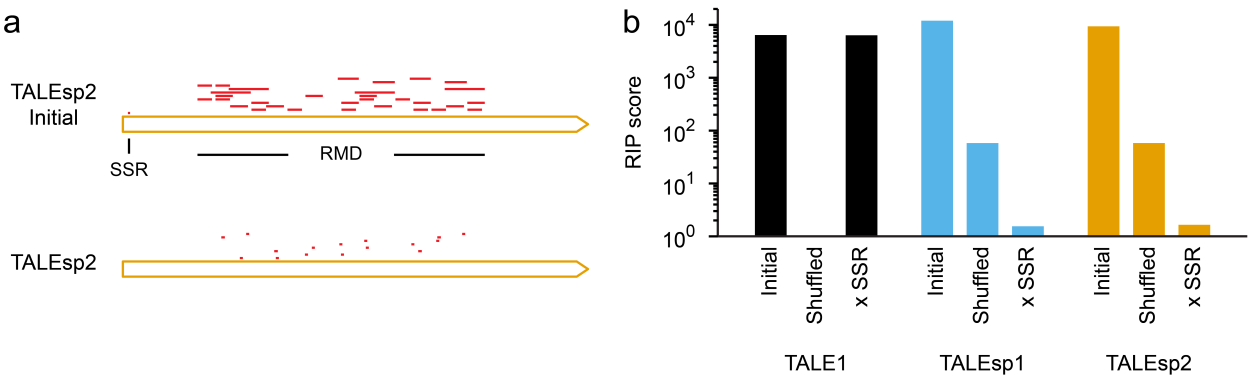


Supplementary Figure 1: Constructs used to test TALE repression.

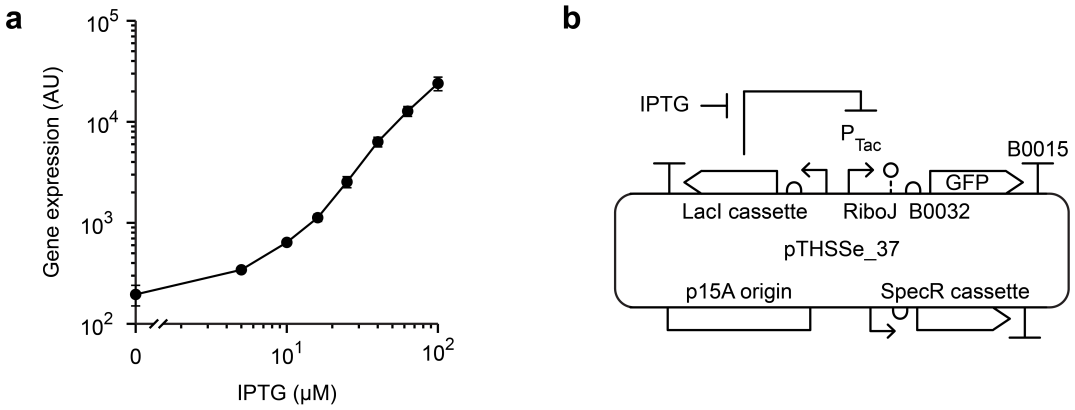
a) Promoter sequences of the TALE repressible promoters are shown along with the constitutive promoters from which they were constructed. $P_{TALE1(-60)F}$ through P_{sp2} were built from promoter PT7A1 and are used in Figure 1f. P_{UPsp1} and P_{UPsp2} are versions of P_{sp1} and P_{sp2} that include more of the native P_{T7A1} UP element and are slightly stronger. They were used in Figure 1g and in the strongest stabilized promoters. Green letters show TALE1 binding sites, blue letters show TALEsp1 binding sites, and orange letters show TALEsp2 binding sites. **b)** Diagram of plasmids used to test TALE repression. pTHSSe_38-40 were used to express each TALE under the control of a P_{Tac} expression system. pTHSSe_2 was used instead when characterizing promoters in the absence of TALE repression. pTHSSe_9-32 were used to measure gene expression from the TALE-repressible promoters shown in (a) using GFP.



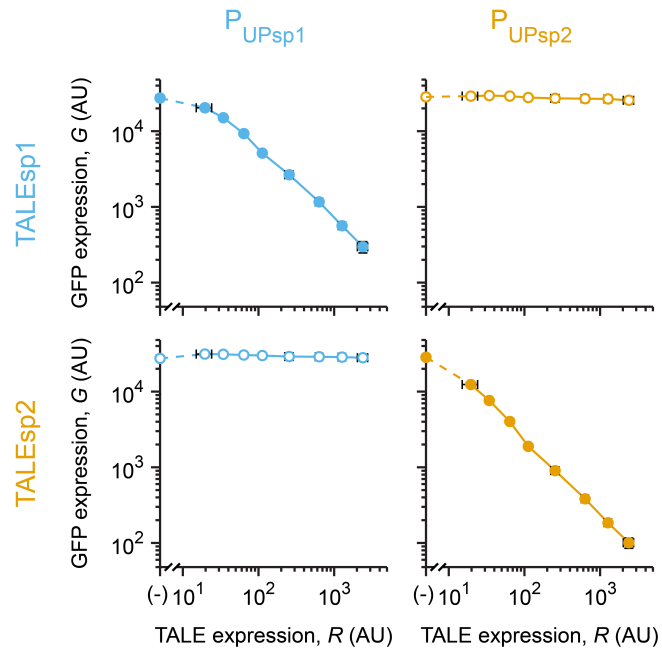
Supplementary Figure 2: Expression levels for TALE repressible promoters.
These data correspond to the fold-change values presented in Figure 1f. Solid bars show gene expression in the absence of the TALE, and hollow bars show gene expression when the TALE is present and induced by 100 μM IPTG. P_{UPsp1} and P_{UPsp2} show the equivalent values from Fig. 1g for comparison. The average of three replicates from different days is shown, and error bars indicate the standard deviation.



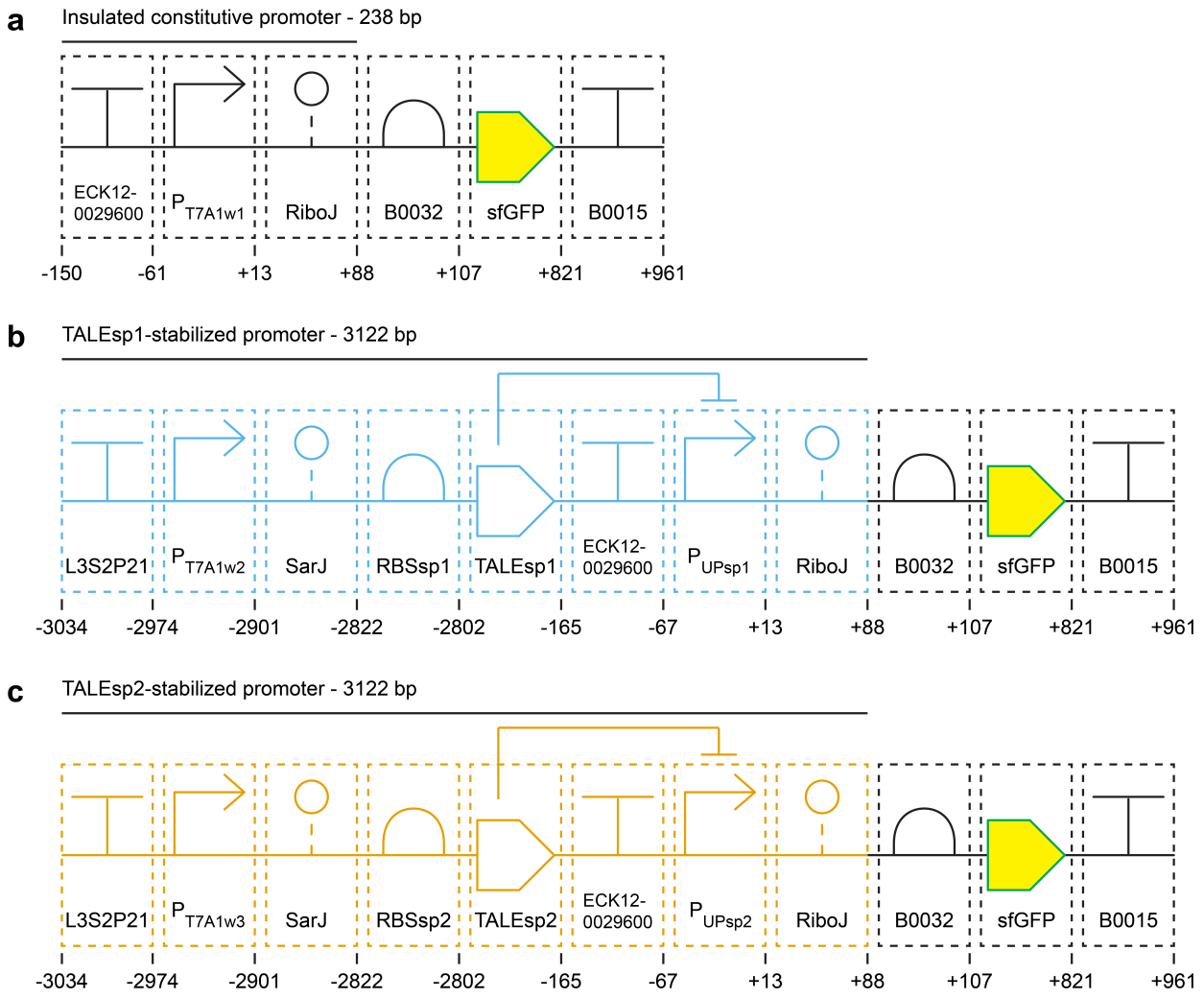
Supplementary Figure 3: Predicted evolutionary stability of TALE genes.
The TALE repressors were initially built using TypeIIs assembly methods, which lead to large repeated sections of DNA sequence. This can make them unsuitable for use in bacteria due to high rates of homologous recombination. The Evolutionary Failure Mode (EFM) calculator[1] predicted that the initial TALE sequences would lead to large evolutionary instabilities in bacteria, due to repeat mediated deletions (RMD) and a simple sequence repeat (SSR). We used Genome Calligrapher[2] and additional manual recoding to disrupt repeated sections and remove the SSR. **a)** The instabilities identified by the EFM calculator are shown for the initial and final versions of the TALEsp2 repressor. Only the worst 10 RMDs are shown. **b)** The relative instability prediction (RIP) score calculated by the EFM calculator is shown for the initial and improved versions of the TALE repressors. This score indicates how much more likely a DNA sequence is to mutate compared to unavoidable random point mutations, with a value of 1 indicating no added mutational propensity. The TALEsp1 and TALEsp2 repressors were re-synthesized with different codons to remove repeats and the SSR was removed. The TALE1 repressor was not re-synthesized, but had the SSR removed so that its N-terminus matches TALEsp1 and TALEsp2. Note that these constructs were designed as a prophylactic against recombination; whether they are more evolutionarily stable was not tested experimentally.



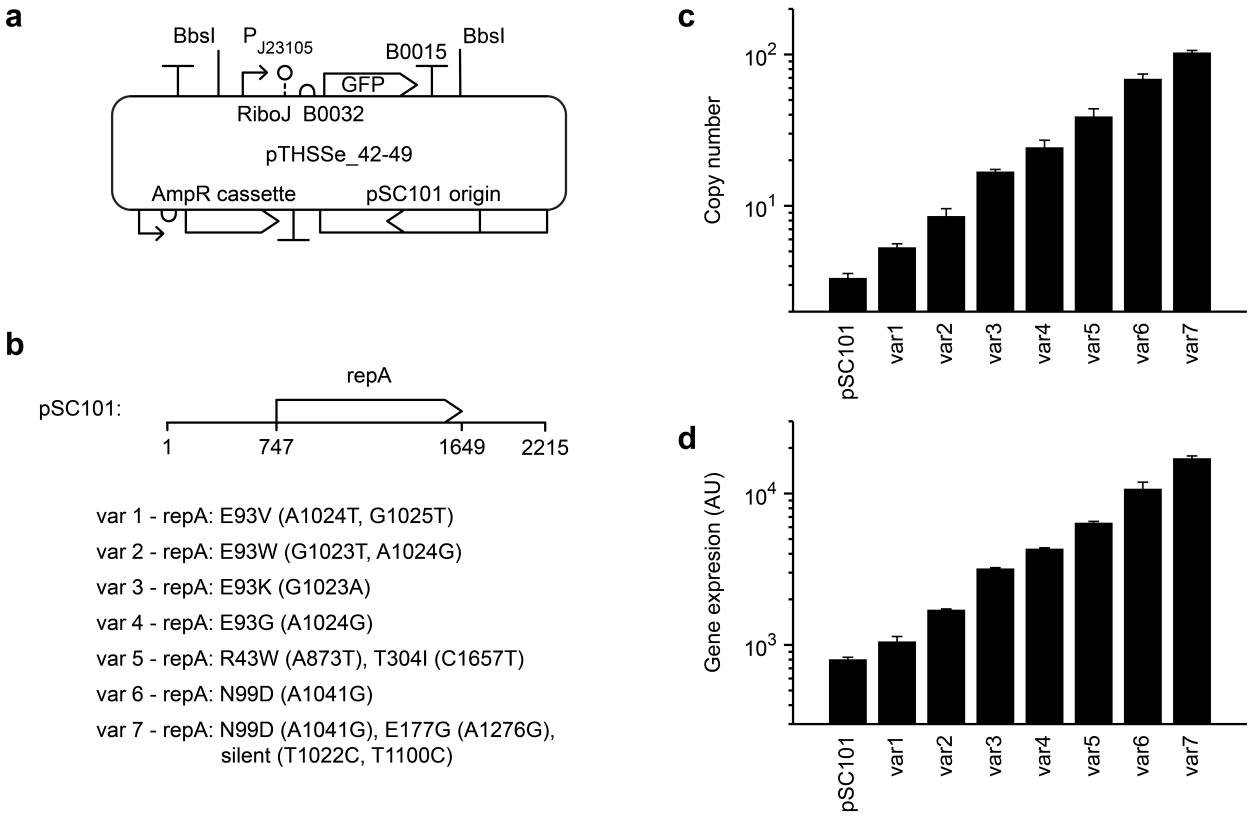
Supplementary Figure 4: Gene expression from the P_{Tac} promoter.
a) GFP expression from the P_{Tac} promoter used to induce TALE expression is shown. These values were used on the x-axis of Figure 1g to compare the relative expression of the TALE at different inducer levels. These data were collected in parallel with the experiments used to build the plots in Figure 1. The average of three replicates from different days is shown, and error bars indicate the standard deviation. **b)** The plasmid used to measure the P_{Tac} promoter. This plasmid is identical to pTHSSe_38-40, except the TALE gene has been replaced with GFP, controlled with a slightly altered RBS.



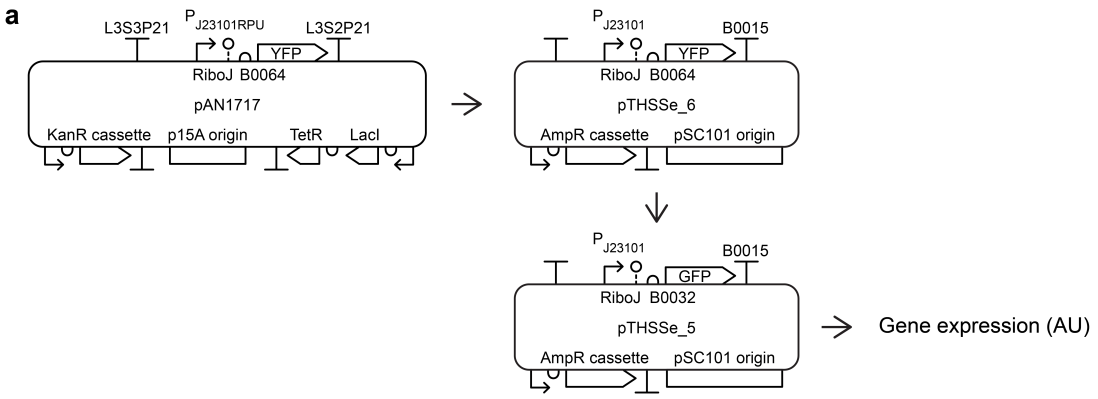
Supplementary Figure 5: Orthogonality of TALE repressors.
The ability of TALEsp1 and TALEsp2 to repress the P_{UPsp1} and P_{UPsp2} promoters is shown. The TALEs are expressed on a separate plasmid from the IPTG-inducible P_{Tac} (left to right, 0, 5, 10, 16, 25, 40, 63, and 100 μM) that was characterized separately in parallel using a fluorescent reporter to generate the x-axis values (Supplementary Fig. 4). On the x-axis, (-) indicates a control with no TALE (Supplementary Fig. 1). The on-target data is identical to what is shown in Fig. 1g. The average of three replicates from different days is shown, and error bars indicate the standard deviation.



Supplementary Figure 6: Design of stabilized and insulated promoters.
Parts diagrams and lengths are shown for the insulated constitutive promoter **(a)**, TALEsp1-stabilized promoter **(b)**, and TALEsp2-stabilized promoter **(c)**. These systems are depicted driving a GFP expression cassette, which was used for most experiments. All base pair numbers are relative to the transcription initiation site of the stabilized promoter. Sequences for the parts can be found in Supplementary Table 4.

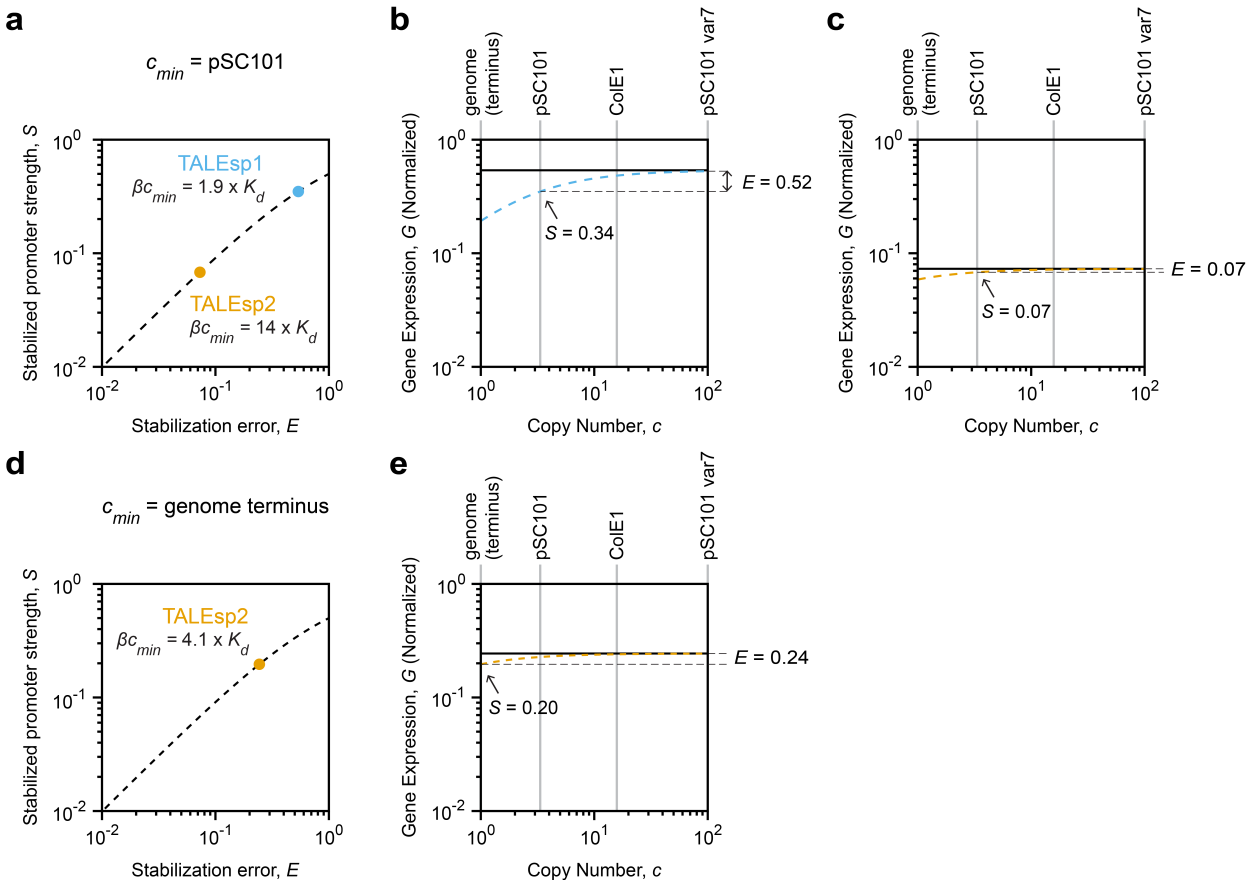


Supplementary Figure 7: pSC101 backbones with varying copy number.
a) Diagram of pSC101 plasmids used to characterize promoter function at different copy numbers. Each backbone contains a constitutive GFP expression cassette. The insulated and stabilized promoters were cloned into the BbsI sites. **b)** A list of pSC101 origin variants that were used in this work. Var 1-4 were constructed by targeted mutagenesis of the E93 codon in the *repA* gene following a previous study[3]. Var 5-7 were constructed through error-prone PCR of the *repA* gene (Methods). **c)** The copy numbers of the pSC101 plasmids, measured by qPCR in exponential growth, are shown (Methods). These data were used as the x-axis in Figure 2a and elsewhere where these plasmids were used. **d)** GFP expression from the constitutive GFP cassette on each plasmid backbone. For all data, the average of three replicates from different days are shown, and error bars indicate the standard deviation.

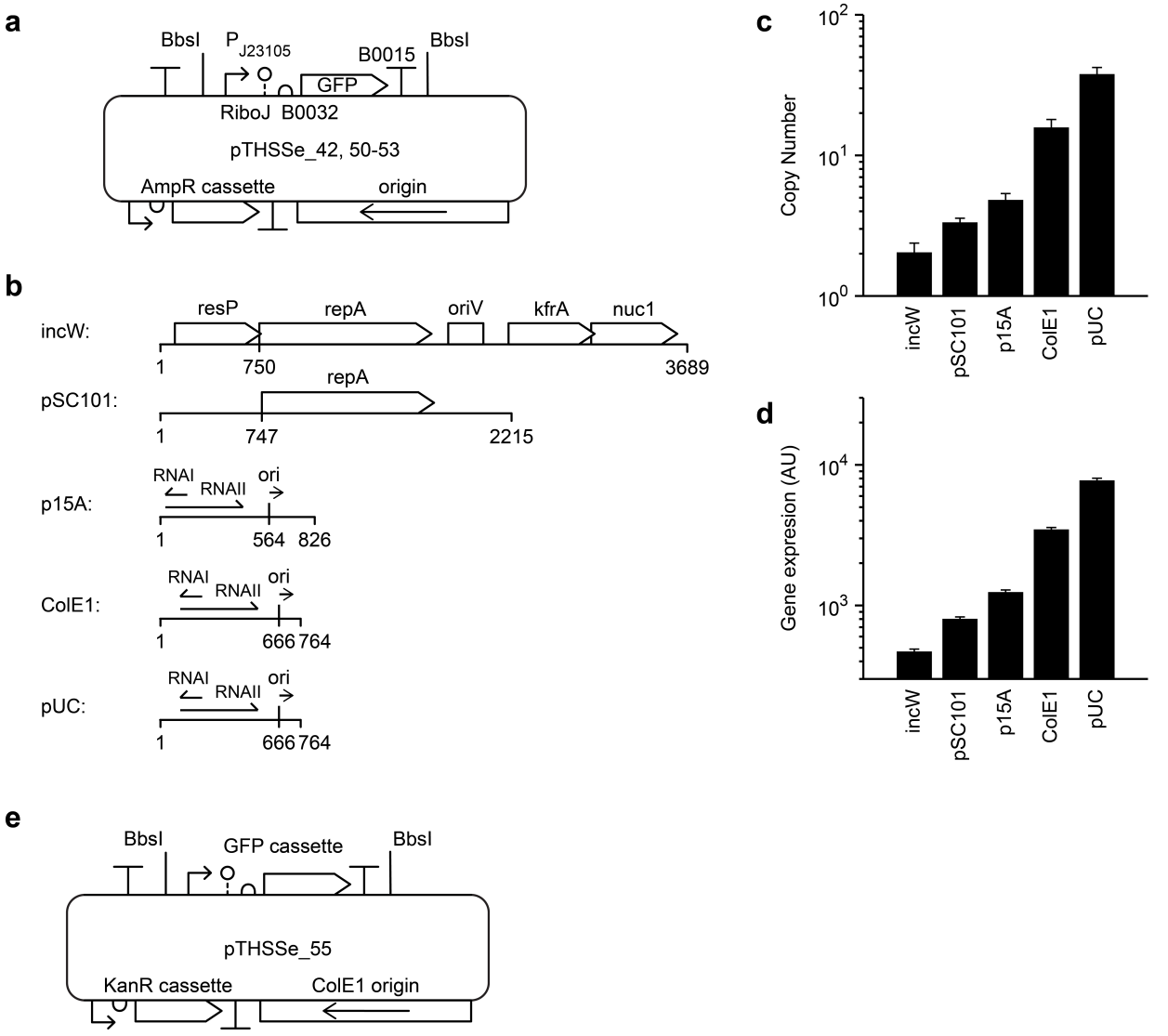


Supplementary Figure 8: RPU standard measurement.

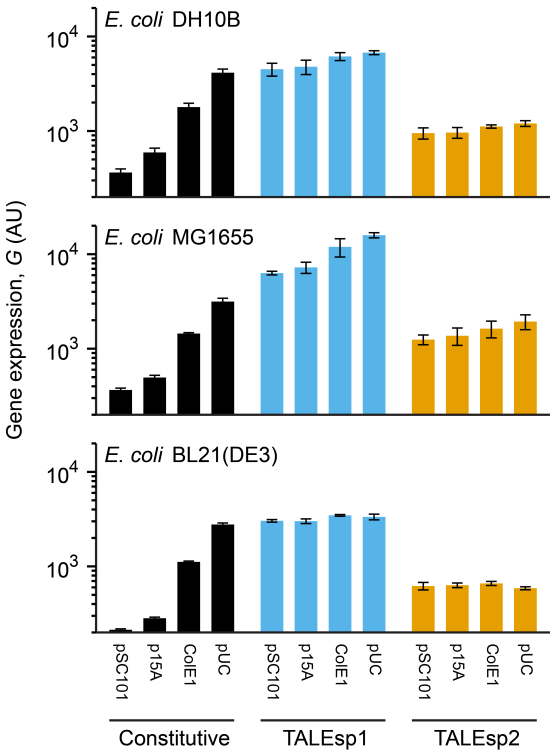
The RPU standard plasmid pAN1717[4] was measured to determine the gene expression level of 1 RPU. This is equivalent to approximately 4.2x the Kelly REU standard[5]. Since pAN1717 uses a different fluorescent protein expression cassette than the systems used in this work, pTHSS_5 and pTHSS_6 were used to convert the RPU measurement to be equivalent to the AUs used elsewhere. These plasmids are identical except for the fluorescent protein and RBS. pTHSS_5 constitutively expresses sfGFP with the B0032 RBS used in this work, pTHSS_6 constitutively expresses EYFP with the B0064 RBS used in pAN1717. To convert the RPU standard to equivalent AUs, the amount of fluorescence produced by pAN1717 was divided by the fluorescence produced by pTHSSe_6, then multiplied by the fluorescence produced by pTHSS_5.



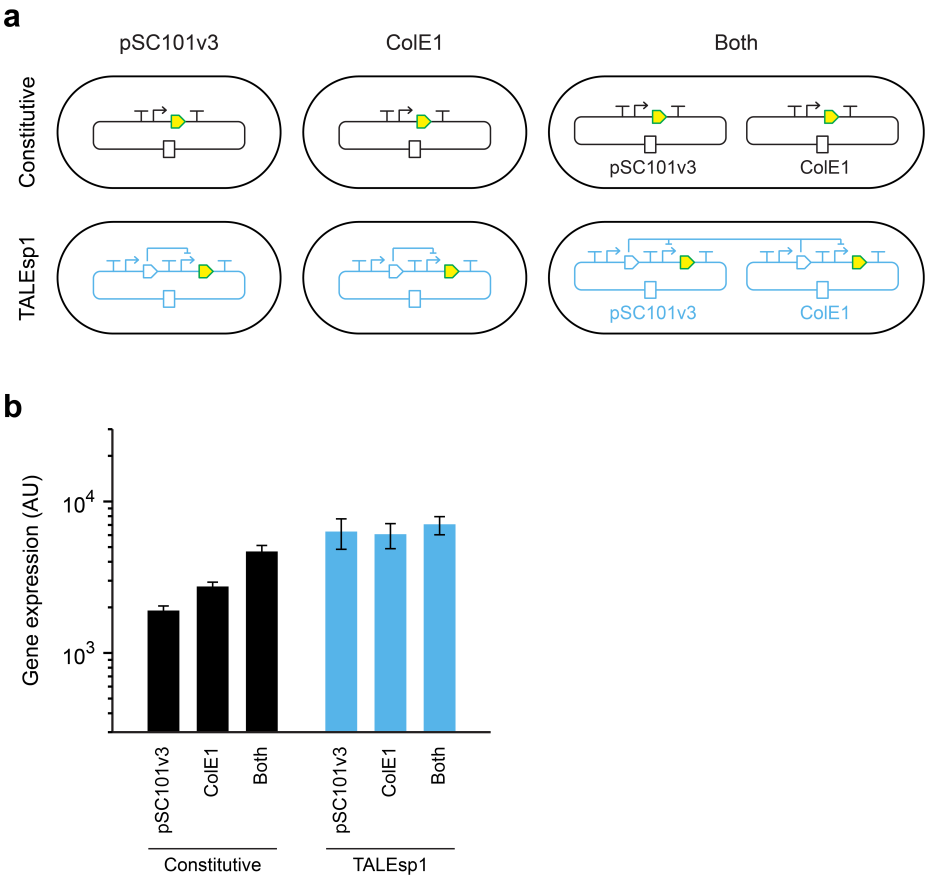
Supplementary Figure 9: Tuning stabilized promoters.
The TALEsp1- and TALEsp2-stabilized promoters were constructed with different promoters and RBSs driving expression of the TALE repressors. Constructs were chosen to balance stabilization and strength at the desired range of copy numbers. The modeling assumes that repression is noncooperative, as in Supplementary Note 1. **a)** The tradeoff between stabilization and strength of the stabilized promoters characterized at a minimum copy number of pSC101. 'Stabilization error' shows the increase in gene expression as copy number is raised from pSC101 divided by gene expression on pSC101. 'Stabilized promoter strength' shows gene expression from the stabilized promoter on pSC101 divided by gene expression from an unrepressed promoter on pSC101. The TALEsp1-stabilized promoter was tuned to have an output of 34% compared to the unrepressed P_{UPsp1} promoter on pSC101 (S = 0.34), implying that it expresses approximately 1.9 x K_d of TALEsp1 on pSC101. This predicts an increase in gene expression of 52% as copy number increases from pSC101 (E = 0.52). The TALEsp2-stabilized promoter was tuned to have an output of 7% compared to the unrepressed P_{UPsp2} promoter on pSC101 (S = 0.07), implying that it expresses approximately 14 x K_d of TALEsp2 on pSC101. This predicts an increase in gene expression of 7% as copy number increases from pSC101 (E = 0.07). **b)** Predicted performance of the TALEsp1-stabilized promoter at different copy numbers (blue dashed line) compared to the ideal power law case (black line). Note that error will increase if the copy number is lowered below the level of pSC101. **c)** Predicted performance of the TALEsp2-stabilized promoter at different copy numbers (orange dashed line) compared to the ideal power law case (black line). In (b) and (c), gene expression is normalized to the unrepressed promoter on pSC101. **d)** The tradeoff between stabilization and strength of the TALEsp2-stabilized promoter using a minimum copy number of the genome terminus. Based on the data shown in (a), the stabilized promoter is expected to express approximately 4.1 x K_d of TALEsp2 on the terminus of the genome, leading to a predicted output of 20% compared to the unrepressed P_{UPsp2} promoter on genome terminus (S = 0.20), and a predicted increase in gene expression of 24% as copy number increases from the genome terminus (E = 0.24). **e)** Predicted performance of the TALEsp2-stabilized promoter at different copy numbers (orange dashed line) compared to the ideal power law case (black line). Gene expression is normalized to the unrepressed promoter on the genome terminus.



Supplementary Figure 10: Plasmid backbones based on different origins of replication.
a) Diagram of the set of common vector backbones used in Figures 2b and 2e. Plasmid architecture is the same as for the pSC101 plasmids shown in Supplementary Figure 7, but with different plasmid origins. **b)** Schematics of the origins used. incW and pSC101 produce proteins required for plasmid replication, whereas p15A, ColE1, and pUC use RNAs (RNAI, RNAII). **c)** Copy number of the plasmids measured by qPCR (Methods). **d)** GFP expression from the constitutive GFP cassette on each plasmid backbone (Methods). For all data, the averages of threereplicates from different days are shown, and error bars indicate the standard deviation. **e)** Diagram of Kanamycin-resistant ColE1 vector used when testing multiple plasmids simultaneously (Supplementary Fig. 12). The GFP cassette is the same as what is shown in (a).

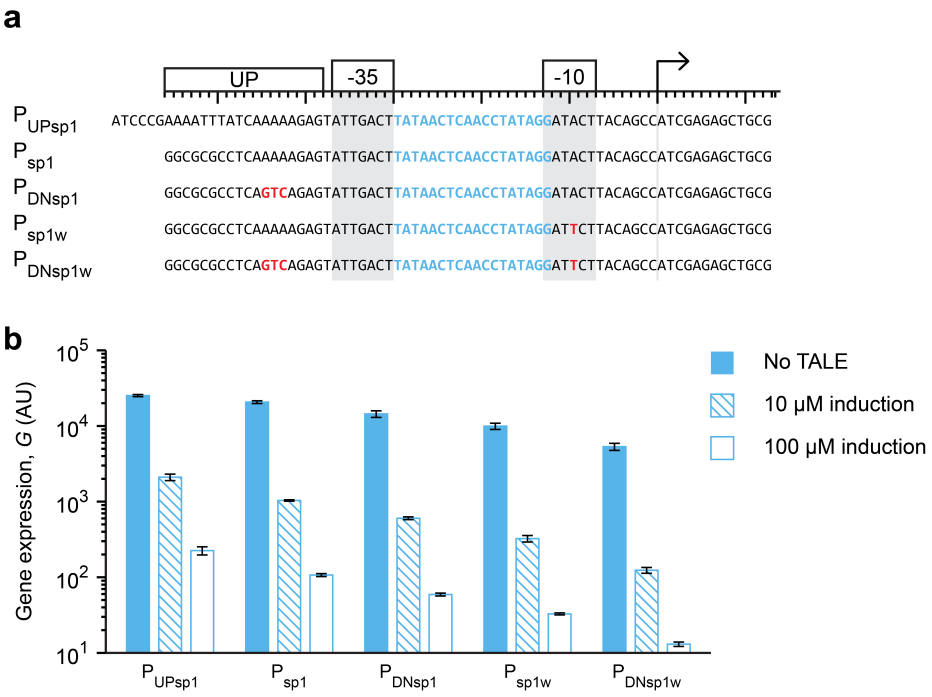


Supplementary Figure 11: Testing stabilized promoters in other strains of *E. coli*.
The constitutive (black) and stabilized (TALEsp1 - blue, TALEsp2 - orange) promoters were tested in a set of four plasmid backbones (pSC101, p15A, ColE1, and pUC) in three different *E. coli* strains (DH10B, MG1655, and BL21(DE3)). The growth assay is described in the Methods. For all data, the averages of three replicates from different days are shown, and error bars indicate the standard deviation.

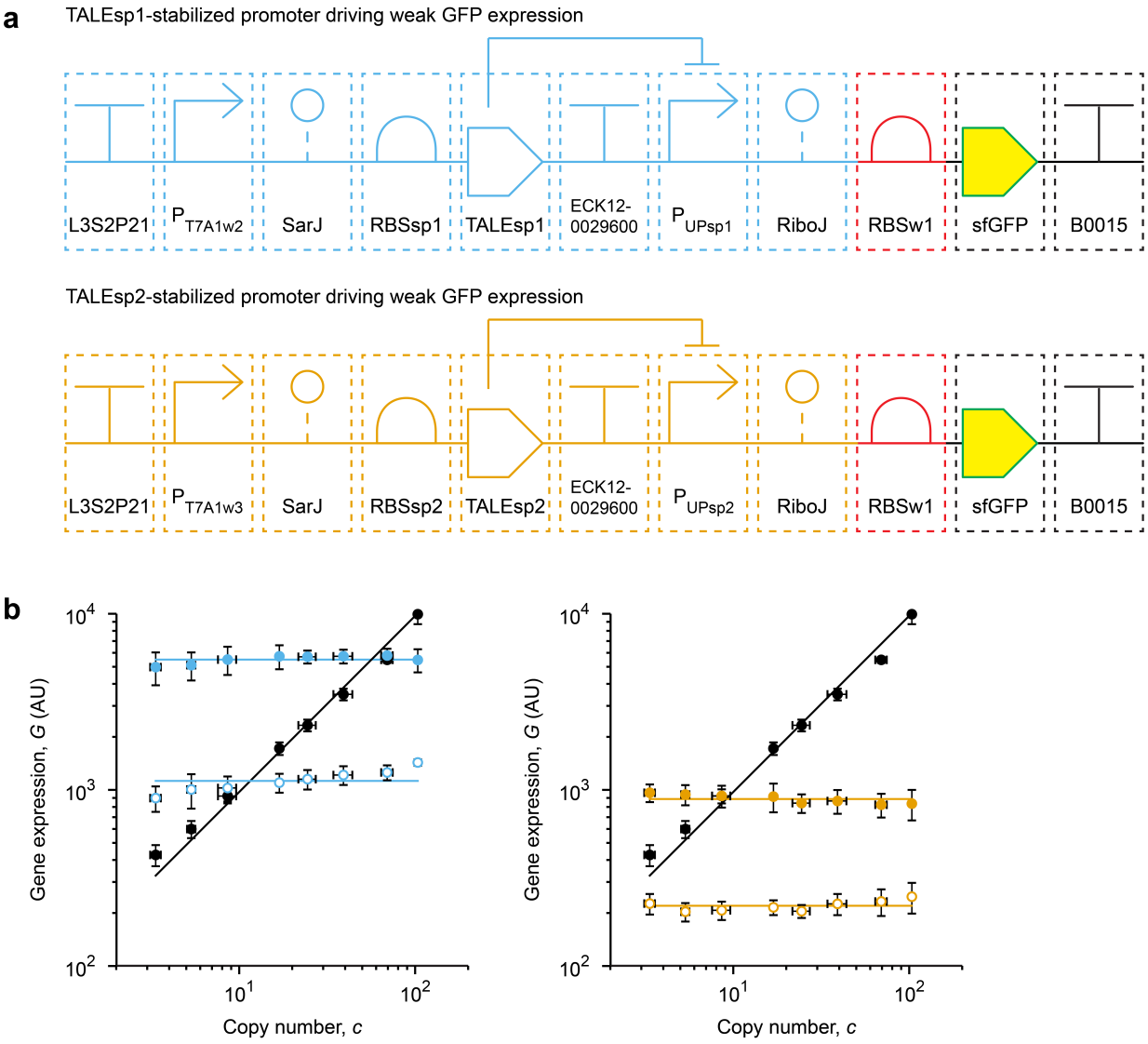


Supplementary Figure 12: Stabilized promoter function on two plasmids.
The TALEsp1-stabilized promoter was tested for its ability to control gene expression when simultaneously expressed from two different plasmid backbones. **a)** Schematic of the assay. An AmpR pSC101v3 plasmid and a KanR ColE1 plasmid that can be maintained together in one cell were used. **b)** Gene expression from cells containing a single plasmid ('pSC101v3' or 'ColE1') or both plasmids ('Both'). Black bars show GFP expression when the insulated constitutive expression cassette was carried on these backbones. Blue bars show GFP expression when the TALEsp1-stabilized promoter was used. The average of three replicates from different days is shown, and error bars indicate the standard deviation.

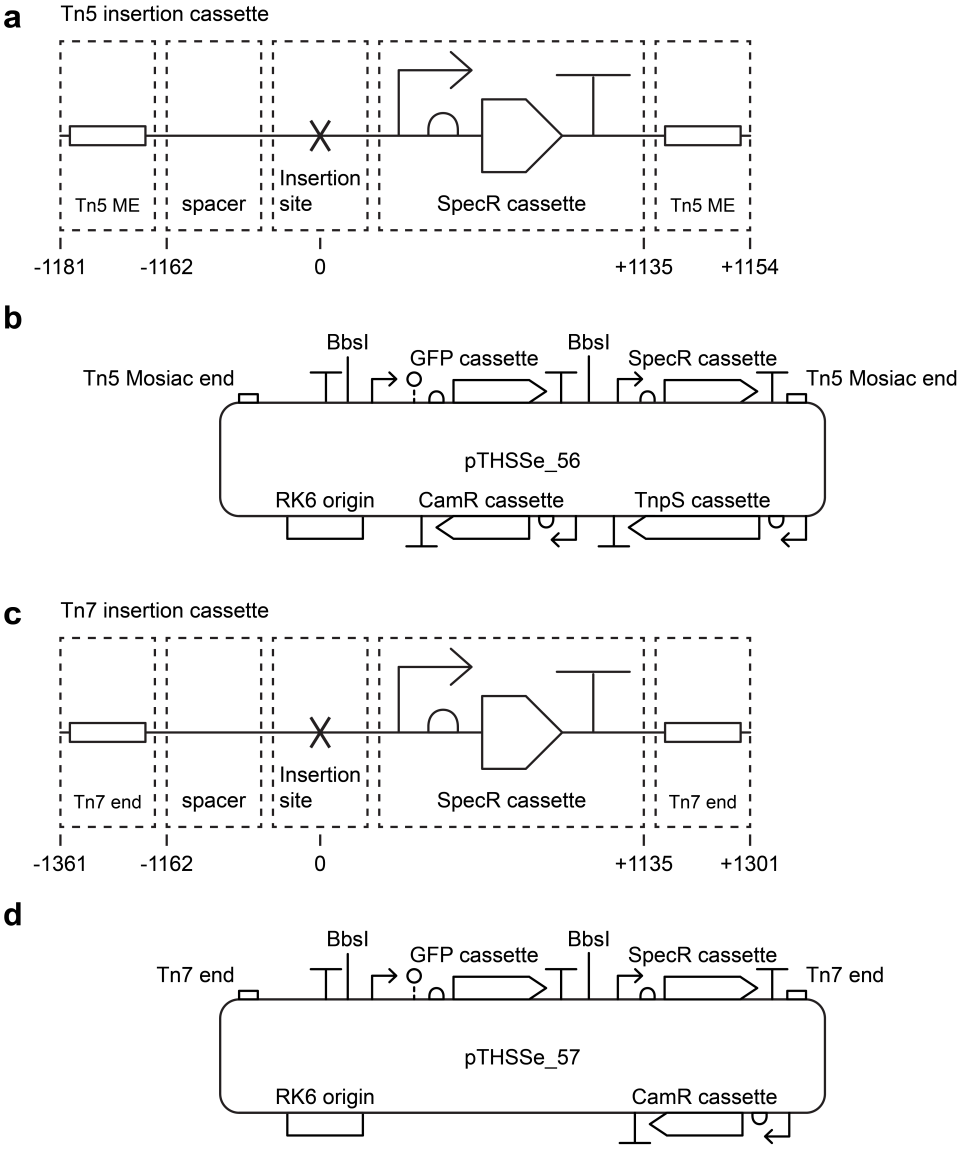
Supplementary Figure 13: Stabilized promoter variants.
Parts diagrams and lengths are shown for the weaker TALEsp1-stabilized promoter **(a)** and TALEsp1-stabilized promoter driving two transcripts **(b)**. 'Var' indicates the TALE-repressible promoter that was mutated to generate stabilized promoters with different levels of gene expression (Supplementary Fig. 14). All base pair numbers are relative to the transcription initiation site of the first stabilized promoter. Sequences for the parts can be found in Supplementary Table 4.



Supplementary Figure 14: Design of stabilized promoters with different strengths.
Variants of the TALEsp1-stabilized promoter with different levels of gene expression were built by mutating the TALEsp1-repressible promoter. **a)** Promoter variants used. Light blue bases show the TALEsp1 binding site, red bases show mutations made to weaken promoter strength. **b)** Comparison of gene expression from these promoters. GFP expression from each of the variant promoters was characterized in the absence of TALE repression, or with TALEsp1 induced from P_{Tac}. The average of three replicates from different days is shown, and error bars indicate the standard deviation.

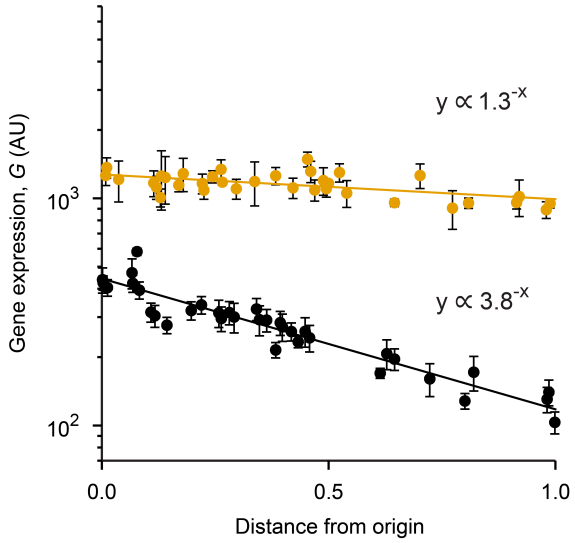


Supplementary Figure 15: Altering the RBSs of genes expressed from stabilized promoters.
The RBSs driving GFP under the control of the stabilized promoters were weakened in order to determine whether stabilization is impacted by the level of downstream expression. **a)** The new constructs for this experiment are shown. A weaker RBS (RBSw1) is used before GFP. **b)** The constitutive (black) and stabilized (TALEsp1 - blue, TALEsp2 - orange) promoters with both RBSs were characterized on the set of pSC101 variant backbones. Solid circles show gene expression with B0032 driving GFP and hollow circles show gene expression with RBSw1 driving GFP. For all data, the averages of three replicates from different days are shown, and error bars indicate the standard deviation.

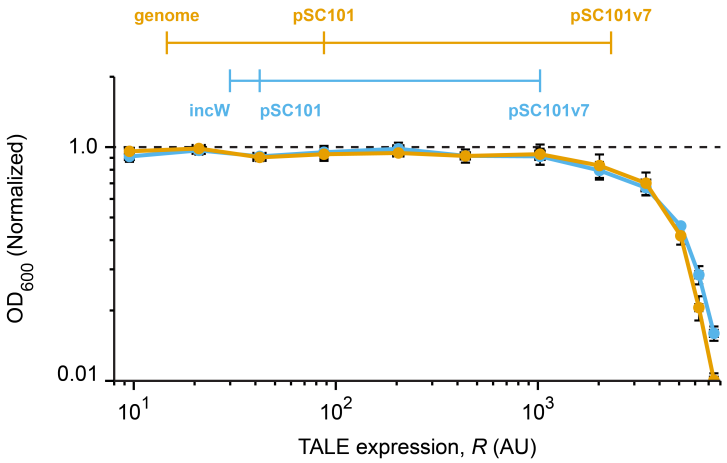


Supplementary Figure 16: Transposition systems.

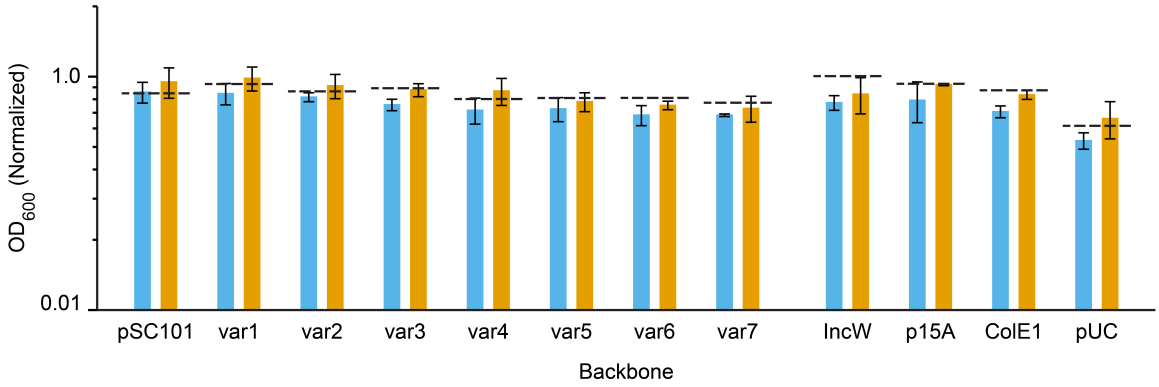
a) Parts diagram of the Tn5 integration cassette. Expression cassettes can be cloned into the insertion site using BbsI. The entire cassette as shown will be randomly integrated into the genome. Base pair numbering counts up and down from the BbsI insertion site. **b)** Diagram of the Tn5 transposition vector used to insert constitutive and stabilized promoters into the genome. This plasmid is a modified form of pBAMD1-4[6], with the resistance cassette changed to CamR and a GFP cassette flanked by BbsI sites inserted. An approximately 1kb-long stretch of DNA was inserted between the first Tn5 mosaic end and the BbsI cloning site to buffer genomic insertions from local context effects. **c)** Parts diagram of the Tn7 integration cassette. Expression cassettes can be cloned into the insertion site using BbsI. The entire cassette as shown will be integrated into the genome at the attTn7 site. Base pair numbering counts up and down from the BbsI insertion site. **d)** Diagram of the Tn7 transposition pasmid used to insert constitutive and stabilized promoters into the attTn7 site on the genome. This plasmid was built from the Tn5 vector above by replacing the Tn5 mosaic ends with Tn7 ends and removing the Tn5 integration gene (*tnpS*). It must be used with an accessory plasmid expressing the necessary Tn7 integration proteins.



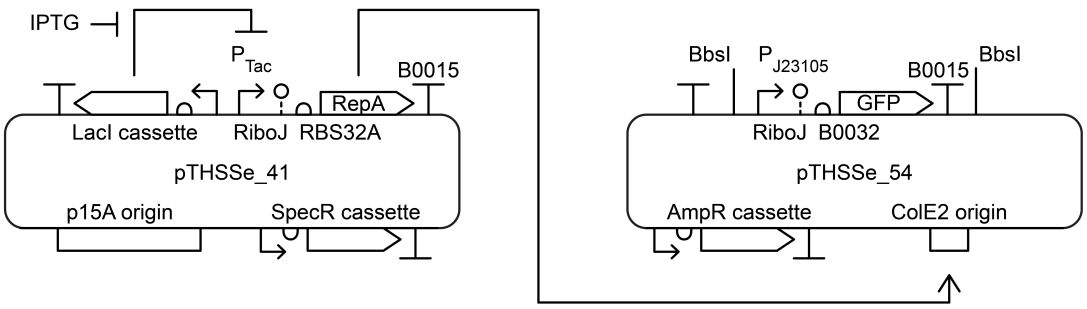
Supplementary Figure 17: Genome position dependent gene expression.
GFP expression from identified insulated constitutive (black) and TALEsp2-stabilized (orange) genomic integrations is shown plotted against the relative distance from the chromosomal origin of replication in *E. coli* DH10B (taxid: 316385, genome ref seq: NC_010473.1, length: 4,686,137). Positions for each integration are given in Supplementary Table 2. The average of three replicates from different days is shown, and error bars indicate the standard deviation.



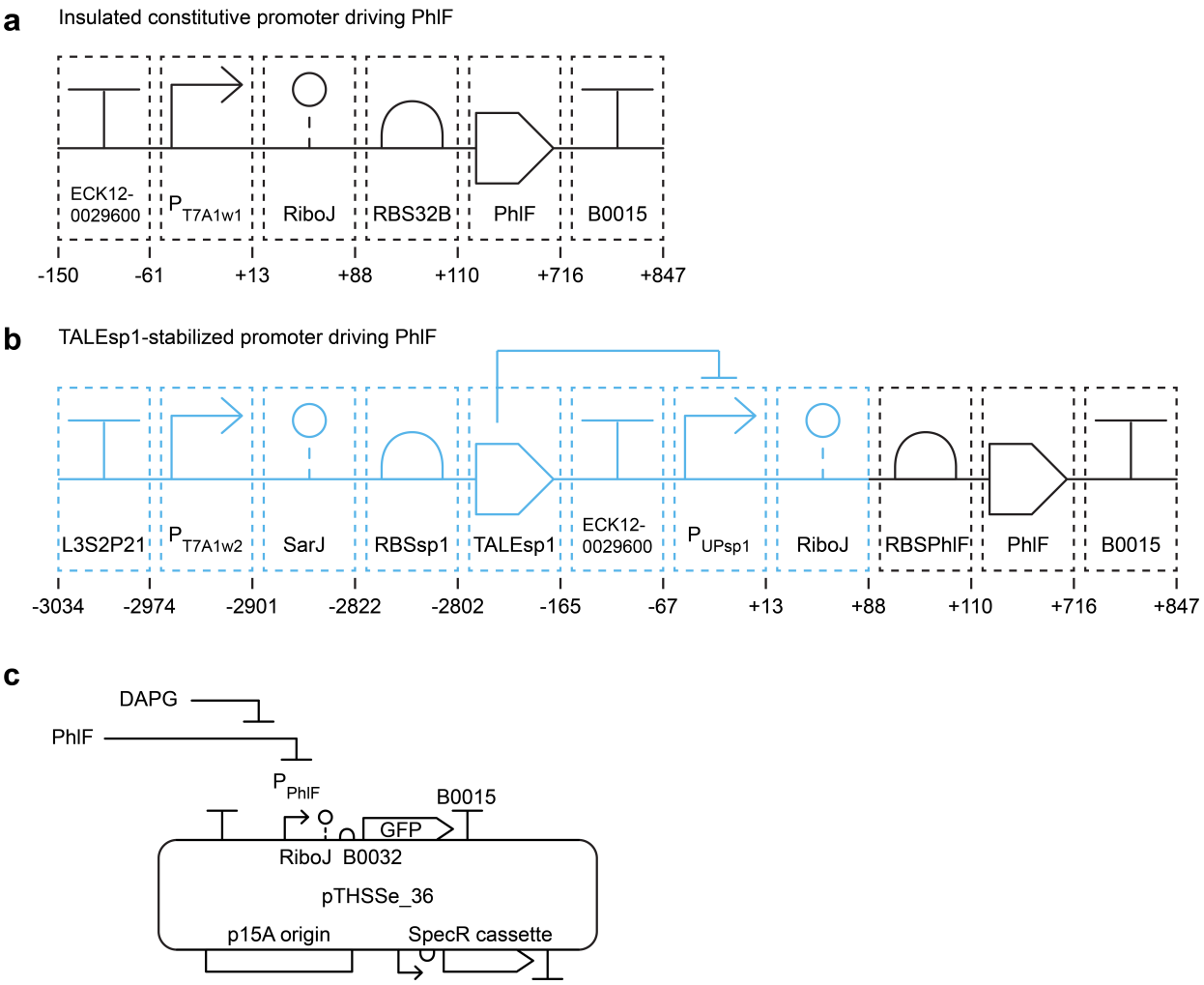
Supplementary Figure 18: TALE induction growth effects.
The TALE expression vectors used to characterize TALE repression (shown in Supplementary Fig.1b) were induced with 0, 5, 10, 16, 25, 40, 63, 100, 160, 250, 400, and 1000 µM IPTG (left to right, shown as the fluorescence when P_{tac} expresses gfp instaed of the TALE). After 6 hrs of induction, the OD_{600} of each TALE expression system was measured and normalized to the growth of an empty vector control grown in the same media and concentration of IPTG. Blue dots show TALEsp1 expression, and orange dots show TALEsp2 expression. The blue and orange bars above the plot show the approximate expression range of the TALEsp1 (blue) and TALEsp2 (orange) repressors in the stabilized promoters. These ranges were calculated by mapping the gene expression of each stabilized promoter on pSC101 to the response functions of the TALE repressors and extrapolating over the full range of gene expression seen in different contexts. The average of three replicates from different days is shown, and error bars indicate the standard deviation.



Supplementary Figure 19: Stabilized promoter growth effects.
The 8 hr growth protocol was used (Methods), and OD_{600} measured. Measurements were normalized to an empty pSC101 vector. The black dashed lines show the growth of the insulated promoter driving GFP in each plasmid backbone. Bars show the growth of the stabilized promoters driving GFP in each plasmid backbone (blue, TALEsp1; orange, TALEsp2). The average of three replicates from different days is shown, and error bars indicate the standard deviation.

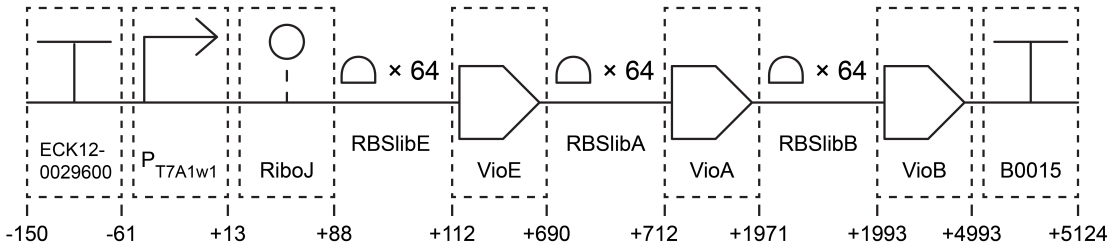


Supplementary Figure 20: Inducible copy number plasmids.
A two-plasmid system was used to test the response of stabilized promoters to a change in copy number (Figure 3c). pTHSSe_41 expresses the ColE2 RepA protein under the control of an inducible P_{Tac} system. This protein regulates the copy number of pTHSSe_54. pTHSSe_54 has a similar architecture to the plasmids shown in Supplementary Figs. 7 and 10. It contains a minimal 40 bp ColE2 origin that only supports plasmid replication when RepA is present.

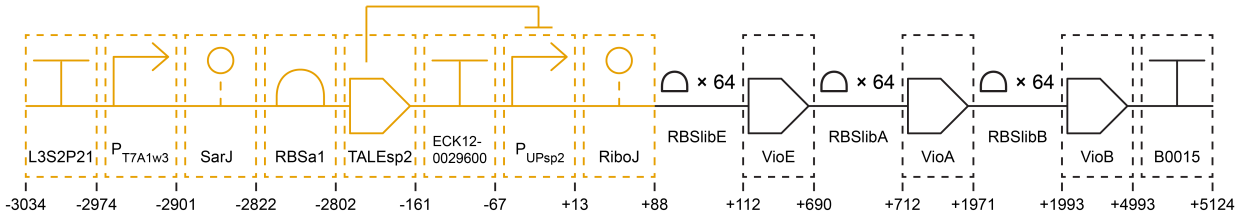


Supplementary Figure 21: Design of PhlF expression systems.
The constructs used to generate the results shown in Figure 4a are shown. **a)** The constitutive PhlF expression cassette. **b)** The TALEsp1-stabilized PhlF expression cassette. **c)** The P_{PhlF} reporter plasmid used to characterize DAPG induction curves.

a Insulated constitutive promoter driving prodeoxyviolacein pathway



b TALEsp2-stabilized promoter driving prodeoxyviolacein pathway



c

RBSlibE: atactcattaNggNggNaaaaaaa
constitutive: atactcatta**G**gg**C**gg**G**aaaaaaa
stabilized: atactcattaGggAggGaaaaaaa

RBSlibA: taacattataNggNggNaaaaaaa
constitutive: taacattata**T**gg**C**gg**A**aaaaaaa
stabilized: taacattataAggTggGaaaaaaa

RBSlibB: taggtcattaNggNggNaaaaaaa
constitutive: taggtcatta**G**gg**T**gg**G**aaaaaaa
stabilized: taggtcattaTggGggTaaaaaaa

Supplementary Figure 22: Design of prodeoxyviolacein metabolic pathway expression systems.
The constructs used to generate the results shown in Figure 4b are shown. Recoded genes from *Chromobacterium violaceum* were obtained from BBa_K274002[7]. **a)** The constitutive prodeoxyviolacein pathway cassette. **b)** The TALEsp2-stabilized prodeoxyviolacein pathway cassette. **c)** RBSs used in these constructs. The RBSlib sequences show the degenerate RBS library used to construct pathway variants. The sequences below show the specific RBSs from the high performing clones selected from these libraries.

Supplementary Table 1: New plasmids used in this work

Name	Origin	Marker	Description
pTHSSe_1	pSC101	Amp	Empty AmpR pSC101 vector
pTHSSe_2	p15A	Spec	Empty SpecR p15A vector
pTHSSe_3	pSC101	Amp	Empty pSC101 AmpR empty vector used in Tn5 transpositions
pTHSSe_4	ColE1	Kan	Empty ColE1 KanR vector
pTHSSe_5	pSC101	Amp	P_{J23101} - B0032 - sfGFP standardization plasmid
pTHSSe_6	pSC101	Amp	P_{J23101} - B0064 - EYFP standardization plasmid
pTHSSe_7	pSC101	Amp	P_{UPsp1} unrepressed control
pTHSSe_8	pSC101	Amp	P_{UPsp2} unrepressed control
pTHSSe_9	pSC101	Amp	$P_{TALE1(-60)F}$ GFP reporter
pTHSSe_10	pSC101	Amp	$P_{TALE1(-60)R}$ GFP reporter
pTHSSe_11	pSC101	Amp	$P_{TALE1(-51)F}$ GFP reporter
pTHSSe_12	pSC101	Amp	$P_{TALE1(-51)R}$ GFP reporter
pTHSSe_13	pSC101	Amp	$P_{TALE1(-30)F}$ GFP reporter
pTHSSe_14	pSC101	Amp	$P_{TALE1(-30)R}$ GFP reporter
pTHSSe_15	pSC101	Amp	$P_{TALE1(-29)F}$ GFP reporter
pTHSSe_16	pSC101	Amp	$P_{TALE1(-28)F}$ GFP reporter
pTHSSe_17	pSC101	Amp	$P_{TALE1(-27)F}$ GFP reporter
pTHSSe_18	pSC101	Amp	$P_{TALE1(-27)R}$ GFP reporter
pTHSSe_19	pSC101	Amp	$P_{TALE1(-7)F}$ GFP reporter
pTHSSe_20	pSC101	Amp	$P_{TALE1(-7)R}$ GFP reporter
pTHSSe_21	pSC101	Amp	$P_{TALE1(-3)R}$ GFP reporter
pTHSSe_22	pSC101	Amp	$P_{TALE1(-1)R}$ GFP reporter
pTHSSe_23	pSC101	Amp	$P_{TALE1(+1)R}$ GFP reporter
pTHSSe_24	pSC101	Amp	$P_{TALE1(+2)F}$ GFP reporter
pTHSSe_25	pSC101	Amp	$P_{TALE1(+2)R}$ GFP reporter
pTHSSe_26	pSC101	Amp	$P_{TALE1(+6)F}$ GFP reporter
pTHSSe_27	pSC101	Amp	$P_{TALE1(+6)R}$ GFP reporter
pTHSSe_28	pSC102	Amp	$P_{TALE1(+14)R}$ GFP reporter
pTHSSe_29	pSC101	Amp	P_{sp1} GFP reporter
pTHSSe_30	pSC101	Amp	P_{sp2} GFP reporter
pTHSSe_31	pSC101	Amp	P_{UPsp1} GFP reporter
pTHSSe_32	pSC101	Amp	P_{UPsp2} GFP reporter
pTHSSe_33	pSC101	Amp	P_{DNsp1} GFP reporter
pTHSSe_34	pSC101	Amp	P_{sp1w} GFP reporter
pTHSSe_35	pSC101	Amp	P_{DNsp1w} GFP reporter
pTHSSe_36	p15A	Spec	P_{PhlF} GFP reporter
pTHSSe_37	p15A	Spec	P_{Tac} driving sfGFP
pTHSSe_38	p15A	Spec	P_{Tac} driving TALE1
pTHSSe_39	p15A	Spec	P_{Tac} driving TALEsp1
pTHSSe_40	p15A	Spec	P_{Tac} driving TALEsp2
pTHSSe_41	p15A	Spec	P_{Tac} driving ColE2 RepA
pTHSSe_42	pSC101	Amp	pSC101 BbsI cloning vector
pTHSSe_43	pSC101*	Amp	pSC101 var1 BbsI cloning vector
pTHSSe_44	pSC101*	Amp	pSC101 var2 BbsI cloning vector
pTHSSe_45	pSC101*	Amp	pSC101 var3 BbsI cloning vector
pTHSSe_46	pSC101*	Amp	pSC101 var4 BbsI cloning vector
pTHSSe_47	pSC101*	Amp	pSC101 var5 BbsI cloning vector
pTHSSe_48	pSC101*	Amp	pSC101 var6 BbsI cloning vector
pTHSSe_49	pSC101*	Amp	pSC101 var7 BbsI cloning vector
pTHSSe_50	incW	Amp	incW BbsI cloning vector
pTHSSe_51	p15A	Amp	p15A BbsI cloning vector
pTHSSe_52	ColE1	Amp	ColE1 BbsI cloning vector
pTHSSe_53	pUC	Amp	pUC BbsI cloning vector
pTHSSe_54	ColE2	Amp	ColE2 BbsI cloning vector
pTHSSe_55	ColE1	Kan	ColE1 KanR cloning vector
pTHSSe_56	RK6	Cam / Spec	BbsI Tn5 transposition vector
pTHSSe_57	RK6	Cam / Spec	BbsI Tn7 transposition vector
pTHSSe_58	p15A	Cam	Insulated constitutive promoter driving GFP
pTHSSe_59	pSC101	Cam	TALEsp1-stabilized promoter driving GFP
pTHSSe_60	pSC101	Cam	TALEsp2-stabilized promoter driving GFP
pTHSSe_61	pSC101	Cam	$TALEsp1_{weak1}(P_{sp1})$-stabilized promoter driving GFP
pTHSSe_62	pSC101	Cam	$TALEsp1_{weak2}(P_{DNsp1})$-stabilized promoter driving GFP
pTHSSe_63	pSC101	Cam	$TALEsp1_{weak3}(P_{sp1w})$-stabilized promoter driving GFP

pTHSSe_64	pSC101	Cam	TALEsp1$_{weak4}$(P_{DNsp1w})-stabilized promoter driving GFP
pTHSSe_65	pSC101	Cam	TALEsp1-stabilized dual transcript promoter driving GFP and RFP
pTHSSe_66	ColE1	Cam	TALEsp1-stabilized promoter driving GFP with RBSB03
pTHSSe_67	ColE1	Cam	TALEsp2-stabilized promoter driving GFP with RBSB03
pTHSSe_68	ColE1	Cam	Insulated constitutive promoter driving PhlF
pTHSSe_69	ColE1	Cam	TALEsp1-stabilized promoter driving PhlF
pTHSSe_70	ColE1	Kan	Insulated, constitutive control driving vio genes in ColE1 KanR
pTHSSe_71	ColE1	Kan	TALEsp2-stabilized promoter driving vio genes in ColE1 KanR
pTHSSf_1	pSC101	Amp	Insulated promoter driving GFP in pSC101
pTHSSf_2	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var1
pTHSSf_3	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var2
pTHSSf_4	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var3
pTHSSf_5	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var4
pTHSSf_6	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var5
pTHSSf_7	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var6
pTHSSf_8	pSC101*	Amp	Insulated promoter driving GFP in pSC101 var7
pTHSSf_9	incW	Amp	Insulated promoter driving GFP in incW
pTHSSf_10	p15A	Amp	Insulated promoter driving GFP in p15A
pTHSSf_11	ColE1	Amp	Insulated promoter driving GFP in ColE1
pTHSSf_12	pUC	Amp	Insulated promoter driving GFP in pUC
pTHSSf_13	pSC101	Amp	TALEsp1-stabilized promoter driving GFP in pSC101
pTHSSf_14	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var1
pTHSSf_15	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var2
pTHSSf_16	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var3
pTHSSf_17	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var4
pTHSSf_18	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var5
pTHSSf_19	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var6
pTHSSf_20	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP in pSC101 var7
pTHSSf_21	incW	Amp	TALEsp1-stabilized promoter driving GFP in incW
pTHSSf_22	p15A	Amp	TALEsp1-stabilized promoter driving GFP in p15A
pTHSSf_23	ColE1	Amp	TALEsp1-stabilized promoter driving GFP in ColE1
pTHSSf_24	pUC	Amp	TALEsp1-stabilized promoter driving GFP in pUC
pTHSSf_25	ColE1	Kan	Insulated promoter driving GFP in ColE1 KanR
pTHSSf_26	ColE1	Kan	TALEsp1-stabilized promoter driving GFP in ColE1 KanR
pTHSSf_27	pSC101	Amp	TALEsp2-stabilized promoter driving GFP in pSC101
pTHSSf_28	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var1
pTHSSf_29	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var2
pTHSSf_30	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var3
pTHSSf_31	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var4
pTHSSf_32	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var5
pTHSSf_33	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var6
pTHSSf_34	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP in pSC101 var7
pTHSSf_35	incW	Amp	TALEsp2-stabilized promoter driving GFP in incW
pTHSSf_36	p15A	Amp	TALEsp2-stabilized promoter driving GFP in p15A
pTHSSf_37	ColE1	Amp	TALEsp2-stabilized promoter driving GFP in ColE1
pTHSSf_38	pUC	Amp	TALEsp2-stabilized promoter driving GFP in pUC
pTHSSf_39	RK6	Cam / Spec	Insulated, constitutive control driving GFP in Tn5 transposition vector
pTHSSf_40	RK6	Cam / Spec	TALEsp2 stabilized promoter driving GFP in Tn5 transposition vector
pTHSSf_41	pSC101	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101
pTHSSf_42	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var1
pTHSSf_43	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var2
pTHSSf_44	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var3
pTHSSf_45	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var4
pTHSSf_46	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var5
pTHSSf_47	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var6
pTHSSf_48	pSC101*	Amp	TALEsp1$_{weak1}$-stabilized promoter driving GFP in pSC101 var7
pTHSSf_49	pSC101	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101
pTHSSf_50	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var1
pTHSSf_51	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var2
pTHSSf_52	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var3
pTHSSf_53	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var4
pTHSSf_54	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var5
pTHSSf_55	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var6
pTHSSf_56	pSC101*	Amp	TALEsp1$_{weak2}$-stabilized promoter driving GFP in pSC101 var7
pTHSSf_57	pSC101	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101
pTHSSf_58	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var1

pTHSSf_59	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var2
pTHSSf_60	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var3
pTHSSf_61	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var4
pTHSSf_62	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var5
pTHSSf_63	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var6
pTHSSf_64	pSC101*	Amp	TALEsp1$_{weak3}$-stabilized promoter driving GFP in pSC101 var7
pTHSSf_65	pSC101	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101
pTHSSf_66	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var1
pTHSSf_67	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var2
pTHSSf_68	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var3
pTHSSf_69	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var4
pTHSSf_70	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var5
pTHSSf_71	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var6
pTHSSf_72	pSC101*	Amp	TALEsp1$_{weak4}$-stabilized promoter driving GFP in pSC101 var7
pTHSSf_73	pSC101	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101
pTHSSf_74	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var1
pTHSSf_75	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var2
pTHSSf_76	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var3
pTHSSf_77	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var4
pTHSSf_78	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var5
pTHSSf_79	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var6
pTHSSf_80	pSC101*	Amp	TALEsp1-stabilized dual transcript promoter driving GFP and RFP in pSC101 var7
pTHSSf_81	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101
pTHSSf_82	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var1
pTHSSf_83	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var2
pTHSSf_84	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var3
pTHSSf_85	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var4
pTHSSf_86	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var5
pTHSSf_87	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var6
pTHSSf_88	pSC101*	Amp	TALEsp1-stabilized promoter driving GFP with RBSB03 in pSC101 var7
pTHSSf_89	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101
pTHSSf_90	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var1
pTHSSf_91	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var2
pTHSSf_92	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var3
pTHSSf_93	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var4
pTHSSf_94	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var5
pTHSSf_95	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var6
pTHSSf_96	pSC101*	Amp	TALEsp2-stabilized promoter driving GFP with RBSB03 in pSC101 var7
pTHSSf_97	ColE2	Amp	Insulated promoter driving GFP in ColE2
pTHSSf_98	ColE2	Amp	TALEsp2-stabilized promoter driving GFP in ColE2
pTHSSf_99	incW	Amp	Insulated constitutive promoter driving PhlF in incW
pTHSSf_100	pSC101	Amp	Insulated constitutive promoter driving PhlF in pSC101
pTHSSf_101	pSC101*	Amp	Insulated constitutive promoter driving PhlF in pSC101var2
pTHSSf_102	ColE1	Amp	Insulated constitutive promoter driving PhlF in ColE1
pTHSSf_103	pUC	Amp	Insulated constitutive promoter driving PhlF in pUC
pTHSSf_104	pSC101*	Amp	Insulated constitutive promoter driving PhlF in pSC101var7
pTHSSf_105	incW	Amp	TALEsp1-stabilized promoter driving PhlF in incW
pTHSSf_106	pSC101	Amp	TALEsp1-stabilized promoter driving PhlF in pSC101
pTHSSf_107	pSC101*	Amp	TALEsp1-stabilized promoter driving PhlF in pSC101var2
pTHSSf_108	ColE1	Amp	TALEsp1-stabilized promoter driving PhlF in ColE1
pTHSSf_109	pUC	Amp	TALEsp1-stabilized promoter driving PhlF in pUC
pTHSSf_110	pSC101*	Amp	TALEsp1-stabilized promoter driving PhlF in pSC101var7
pTHSSf_111	RK6	Cam / Spec	Insulated, constitutive control driving vio genes in Tn7 transposition vector
pTHSSf_112	RK6	Cam / Spec	TALEsp2-stabilized promoter driving vio genes in Tn7 transposition vector

Supplementary Table 2: Genomic insertion locations

Insulated			Stabilized		
Insertion #	Location[a]	Orientation[b]	Insertion #	Location[a]	Orientation[b]
1	604394	Fwd	1	-327531	Rev
2	-2300253	Fwd	2	1811424	Fwd
3	1050060.5[c]	Rev	3	1099717	Rev
4	181487	Fwd	4	-19881	Fwd
5	898113	Fwd	5	-789422	Fwd
6	-28933	Fwd	6	-1227525	Fwd
7	-253564	Rev	7	897969	Rev
8	-1921234	Fwd	8	86844	Rev
9	-851326	Rev	9	1159469	Fwd
10	-814174	Rev	10	-1062333	Fwd
11	-1073034	Fwd	11	2142751	Rev
12	-193907	Rev	12	281986	Fwd
13	979066	Fwd	13	-420370	Fwd
14	514408	Rev	14	571851	Rev
15	6633	Rev	15	615939	Rev
16	-1471863	Rev	16	518866	Rev
17	617018	Fwd	17	-2295964	Rev
18	932461	Fwd	18	987467	Rev
19	-1511916	Fwd	19	-2315671	Fwd
20	-798985	Fwd	20	-1894334	Rev
21	-1014260	Fwd	21	307588	Fwd
22	336123	Fwd	22	-1144415	Rev
23	2340069	Rev	23	1643979	Rev
24	-155749	Rev	24	-694590	Fwd
25	159839	Fwd	25	-302635	Fwd
26	-658535	Fwd	26	-268017	Fwd
27	461690	Rev	27	527321	Rev
28	-1694070	Rev	28	-1264993	Rev
29	-273957	Fwd	29	2156245	Fwd
30	-1437949	Fwd	30	1078956	Rev
31	-3599	Fwd	31	397309	Fwd
32	-921586	Rev	32	1511815	Fwd
33	-683477	Rev	33	-24882	Rev
34	-1875522	Rev	34	-1170877	Fwd
35	2309556	Fwd	35	622300	Rev

a) The location of each genomic insertion is given relative to the center of the genomic origin of replication identified using OriDB[8]. (*E. coli* DH10B taxid: 316385, genome ref seq: NC_010473.1) The location corresponds to the middle bp of the 9bp replicated during Tn5 transposition. When sequencing reads were not available for each end of the transposition, a 9bp duplication was assumed.

b) The orientation of the integration, relative to the orientation of the GFP gene. Fwd: in the direction of leading strand replication, Rev: in the direction of lagging strand replication.

c) This integration had reads on both ends of the transposition revealing a 10bp duplication. The position reported is between the 5th and 6th bp of the duplication.

Supplementary Table 3: Genetic parts list

Name	Sequence
Promoters	
P_{J23105}	ggcgcgccTTTACGgctagctcagtcctaggTACTATgctagcaAggt
$P_{TacSymO}$	tgttgacaattaatcatcggctcgtataatgtgtggAATTGTGAGCGCTCACAATTctatggactatgttt
P_{T7A1w1}	ggcgcgcctcagtcagagtATTGACTtaaagtctaacctatagGACCTTtacagccAtcgagagctgcg
P_{T7A1w2}	ggcgcgcctcagtcagagtATTGACTtaaagtctaacctatagGACCGTtacagccAtcgagagctgcg
P_{T7A1w3}	ggcgcgcctcagtcagagtATTGACTtaaagtctaacctatagGAGATCtacagccAtcgagagctgcg
$P_{TALE1(-60)F}$	GGCGCGCCTCTACGACTCACTATAAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-60)R}$	GGCGCGCCTCTATAGTGAGTCGTAAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-51)F}$	GGCGCGCCTCAAAAAGAGTTACGACTCACTATAATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-51)R}$	GGCGCGCCTCAAAAAGAGTTATAGTGAGTCGTAATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-30)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTACGACTCACTATATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-30)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTATAGTGAGTCGTATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-29)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTTACGACTCACTATAAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-28)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTATACGACTCACTATAGGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-27)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTAGTACGACTCACTATAGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-27)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAATATAGTGAGTCGTAGATACTTACAGCCATCGAGAGCTGCG
$P_{TALE1(-7)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACGACTCACTATA
$P_{TALE1(-7)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTATAGTGAGTCGTA
$P_{TALE1(-3)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACATATAGTGAGTCGTA
$P_{TALE1(-1)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCTATAGTGAGTCGTA
$P_{TALE1(+1)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCTATAGTGAGTCGTA
$P_{TALE1(+2)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATACGACTCACTATA
$P_{TALE1(+2)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATATAGTGAGTCGTA
$P_{TALE1(+6)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGATACGACTCACTATA
$P_{TALE1(+6)R}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGATATAGTGAGTCGTA
$P_{TALE1(+14)F}$	GGCGCGCCTCAAAAAGAGTATTGACTTAAAGTCTAACCTATAGGATACTTACAGCCATCGAGAGCTGCGTATAGTGAGTCGTA
P_{sp1}	GGCGCGCCTCAAAAAGAGTATTGACTTATAACTCAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
P_{sp2}	GGCGCGCCTCAAAAAGAGTATTGACTTATATTGAGTCGTATAGGATACTTACAGCCATCGAGAGCTGCG
P_{UPsp1}	ATCCCGAAAATTTATCAAAAAGAGTATTGACTTATAACTCAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
P_{UPsp2}	ATCCCGAAAATTTATCAAAAAGAGTATTGACTTATATTGAGTCGTATAGGATACTTACAGCCATCGAGAGCTGCG
P_{DNsp1}	GGCGCGCCTCAGTCAGAGTATTGACTTATAACTCAACCTATAGGATACTTACAGCCATCGAGAGCTGCG
P_{sp1w}	GGCGCGCCTCAAAAAGAGTATTGACTTATAACTCAACCTATAGGATTCTTACAGCCATCGAGAGCTGCG
P_{DNsp1w}	GGCGCGCCTCAGTCAGAGTATTGACTTATAACTCAACCTATAGGATTCTTACAGCCATCGAGAGCTGCG
P_{PhlF}	GATTCGTTACCAATTGACATGATACGAAACGTACCGTATCGTTAAGGTT
Ribosome Binding Sites	
B0032	tcacacaggaaagtactag
RBS32A	tcacacaggaaagtactaa
RBS32B	tactcacacaggaaagtactag
B0034	aaagaggagaaatactag
B0064	tactagagaaagaggggaaatactag
RBSsp1	tcaattcacctgcgtgaaa
RBSsp2	atcaattcatcgacgtgaaa
RBSw1	tcagcgcggaaagtactag
RBSPhLF	TACTCATTGGTGAAAG
RBSVioEc	atactcattaGggCggGaaaaaaa
RBSVioAc	taacattataTggCggAaaaaaaa
RBSVioBc	taggtcattaGggTggGaaaaaaa
RBSVioEs	atactcattaGggAggGaaaaaaa
RBSVioAs	taacattataAggTggGaaaaaaa
RBSVioBs	taggtcattaTggGggTaaaaaaa
Terminators	
L3S2P21	TCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC
ECK120029600	TTCAGCCAAAAAACTTAAGACCGCCGGTCTTGTCCACTACCTTGCAGTAATGCGGTGGACAGGATCGGCGGTTTTCTTTTCTCTTCTCAA
B0015	tactagagCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCtactagagTCACACTGGCTCA CCTTCGGGTGGGCCTTTCTGCGTTTATA

Ribozymes	
RiboJ	agCTGTcACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGcctctacaaataattttgtttaa
SarJ	agaCTGTcGCCGGATGTGTATCCGACCTGACGATGGCCCAAAAGGGCCGAAACAGTcctctacaaataattttgtttaa
Genes	
sfGFP	ATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATG CTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTTCCCG TTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGT GCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTA ACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTA TCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATG GTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAA
mRFP	ATGGCTTCCTCCGAGGACGTGATCAAAGAGTTCATGCGTTTTAAAGTTCGTATGGAAGGTTCCGTTAACGGTCACGAGTTCGAAATCGAAGGTGAAGGTGAAGGTCGTC CGTACGAAGGTACCCAGACCGCTAAACTGAAAGTTACCAAAGGTGGTCCGCTGCCGTTCGCTTGGGACATCCTGTCCCCGCAGTTCCAGTACGGTTCCAAAGCTTACGT TAAACACCCGGCTGACATCCCGGACTACCTGAAACTGTCCTTCCCGGAAGGTTTCAAATGGGAACGTGTTATGAACTTCGAAGATGGTGGTGTTGTTACCGTTACCCAG GACTCCTCCCTGCAAGACGGTGAGTTCATCTACAAAGTTAAACTGCGTGGTACCAACTTCCCGTCCGACGGTCCGGTTATGCAGAAAAAAACCATGGGTTGGGAAGCTT CCACCGAACGTATGTACCCGGAAGATGGTGCTCTGAAAGGTGAAATCAAAATGCGTCTGAAACTGAAAGACGGTGGTCACTACGACGCTGAAGTTAAAACCACCTACAT GGCTAAAAAACCGGTTCAGCTGCCGGGTGCTTACAAAACCGACATCAAACTGGACATCACCTCCCACAACGAAGATTACACCATCGTTGAACAGTACGAACGTGCTGAA GGTCGTCACTCCACCGGTGCT
EYFP 3snp	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG ATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACAGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTGCAATGCTTCGC CCGCTACCCCGACCACATGAAGCTGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCA CTACCAGCAGAACACCCCAATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTTAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCAC ATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
TALE1	ATGGTAGATTTAAGAACTTTAGGATATTCACAGCAGCAACAGGAAAAGATCAAGCCCAAAGTTAGGTCGACAGTCGCGCAGCATCACGAAGCGCTGGTTGGTCATGGGT TTACACATGCCCACATCGTAGCCTTATCGCAGCACCCTGCAGCCCTTGGCACGGTCGCCGTCAAGTACCAGGACATGATTGCGGCGTTGCCGGAAGCCACACATGAGGC GATCGTCGGTGTGGGGAAACAGTGGAGCGGAGCCCGAGCGCTTGAGGCCCTGTTGACGGTCGCGGGAGAGCTGAGAGGGCCTCCCCTTCAGCTGGACACGGGCCAGTTG CTGAAGATCGCGAAGCGGGGAGGAGTCACGGCGGTCGAGGCGGTGCACGCGTGGCGCAATGCGCTCACGGGAGCACCCCTCAACCTGACCCCAGAGCAGGTCGTGGCAA TCGCCTCCAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTGTGCCAAGCGCACGGACTCACCCCAGAGCAGGTCGTGGCGATCGCAAG CCACGACGGAGGAAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTGTGCCAAGCGCACGGCCTCACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAAC GGCGGAAAACAGGCTTTGGAAACGGTGCAGAGGCTCCTTCCAGTGCTGTGCCAAGCGCACGGATTAACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGGGA AACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTGTGCCAAGCGCACGGCTTAACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAGGAAAGCAAGC CTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTGTGCCAAGCGCACGGACTTACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGGCAAACAGGCGTTGGAA ACCGTACAACGATTGCTGCCGGTGCTGTGCCAAGCGCACGGCCTTACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAGGAAAGCAAGCCTTGGAAACAGTAC AGAGGCTGTTGCCTGTGCTGTGCCAAGCGCACGGACTAACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCT GCTTCCCGTGCTGTGCCAAGCGCACGGGCTCACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAGGAAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCT GTGCTGTGCCAAGCGCACGGGCTAACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGGCAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTGT GCCAAGCGCACGGCCTAACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTGTGCCAAGC GCACGGGTTAACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGGCAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTGTGCCAAGCGCACGGA CTCACGCCTGAGCAGGTAGTGGCTATTGCATCCAATATCGGGGGCAGACCCGCACTGGAGTCAATCGTGGCCCAGCTTTCGAGGCCGGACCCCGCGCTGGCCGCACTCA CTAATGATCATCTTGTAGCGCTGGCCTGCCTCGGCGGACGACCCGCCTTGGATGCGGTGAAGAAGGGGCTCCCGCACGCGCCTGCATTGATTAAGCGGACCAACAGAAG GATTCCCGAGAGGACATCACATCGAGTGGCAGATCACGCGCAAGTGGTCCGCGTGCTCGGATTCTTCCAGTGTCACTCCCACCCCGCACAAGCGTTCGATGACGCCATG ACTCAATTTGGTATGTCGAGACACGGACTGCTGCAGCTCTTTCGTAGAGTCGGTGTCACAGAACTCGAGGCCCGCTCGGGCACACTGCCTCCCGCCTCCCAGCGGTGGG ACAGGATTCTCCAAGCGAGCGGTATGAAACGCGCGAAGCCTTCACCTACGTCAACTCAGACACCTGACCAGGCGAGCCTTCATGCGTTCGCAGACTCGCTGGAGAGGGA TTTGGACGCGCCCTCGCCCATGCATGAAGGGGACCAAACTCGCGCGTCA
TALEsp1	ATGGTAGATTTAAGAACTTTAGGATATTCACAGCAGCAACAGGAAAAGATCAAGCCCAAAGTTAGGTCGACAGTCGCGCAGCATCACGAAGCGCTGGTTGGTCATGGGT TTACACATGCCCACATCGTAGCCTTATCGCAGCACCCTGCAGCCCTTGGCACGGTCGCCGTCAAGTACCAGGACATGATTGCGGCGTTGCCGGAAGCCACACATGAGGC GATCGTCGGTGTGGGGAAACAGTGGAGCGGAGCCCGAGCGCTTGAGGCCCTGTTGACGGTCGCGGGAGAGCTGAGAGGGCCTCCCCTTCAGCTGGACACGGGCCAGTTG CTGAAGATCGCGAAGCGGGGAGGAGTCACGGCGGTCGAGGCGGTGCACGCGTGGCGCAATGCGCTCACGGGAGCACCCCTCAATCTGACCCCGGATCAGGTGGTTGCGA TCGCCAGTAATGGCGGAGGCAAACAAGCACTCGAAACTGTACAGCGCCTCCTGCCGGTACTGTGCCAAGATCATGGCTTGACGCCTGAGCAAGTCGTAGCTATTGCATC AAACATCGGTGGTAAACAGGCGCTGGAAACCGTACAACGATTACTCCCTGTCTTATGCCAGGCACACGGTCTGACCCCCGACCAAGTTGTAGCCATTGCGTCTAATGGC GGCGGGAAACAGGCCCTGGAAACGGTCCAACGTCTGTTACCCGTTCTGTGTCAGGCTCACGGTCTGACCCCTGCCCAGGTAGTTGCAATTGCCAGCAACATCGGCGGGA AACAAGCGCTGGAGACTGTGCAGCGTCTGCTCCCTGTGTTATGCCAAGATCATGGGCTCACTCCGGATCAGGTGGTGGCCATCGCTTCCAATATTGGCGGTAAACAGGC GCTGGAGACAGTGCAACGACTTTTACCTGTTCTCTGCCAGGATCATGGTCTAACTCCCGAGCAGGTCGTCGCCATCGCCTCTCATGACGGCGGGAAACAAGCGTTGGAA ACTGTCCAGCGACTCCTGCCGGTTTTGTGCCAGGCCCACGGGCTTACTCCTGACCAGGTAGTTGCGATCGCGTCAAATGGGGGTGGCAAACAAGCCCTCGAAACCGTGC AACGCCTGCTGCCCGTCTTGTGCCAAGCTCATGGGCTGACTCCGGCGCAAGTAGTCGCGATTGCGAGCCACGATGGCGGTAAGCAGGCACTGGAAACGGTTCAGCGCCT GCTCCCGGTTCTATGCCAGGATCACGGCCTGACCCCGGACCAGGTCGTCGCGATCGCGTCAAATATCGGTGGCAAACAAGCTTTGGAGACAGTACAGCGCCTGTTACCA GTGCTTTGCCAGGACCATGGTCTGACCCCTGAGCAAGTAGTGGCGATCGCTTCTAATATTGGGGGCAAACAAGCGCTGGAAACAGTACAGCGTCTGTTACCGGTCCTAT GCCAGGCACATGGCCTGACCCCTGATCAGGTGGTAGCCATTGCCAGTCATGATGGCGGTAAACAGGCGCTTGAGACTGTCCAACGTCTGCTGCCGGTCCTCTGTCAGGC TCATGGCCTGACGCCAGCTCAAGTCGTGGCTATCGCTTCGCATGATGGCGGAAAACAGGCACTGGAGACTGTGCAGCGACTGTTGCCAGTTCTGTGTCAGGATCACGGT TTAACTCCGGACCAGGTGGTCGCTATTGCGTCGAATGGGGGCGGTAAACAAGCGCTGGAAACTGTGCAACGTTTGCTCCCAGTTCTGTGCCAGGACCATGGGCTGACTC CGGAACAGGTAGTGGCCATTGCTTCTAATATTGGTGGGAAACAGGCGCTGGAAACCGTGCAGCGCCTGCTTCCAGTGCTTTGCCAGGCCCATGGCCTGACGCCAGATCA GGTGGTTGCTATAGCCAGCAATGGCGGCGGTAAACAGGCCCTCGAAACCGTCCAGCGCCTGCTCCCTGTGCTGTGCCAGGCCCATGGGCTTACCCCAGCGCAAGTAGTG GCGATTGCGTCTAATATTGGTGGTAAACAGGCGTTGGAGACTGTACAACGCCTGCTGCCAGTTTTATGCCAAGATCATGGTCTGACCCCTGAGCAGGTAGTGGCTATTG CATCCAACAACGGGGGCAGACCCGCACTGGAGTCAATCGTGGCCCAGCTTTCGAGGCCGGACCCCGCGCTGGCCGCACTCACTAATGATCATCTTGTAGCGCTGGCCTG CCTCGGCGGACGACCCGCCTTGGATGCGGTGAAGAAGGGGCTCCCGCACGCGCCTGCATTGATTAAGCGGACCAACAGAAGGATTCCCGAGAGGACATCACATCGAGTG GCAGATCACGCGCAAGTGGTCCGCGTGCTCGGATTCTTCCAGTGTCACTCCCACCCCGCACAAGCGTTCGATGACGCCATGACTCAATTTGGTATGTCGAGACACGGAC TGCTGCAGCTCTTTCGTAGAGTCGGTGTCACAGAACTCGAGGCCCGCTCGGGCACACTGCCTCCCGCCTCCCAGCGGTGGGACAGGATTCTCCAAGCGAGCGGTATGAA ACGCGCGAAGCCTTCACCTACGTCAACTCAGACACCTGACCAGGCGAGCCTTCATGCGTTCGCAGACTCGCTGGAGAGGGATTTGGACGCGCCCTCGCCCATGCATGAA GGGGACCAAACTCGCGCGTCA

TALEsp2	ATGGTAGATTTAAGAACTTTAGGATATTCACAGCAGCAACAGGAAAAGATCAAGCCCAAAGTTAGGTCGACAGTCGCGCAGCATCACGAAGCGCTGGTTGGTCATGGGT TTACACATGCCCACATCGTAGCCTTATCGCAGCACCCTGCAGCCCTTGGCACGGTCGCCGTCAAGTACCAGGACATGATTGCGGCGTTGCCGGAAGCCACACATGAGGC GATCGTCGGTGTGGGGAAACAGTGGAGCGGAGCCCGAGCGCTTGAGGCCCTGTTGACGGTCGCGGGAGAGCTGAGAGGGCCTCCCCTTCAGCTGGACACGGGCCAGTTG CTGAAGATCGCGAAGCGGGGAGGAGTCACGGCGGTCGAGGCGGTGCACGCGTGGCGCAATGCGCTCACGGGAGCACCCCTCAACCTGACCCCGGACCAGGTAGTCGCGA TTGCTTCACATGACGGGGGTAAACAAGCGCTGGAAACGGTGCAGCGTCTGCTACCGGTGTTATGTCAGGATCATGGGCTCACGCCGGAACAGGTAGTGGCAATTGCGAG TCATGACGGTGGCAAACAGGCCCTGGAAACCGTACAGCGGCTGCTACCGGTGCTGTGTCAAGCGCATGGCCTGACTCCGGACCAAGTTGTAGCCATTGCCTCGAACGGG GGCGGCAAGCAGGCGCTGGAGACTGTTCAACGTCTGCTCCCCGTTCTGTGTCAGGCGCATGGCCTGACGCCTGCGCAGGTCGTGGCGATCGCTTCAAACATCGGTGGGA AGCAAGCCCTGGAGACTGTCCAAAGACTGTTGCCAGTGTTGTGTCAAGATCATGGCTTAACCCCAGATCAGGTGGTTGCGATTGCATCAAATGGAGGTGGTAAACAGGC GCTGGAGACTGTGCAGCGCCTGTTGCCGGTTCTGTGCCAAGATCATGGGCTGACTCCGGAACAGGTTGTGGCTATCGCAAGCAATATTGGTGGCAAGCAGGCCCTGGAA ACAGTACAGCGCCTGCTGCCTGTATTGTGTCAAGCCCACGGTCTTACCCCCGATCAGGTAGTCGCCATAGCATCGCACGACGGCGGGAAGCAGGCCCTTGAGACTGTAC AACGCCTCCTGCCGGTTTTGTGCCAGGCGCACGGCCTGACGCCAGCCCAGGTGGTTGCGATAGCCAGTAATAACGGCGGTAAGCAAGCCCTTGAAACCGTTCAACGTTT GCTGCCAGTGCTGTGCCAGGATCACGGCCTGACCCCGGATCAGGTAGTCGCCATTGCTAGCAACATTGGTGGCAAACAAGCACTGGAGACAGTTCAACGCTTACTGCCC GTGCTTTGCCAGGATCATGGACTGACCCCAGAGCAAGTGGTCGCGATTGCCTCGCATGACGGAGGTAAACAGGCCCTTGAGACTGTCCAGCGTCTGCTGCCGGTCCTTT GCCAGGCTCATGGGCTGACGCCCGACCAGGTGGTAGCAATCGCTTCGAACGGCGGAGGCAAACAGGCGTTAGAAACCGTTCAACGTCTGTTACCGGTGCTGTGCCAAGC TCATGGCTTAACCCCGGCCCAGGTAGTCGCAATCGCTAGTCATGATGGTGGGAAACAGGCATTAGAAACAGTACAACGTCTGCTCCCGGTCCTCTGTCAGGATCACGGT CTGACCCCGGATCAGGTTGTAGCAATCGCTAGCAATATTGGTGGTAAACAGGCGCTGGAAACAGTGCAAAGATTATTACCAGTTCTGTGTCAGGACCATGGCCTGACAC CTGAGCAGGTCGTAGCGATCGCAAGTAATATTGGAGGTAAACAGGCCCTGGAAACCGTGCAGCGTCTGCTGCCTGTGCTCTGTCAAGCGCATGGTCTGACTCCGGATCA GGTTGTCGCGATCGCCAGTAACGGGGGAGGGAAACAGGCACTCGAGACTGTGCAGAGATTGCTGCCGGTCCTGTGTCAGGCGCATGGTCTGACCCCAGCGCAGGTCGTG GCAATCGCTTCCAACATAGGTGGTAAACAGGCCCTCGAAACTGTCCAACGGCTGTTACCGGTACTGTGCCAGGATCATGGTCTGACCCCTGAGCAGGTAGTGGCTATTG CATCCAACGGAGGGGGCAGACCCGCACTGGAGTCAATCGTGGCCCAGCTTTCGAGGCCGGACCCCGCGCTGGCCGCACTCACTAATGATCATCTTGTAGCGCTGGCCTG CCTCGGCCGGACGACCCGCCTTGGATGCGGTGAAGAAGGGGCTCCCGCACGCGCCTGCATTGATTAAGCGGACCAACAGAAGGATTCCCGAGAAGGACATCACATCGAGTG GCAGATCACGCGCAAGTGGTCCGCGTGCTCGGATTCTTCCAGTGTCACTCCCACCCCGCACAAGCGTTCGATGACGCCATGACTCAATTTGGTATGTCGAGACACGGAC TGCTGCAGCTCTTTCGTAGAGTCGGTGTCACAGAACTCGAGGCCCGCTCGGGCACACTGCCTCCCGCCTCCCAGCGGTGGGACAGGATTCTCCAAGCGAGCGGTATGAA ACGCGCGAAGCCTTCACCTACGTCAACTCAGACACCTGACCAGGCGAGCCTTCATGCGTTCGCAGACTCGCTGGAGAGGGATTTGGACGCGCCCTCGCCCATGCATGAA GGGGACCAAACTCGCGCGTCA
repA	ATGAGCGCCGTACTTCAACGCTTCCGTGAAAAATTACCGCACAAACCGTACTGTACTAACGATTTCGCGTACGGCGTTCGCATTCTGCCGAAAAACATTGCCATTCTTG CCCGTTTCATCCAGCAGAACCAGCCACATGCACTGTACTGGCTTCCCTTTGACGTGGACCGGACGGGGGCATCAATCGACTGGAGCGACCGGAATTGTCCGGCCCCGAA CATCACCGTAAAAAATCCCCGTAACGGGCACGCGCATCTGCTCTACGCGCTCGCCCTTCCTGTGAGAACTGCGCCGGATGCATCGGCTTCGGCGCTCAGATACGCTGCC GCTATTGAGCGTGCGTTGTGTGAAAAACTGGGCGCGGATGTGAATTACAGCGGCCTGATCTGCAAAAATCCGTGCCACCCTGAATGGCAGGAAGTGGAATGGCGCGAGG AACCCTACACTCTCGACGAACTGGCTGATTATCTGGATCTGAGCGCCTCAGCGCGCCGTAGCGTCGATAAAAATTACGGGCTGGGGCGAAACTACCATCTGTTCGAAAA GGTCCGTAAATGGGCCTACAGAGCGATTCGTCAGGGCTGGCCTGTATTCTCACAATGGCTTGATGCCGTTATCCAGCGTGTCGAAATGTACAACGCTTCGCTTCCCGTT CCGCTTTCTCCGGCTGAATGTCGGGCTATTGGCAAGAGCATTGCGAAATATACACACAGGAAATTCTCACCAGAGGGATTTTCCGCTGTACAGGCCGCTCGCGGTCGCA AGGGCGGAACTAAATCTAAGCGCGCAGCAGTTCCTACATCAGCACGTTCGCTGAAACCGTGGGAGGCATTAGGCATCAGTCGAGCGACGTACTACCGAAAATTAAAATG TGACCCAGACCTCGCAAAA
PhlF	ATGGCACGTACCCCGAGCCGTAGCAGCATTGGTAGCCTGCGTAGTCCGCATACCCATAAAGCAATTCTGACCAGCACCATTGAAATCCTGAAAGAATGTGGTTATAGCG GTCTGAGCATTGAAAGCGTTGCACGTCGTGCCGGTGCAAGCAAACCGACCATTTATCGTTGGTGGACCAATAAAGCAGCACTGATTGCCGAAGTGTATGAAAATGAAAG CGAACAGGTGCGTAAATTTCCGGATCTGGGTAGCTTTAAAGCCGATCTGGATTTTCTGCTGCGTAATCTGTGGAAAGTTTGGCGTGAAACCATTTGTGGTGAAGCATTT CGTTGTGTTATTGCAGAAGCACAGCTGGACCCTGACCCCTGACCCAGCTGAAAGATCAGTTTATGGAACGTCGTCGTGAGATGCCGAAAAAACTGGTTGAAAATGCCA TTAGCAATGGTGAACTGCCGAAAGATACCAATCGTGAACTGCTGCTGGATATGATTTTTGGTTTTTGTTGGTATCGCCTGCTGACCGAACAGCTGACCGTTGAACAGGA TATTGAAGAATTTACCTTCCTGCTGATTAATGGTGTTTGTCCGGGTACACAGCGT
VioA	ATGAAACATTCTTCCGATATCTGCATTGTTGGTGCTGGTATTTCTGGTTTGACGTGCGCAAGCCATCTGCTGGACAGCCCGGCATGCCGTGGTCTGAGCCTGCGTATCT TTGACATGCAGCAAGAAGCCGGTGGCCGTATCCGCAGCAAAATGCTGGATGGTAAGGCAAGCATTGAACTGGGCGCAGGTCGCTACTCCCCTCAGTTGCACCCGCATTT CCAAAGCGCAATGCAGCACTATAGCCAAAAGAGCGAAGTCTATCCGTTCACCCAGTTGAAGTTCAAATCTCACGTGCAGCAAAAGCTGAAGCGCGCCATGAATGAACTG TCCCCGCGTCTGAAAGAGCATGGTAAAGAGAGCTTTTTGCAGTTTGTCAGCCGTTATCAAGGTCACGATAGCGCGGTTGGTATGATCCGCTCTATGGGTTACGACGCAC TGTTCCTGCCGGATATCAGCGCAGAAATGGCCTACGACATTGTGGGTAAGCACCCGGAGATCCAGAGCGTGACGGACAACGACGCGAACCAATGGTTTGCAGCGGAAAC GGGCTTTGCTGGTCTGATTCAGGGCATCAAGGCTAAGGTTAAGGCGGCAGGTGCGCGTTTTAGCCTGGGTTATCGTCTGCTGAGCGTCCGTACCGACGGTGACGGCTAC CTGCTGCAACTGGCAGGTGACGACGGCTGGAAACTGGAGCACCGTACCCGCCATCTGATTCTGGCGATTCCGCCGAGCGCGATGGCGGGTTTGAATGTTGATTTTCCAG AAGCCTGGTCCGGTGCGCGCTATGGCAGCCTGCCGCTGTTTAAGGGCTTTCTGACGTACGGTGAGCCGTGGTGGTTGGACTACAAACTGGACGATCAGGTGCTGATTGT TGACAACCCGCTGCGCAAAATCTATTTCAAAGGCGATAAGTACCTGTTCTTCTATACCGATAGCGAGATGGCGAATTACTGGCGCGGTTGTGTCGCGGAGGGCGAGGAC GGTTACCTGGAGCAAATTCGCACCCATTTGGCTAGCGCACTGGGTATCGTCCGTGAACGTATCCCGCAACCGCTGGCACACGTTCACAAGTATTGGGCGCACGGCGTTG AGTTTTGCCGTGATTCTGATATTGACCACCCGAGCGCACTGTCTCATCGCGACAGCGGTATCATCGCGTGCTCCGATGCGTACACGGAGCATTGTGGTTGGATGGAGGG CGGTCTGCTGAGCGCCCGTGAGGCAAGCCGTCTGCTGTTGCAGCGTATCGCCGCG
VioB	ATGAGCATTCTGGATTTCCCGCGTATCCACTTCCGTGGCTGGGCCCGTGTCAATGCGCCGACCGCGAACCGCGATCCGCACGGCCACATCGATATGGCCAGCAATACCG TGGCGATGGCGGGTGAGCCGTTCGACCTGGCACGCCATCCTACGGAGTTCCACCGTCACCTGCGCTCCCTGGGTCCGCGCTTCGGCTTGGATGGTCGTGCTGACCCGGA AGGCCCGTTCAGCCTGGCCGAGGGCTACAACGCTGCCGGTAACAACCACTTTTCGTGGGAGAGCGCAACCGTTAGCCACGTGCAATGGGATGGCGGTGAGGCGGATCGT GGTGACGGTCTGGTCGGTGCTCGTTTGGCACTGTGGGGTCACTACAATGATTATCTGCGTACCACCTTCAATCGTGCTCGTTGGGTCGACAGCGACCCGACGCGCCGTG ACGCTGCACAAATCTATGCGGGCCAATTCACCATTAGCCCGGCTGGTGCCGGTCCGGGTACGCCGTGGCTGTTTACGGCAGACATTGATGATAGCCATGGTGCACGTTG GACGCGTGGCGGCCACATTGCAGAGCGTGGCGGCCACTTCTTGGATGAAGAGTTTGGTCTGGCACGCCTGTTTCAGTTCTCTGTGCCGAAAGATCACCCACATTTTCTG TTTCACCCGGGTCCGTTTGATTCCGAGGCCTGGCGTCGTCTGCAATTGGCTCTGGAGGATGACGACGTTCTGGGTCTGACCGTGCAATATGCGTTGTTCAATATGAGCA CCCCGCCTCAGCCGAACAGCCCGGTTTTTCACGATATGGTCGGTGTTGTCGGTCTGTGGCGTCGTGGTGAACTGGCGAGCTACCCGGCTGGTCGTCTGCTGCGTCCGCG TCAACCGGGTCTGGGTGACCTGACCCTGCGCGTCAACGGTGGTCGCGTTGCGCTGAATTTGGCGTGTGCCATTCCGTTCAGCACTCGTGCCGCGCAGCCAAGCGCACCG GACCGCCTGACCCCGGACCTGGGTGCCAAACTGCCGCTGGGCGATCTGCTGCTGCGTGATGAGGACGGCGCACTGTTGGCACGTGTGCCGCAGGCTCTGTACCAAGACT ATTGGACGAATCACGGTATTGTGGACCTGCCGCTGCTGCGCGAACCGCGTGGTAGCTTGACCCTGAGCAGCGAACTGGCGGAGTGGCGTGAGCAAGACTGGGTCACCCA AAGCGACGCGTCTAACCTGTACCTGGAGGCACCGGATCGCCGTCACGGTCGCTTTTTCCCTGAGAGCATCGCGCTGCGCAGCTACTTTCCGCGGTGAAGCCGCGTGCGCGT CCGGATATCCCGCATCGTATCGAGGGCATGGGCCTGGTCGGCGTCGAATCTCGTCAGGATGGCGACGCTGCGGAATGGCGTCTGACGGGTCTGCGTCCGGGTCCGGCAC GCATTGTTCTGGACGATGGTGCCGAGGCGATCCCTCTGCGTGTTCTGCCTGACGATTGGGCGCTGGATGACGCGACCGTCGAAGAAGTGGATTACGCCTTTTTGTACCG CCACGTTATGGCGTATTACGAGCTGGTGTATCCATTCATGAGCGACAAGGTGTTTTCCCTGGCTGATCGTTGCAAATGTGAAACGTACGCACGTCTGATGTGGCAGATG TGTGATCCGCAGAACCGCAACAAGTCCTATTACATGCCGAGCACCCGCGAACTGTCGGCACCGAAAGCTCGTTTGTTCTTGAAGTATCTGGCCCACGTGGAAGGCCAGG CACGCCTGCAAGCACCTCCGCCAGCGGGTCCGGCACGCATTGAATCTAAAGCCCAGTTGGCGGCAGAGCTGCGTAAAGCCGTCGACCTGGAGCTGTCTGTGATGCTGCA ATACCTGTACGCGGCGTATAGCATTCCGAACTATGCACAGGGCCAACAACGTGTTCGTGACGGTGCGTGGACCGCCGAGCAGCTGCAACTGGCGTGCGGTAGCGGTGAC CGTCGCCGTGATGGCGGTATTCGTGCAGCACTGCTGGAAATTGCTCATGAAGAAATGATTCATTACCTGGTCGTTAACAACCTGCTGATGGCCCTGGGCGAGCCGTTCT ACGCGGGTGTCCCGCTGATGGGCGAAGCGGCACGTCAGGCGTTTGGCCTGGACACCGAGTTCGCTCTGGAACCGTTTAGCGAAAGCACGCTGGCACGTTTTGTTCGTCT GGAATGGCCGCACTTTATCCCAGCACCGGGCAAATCCATCGCGGACTGCTATGCCGCCATTCGTCAGGCGTTTTTGGATCTGCCGGACTTGTTTGGTGGCGAGGCAGGT AAGCGTGGCGGTGAACACCACCTGTTCCTGAATGAGCTGACCAACCGTGCGCATCCGGGTTATCAACTGGAAGTTTTCGATCGCGACTCGGCGCTGTTTGGTATTGCAT TTGTGACCGATCAGGGCGAAGGTGGCGCTCTGGACAGCCCGCACTACGAACATAGCCATTTTCAACGTCTGCGTGAAATGAGCCGCGTATCATGGCTCAAAGCGCACC GTTCGAACCGGCGCTGCCGGCGTTGCGTAATCCGGTTCTGGATGAGAGCCCGGGTTGCCAACGTGTCGCAGACGGTCGTGCGCGTGCGCTGATGGCATTGTACCAAGGC GTTTATGAGCTGATGTTTGCGATGATGGCGCAGCACTTCGCCGTGAAACCGCTGGGTAGCTTGCGTCGCAGCCGCCTGATGAACGCAGCAATCGATCTGATGACCGGTC TGTTGCGTCCGCTGAGCTGCGCGCTGATGAACCTGCCAAGCGGCATCGCCGGTCGCACGGCCGGTCCGCCGCTGCCGGGTCCGGTTGACACCCGTAGCTATGACGACTA CGCGCTGGGCTGTCGCATGCTGGCACGCCGTTGCGAGCGTCTGCTGGAGCAGGCGAGCATGCTGGAACCGGGTTGGCTGCCGGATGCGCAGATGGAGCTGCTGGATTTC TATCGTCGCCAAATGCTGGACTTGGCGTGCGGCAAACTGAGCCGCGAGGCC
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Composite Parts	
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TALEsp2-stabilized promoter	CTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCCTACTGGCGCGCCTCAGTCAGAGTATTGACTTAAAGTCTAACCTATAGG AGATCTACAGCCATCGAGAGCTGCGAGACTGTCGCCGGATGTGTATCCGACCTGACGATGGCCCAAAAGGGCCGAAACAGTCCTCTACAAATAATTTTGTTTAAATCAA TTCATCGACGTGAAAATGGTAGATTTAAGAACTTTAGGATATTCACAGCAGCAACAGGAAAAGATCAAGCCCAAAGTTAGGTCGACAGTCGCGCAGCATCACGAAGCGC TGGTTGGTCATGGGTTTACACATGCCCACATCGTAGCCTTATCGCAGCACCCTGCAGCCCTTGGCACGGTCGCCGTCAAGTACCAGGACATGATTGCGGCGTTGCCGGA AGCCACACATGAGGCGATCGTCGGTGTGGGGAAACAGTGGAGCGGAGCCCGAGCGCTTGAGGCCCTGTTGACGGTCGCGGGAGAGCTGAGAGGGCCTCCCCTTCAGCTG GACACGGGCCAGTTGCTGAAGATCGCGAAGCGGGGAGGAGTCACGGCGGTCGAGGCGGTGCACGCGTGGCGCAATGCGCTCACGGGAGCACCCCTCAACCTGACCCCGG ACCAGGTAGTCGCGATTGCTTCACATGACGGGGGTAAACAAGCGCTGGAAACGGTGCAGCGTCTGCTACCGGTGTTATGTCAGGATCATGGGCTCACGCCGGAACAGGT AGTGGCAATTGCGAGTCATGACGGTGGCAAACAGGCCCTGGAAACCGTACAGCGGCTGCTACCGGTGCTGTGTCAAGCGCATGGCCTGACTCCGGACCAAGTTGTAGCC ATTGCCTCGAACGGGGGCGGCAAGCAGGCGCTGGAGACTGTTCAACGTCTGCTCCCCGTTCTGTGTCAGGCGCATGGCCTGACGCCTGCGCAGGTCGTGGCGATCGCTT CAAACATCGGTGGGAAGCAAGCCCTGGAGACTGTCCAAAGACTGTTGCCAGTGTTGTGTCAAGATCATGGCTTAACCCCAGATCAGGTGGTTGCGATTGCATCAAATGG AGGTGGTAAACAGGCGCTGGAGACTGTGCAGCGCCTGTTGCCGGTTCTGTGCCAAGATCATGGGCTGACTCCGGAACAGGTTGTGGCTATCGCAAGCAATATTGGTGGC AAGCAGGCCCTGGAAACAGTACAGCGCCTGCTGCCTGTATTGTGTCAAGCCCACGGTCTTACCCCCGATCAGGTAGTCGCCATAGCATCGCACGACGGCGGGAAGCAGG CCCTTGAGACTGTACAACGCCTCCTGCCGGTTTTGTGCCAGGCGCACGGCCTGACGCCAGCCCAGGTGGTTGCGATAGCCAGTAATAACGGCGGTAAGCAAGCCCTTGA AACCGTTCAACGTTTGCTGCCAGTGCTGTGCCAGGATCACGGCCTGACCCCGGATCAGGTAGTCGCCATTGCTAGCAACATTGGTGGCAAACAAGCACTGGAGACAGTT CAACGCTTACTGCCCGTGCTTTGCCAGGATCATGGACTGACCCCAGAGCAAGTGGTCGCGATTGCCTCGCATGACGGAGGTAAACAGGCCCTTGAGACTGTCCAGCGTC TGCTGCCGGTCCTTTGCCAGGCTCATGGGCTGACGCCCGACCAGGTGGTAGCAATCGCTTCGAACGGCGGAGGCAAACAGGCGTTAGAAACCGTTCAACGTCTGTTACC GGTGCTGTGCCAAGCTCATGGCTTAACCCCGGCCCAGGTAGTCGCAATCGCTAGTCATGATGGTGGGAAACAGGCATTAGAAACAGTACAACGTCTGCTCCCGGTCCTC TGTCAGGATCACGGTCTGACCCCGGATCAGGTTGTAGCAATCGCTAGCAATATTGGTGGTAAACAGGCGCTGGAAACAGTGCAAAGATTATTACCAGTTCTGTGTCAGG ACCATGGCCTGACACCTGAGCAGGTCGTAGCGATCGCAAGTAATATTGGAGGTAAACAGGCCCTGGAAACCGTGCAGCGTCTGCTGCCTGTGCTCTGTCAAGCGCATGG TCTGACTCCGGATCAGGTTGTCGCGATCGCCAGTAACGGGGGAGGGAAACAGGCACTCGAGACTGTGCAGAGATTGCTGCCGGTCCTGTGTCAGGCGCATGGTCTGACC CCAGCGCAGGTCGTGGCAATCGCTTCCAACATAGGTGGTAAACAGGCCCTCGAAACTGTCCAACGGCTGTTACCGGTACTGTGCCAGGATCATGGTCTGACCCCTGAGC AGGTAGTGGCTATTGCATCCAACGGAGGGGGCAGACCCGCACTGGAGTCAATCGTGGCCCAGCTTTCGAGGCCGGACCCCGCGCTGGCCGCACTCACTAATGATCATCT TGTAGCGCTGGCCTGCCTCGGCGGACGACCCGCCTTGGATGCGGTGAAGAAGGGGCTCCCGCACGCGCCTGCATTGATTAAGCGGACCAACAGAAGGATTCCCGAGAGG ACATCACATCGAGTGGCAGATCACGCGCAAGTGGTCCGCGTGCTCGGATTCTTCCAGTGTCACTCCCACCCCGCACAAGCGTTCGATGACGCCATGACTCAATTTGGTA TGTCGAGACACGGACTGCTGCAGCTCTTTCGTAGAGTCGGTGTCACAGAACTCGAGGCCCGCTCGGGCACACTGCCTCCCGCCTCCCAGCGGTGGGACAGGATTCTCCA AGCGAGCGGTATGAAACGCGCGAAGCCTTCACCTACGTCAACTCAGACACCTGACCAGGCGAGCCTTCATGCGTTCGCAGACTCGCTGGAGAAGGGATTTGGACGCGCCC TCGCCCATGCATGAAGGGGACCAAACTCGCGCGTCATAATAGGTTTCAGCCAAAAAACTTAAGACCGCCGGTCTTGTCCACTACCTTGCAGTAATGCGGTGGACAGGAT CGGCGGTTTTCTTTTCTCTTCTCAAGCTTATCCCGAAAATTTATCAAAAAGAGTATTGACTTATATTGAGTCGTATAGGATACTTACAGCCATCGAGAGCTGCGAGCTG TCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTTTGTTTAA

Tn5 integration cassette	CTGTCTCTTATACACATCTttgtgtctcaggccgcctaggccagacttaagccagttcattgcttcaaccgcagctaccaccgtcacgctcacagatttcccaaatcagc ttagccatgtatccagcagcctgattcggctgagtgaacatcagacccttgccggaatcaatagctggctgaatggtatcttccagcacttgttgacggaagccgaact ctttggacccgtaagccagcgtcatgactgaactcttagtcacactgcgagtaacaccgtaagccagccattgaccagccagtgccttagtgcccagcttgactttctc agagatttcaccagtgttctcatcggtcacggtaactacttcgttatcggtcccattgattgcgtctgcttgtagaatctcgttgactttctagcaacaatcccgtag atgtcctgaacggtttcactaggaagcaagttaaccgcgcgaccacctacctcatctcggagcatcgcggagaagtgctggatgccagagcaagacccgtcaaattacg ccagcggaagggagcagttatagctcaggccgtggtgctgtaccccagcgtactcaaagcagaacgcaaggaagcagaacggagaatcttgctcagcccaccaagtgtt ctccagtggagacttagcgcaagccatgatgttctcgtggttttcctcaatgaacttgatgcgctcagggaacggaaccttgtcgacacccgcacagtttgcaccgtgg attttcagccagtagtaaccttcctttaccgattggtttacctttcgccagcgtaagcagtcctttggtcatatcgttaccttgcgggttgaacattgacacagcgtaaa cacgaccgcgccagtccatgttgtaagggaaccagatggccttatggttagcaaacttattggcttgctcaagcatgaactcaaggctgatacggcgagacttgcgagc cttgtccttgcggtacacagcagcggcagcacgtttccacgcggtgagagcctcaggatttgccacctgacgtctaagaaaccattattatcatgacattaacctataa aaataggcgtatcacgaggcagaatttcagataaaaaaaatccttagctttcgctaaggatgatttctggaattcgcggccgcttctagagGGAGgtgtcttcggcgcg cctttacggctagctcagtcctaggtactatgctagcaaggtagctgtcaccggatgtgctttccggtctgatgagtccgtgaggacgaaacagcctctacaaataatt ttgtttaatcacacaggaaagtactagatgagcaaaggagaagaacttttcactggagttgtcccaattcttgttgaattagatggtgatgttaatgggcacaaatttt ctgtccgtggagagggtgaaggtgatgctacaaacggaaaactcacccttaaatttatttgcactactggaaaactacctgttccgtggccaacacttgtcactactct gacctatggtgttcaatgcttttcccgttatccggatcacatgaaacggcatgactttttcaagagtgccatgcccgaaggttatgtacaggaacgcactatatctttc aaagatgacgggacctacaagacgcgtgctgaagtcaagtttgaaggtgataccctt gttaatcgtatcgagttaaagggtattgatttaaagaagatggaaacattc ttggacacaaactcgagtacaactttaactcacacaatgtatacatcacggcagacaaacaaaagaatggaatcaaagctaacttcaaaattcgccacaacgttgaaga tggttccgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccagacaaccattacctgtcgacacaatctgtcctttcgaaa gatcccaacgaaaagcgtgaccacatggtccttcttgagtttgtaactgctgctgggattacacatggcatggatgagctctacaaaatatactagagccaggcatcaa ataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgctctctactagagtcacactggctcaccttcgggtgggcctttct gcgtttatagaagacatCGCTtactagtagcggccgccaaagcccgccgaaaggcgggcttttctgtatttaaatgaaccttgaccgaacgcagcggtggtaacggcgc agtggcggttttcatggcttgttatgactgttttttggggtacagtctatgcctcgggcatccaagcagcaagcgcgttacgccgtgggtcgatgtttgatgttatgg agcagcaacgatgttacgcagcagggcagtcgccctaaaacaaagttaaacatcatgagggaagcggtgatcgccgaagtatcgactcaactatcagaggtagttggcg tcatcgagcgccatctcgaaccgacgttgctggccgtacatttgtacggctccgcagtggatggcggcctgaagccacacagtgatattgatttgctggttacggtgac cgtaaggcttgatgaaacaacgcggcgagctttgatcaacgacctttttggaaacttcggcttcccctggagagagcgagattctccgcgctgtagaaagtcaccattgtt gtgcacgacgacatcattccgtggcgttatccagctaagcgcgaactgcaatttggagaatggcagcgcaatgacattcttgcaggtatcttcgagccagccacgatcg acattgatctggctatcttgctgacaaaagcaagagaacatagcgttgccttggtaggtccagcggcggaggaactctttgatccggttcctgtcaaggatctatttga ggcgctaaatgaaaccttaacgctatggaactcgccgcccgactgggctggcgatgagcgaaatgtagtgcttacgttgtcccgcatttggtacagcgcagtaaccggc aaaatcgccgcgaaggatgtcgctgccgactgggcaatggagcgcctgccggcccagtatcagcccgtcatacttgaagctagacaggcttatcttggacaagaagaag atcgcttggcctcgcgcgcagatcagttggaagaatttgtccactacgtgaaaggcgagatcaccaaggtagtcggcaaataagacaattgtctaattaattgcggacc ctagaggtccccttttttatttttaaaaatttttttcacaaaacggtttacaagcataaaatctctgaAGATGTGTATAAGAGACAG
Tn7 integration cassette	TGTGGGCGGACAATAAAGTCTTAAACTGAACAAAATAGATCTAAACTATGACAATAAAGTCTTAAACTAGACAGAATAGTTGTAAACTGAAATCAGTCCAGTTATGCTG TGAAAAAGCATACTGGACTTTTGTTATGGCTAAAGCAAACTCTTCATTTTCTGAAGTGCAAATTGCCCGTCGTATTAAAGAGGGGCGTGGttgtgtctcaggccgccta ggccagacttaagccagttcattgcttcaaccgcagctaccaccgtcacgctcacagattcccaaatcagcttagccatgtatccagcagcctgattcggctgagtgaa catcagacccttgccggaatcaatagctggctgaatggtatcttccagcacttgttgacggaagccgaactctttggacccgtaagccagcgtcatgactgaactctta gtcacactgcgagtaacaccgtaagccagccattgaccagccagtgccttagtgcccagcttgactttctcagagatttcaccagtgttctcatcggtcacggtaacta cttcgttatcggtcccattgattgcgtctgcttgtagaatctcgttgacttttcttagcaacaatcccgtagatgtcctgaacggtttcactaggaagcaagttaaccgc gcgaccacctacctcatctcggagcatcgcggagaagtgctggatgccagagcaagacccgtcaaattacgccagcggaagggagcagttatagctcaggccgtggtgc tgtaccccagcgtactcaaagcagaacgcaaggaagcagaacggagaatcttgctcagccccaccaagtgttctccagtggagacttagcgcaagccatgatgttctcgt ggttttcctcaatgaacttgatgcgctcagggaacggaaccttgtcgacacccgcacagtttgcaccgtggattttcagccagtagtaaccttccttaccgattggttt accttcgccagcgtaagcagtcctttggtcatatcgttaccttgcgggttgaacattgacacagcgtaaacacgaccgccgccagtccatgttgtaagggaaccagatg gccttatggttagcaaacttattggcttgctcaagcatgaactcaaggctgatacggcgagacttgcgagccttgtccttgcggtacacagcagcggcagcacgtttcc acgcggtgagagcctcaggatttgccacctgacgtctaagaaaccattattatcatgacattaacctataaaaaataggcgtatcacgaggcagaatttcagataaaaaa aatccttagctttcgctaaggatgatttctggaattcgcggccgcttctagagGGAGgtgtcttcggcgcgccttacggctagctcagtcctaggtactatgctagca aggtagctgtcaccggatgtgctttccggtctgatgagtccgtgaggacgaaacagcctctacaaataatttttgtttaatcacacaggaaagtactagatgagcaaagg agaagaacttttcactggagttgtcccaattcttgttgaattagatggtgatgttaatgggcacaaatttttctgtccgtggagagggtgaaggtgatgctacaaacgga aaactcaccctaaatttatttgcactactggaaaactacctgttccgtggccaacacttgtcactactctgacctatggtgttcaatgcttttcccgttatccggatc acatgaaacggcatgacttttttcaagagtgccatgcccgaaggttatgtacaggaacgcactatatctttcaaagatgacgggacctacaagacgcgtgctgaagtcaa gtttgaaggtgataccctt gttaatcgtatcgagttaaagggtattgatttaaagaagatggaaacattcttggacacaaactcgagtacaacttaactcacacaat gtatacatcacggcagacaaacaaaagaatggaatcaaagctaacttcaaaattcgccacaacgttgaagatggttccgttcaactagcagaccattatcaacaaaata ctccaattggcgatggccctgtccttttaccagacaaccattacctgtcgacacaatctgtccttccgaaagatcccaacgaaaagcgtgaccacatggtccttcttga gtttgtaactgctgctgggattacacatggcatggatgagctctacaaaatatactagagccaggcatcaaatacaaaacgaaaggctcagtcgaaagactgggcctttcg ttttatctgttgtttgtcggtgaacgctctctactagagtcacactggctcaccttcgggtgggcctttctgcgtttatagaagacatCGCTtactagtagcggccgcc aaagcccgccgaaaggcgggcttttctgtatttaaatgaaccttgaccgaacgcagcggtggtaacggcgcagtggcggttttcatggcttgttatgactgttttttg gggtacagtctatgcctcgggcatccaagcagcaagcgcgttacgccgtgggtcgatgtttgatgttatggagcagcaacgatgttacgcagcagggcagtcgccctaa aacaaagttaaacatcatgagggaagcggtgatcgccgaagtatcgactcaactatcagaggtagttggcgtcatcgagcgccatctcgaaccgacgttgctggccgta catttgtacggctccgcagtggatggcggcctgaagccacacagtgatattgatttgctggttacggtgaccgtaaggcttgatgaaacaacgcggcgagctttgatca acgacctttggaaacttcggcttcccctggagagagcgagattctccgcgctgtagaagtcaccattgttgtgcacgacgacatcattccgtggcgttatccagctaa gcgcgaactgcaatttggagaatggcagcgcaatgacattcttgcaggtatcttcgagccagccacgatcgacattgatctggctatcttgctgacaaaagcaagagaa catagcgttgccttggtaggtccagcggcggaggaactctttgatccggttcctgaacaggatctatttgaggcgctaaatgaaaccttaacgctatggaactcgccgc ccgactgggctggcgatgagcgaaatgtagtgcttacgttgtcccgcatttggtacagcgcagtaaccggcaaaatcgcgccgaaggatgtcgctgccgactgggcaat ggagcgcctgccggcccagtatcagcccgtcatacttgaagctagacaggcttatcttggacaagaagaagatcgcttggcctcgcgcgcagatcagttggaagaatttt gtccactacgtgaaaggcgagatcaccaaggtagtcggcaaataagacaattgtctaattaattgcggacccctagaggtcccctttttatttttaaaaatttttcaca aaacggtttacaagcataaaatctctgaAACCAGATAAGTGAAATCTAGTTCCAAACTATTTTGTCATTTTTAATTTTCGTATTAGCTTACGACGCTACACCCAGTTCC CATCTATTTTGTCACTCTTCCCTAAATAATCCTTAAAAACTCCATTTCCACCCCTCCCAGTTCCCAACTATTTTGTCCGCCCACA
Plasmid origins	
pSC101	GACAGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCA GAGGGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTT TCCTTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTG CCTGATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGT TTGCGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATT ATAACCACTTGAATATAAACAAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGA TACCCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGT TGTTTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCT ACAAGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGA TGACGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTGAGATTTTCCAGTGGACAAACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGA AGAGATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGATGAGGAATCAAATACTCTATGAGGATTTATGAGTGGTTATTA AAAGAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTA ACCAATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGA ACTAGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGA CTAAGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCT CATGGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAA CAAACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGT TTTTGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAA CATGAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGC CAGTGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAA CACCAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC

pSC101 var1	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTTTCC TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA AGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTGTTATTTTCCAGTGGACAAACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC
pSC101 var2	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTTTCC TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA AGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTTGGATTTTCCAGTGGACAAACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC
pSC101 var3	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA AGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTAAGATTTTCCAGTGGACAAACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC
pSC101 var4	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTTTCC TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA AGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTGGGATTTTCCAGTGGACAAACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC

pSC101 var5	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTTTCC TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA TGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTGAGATTTTCCAGTGGACAAACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAATCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC
pSC101 var6	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTTTCC TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA AGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTTGAGATTTTCCAGTGGACAGACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCTTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGAAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC
pSC101 var7	AGTAAGACGGGTAAGCCTGTTGATGATACCGCTGCCTTACTGGGTGCATTAGCCAGTCTGAATGACCTGTCACGGGATAATCCGAAGTGGTCAGACTGGAAAATCAGAG GGCAGGAACTGCTGAACAGCAAAAAGTCAGATAGCACCACATAGCAGACCCGCCATAAAACGCCCTGAGAAGCCCGTGACGGGCTTTTCTTGTATTATGGGTAGTTTCC TTGCATGAATCCATAAAAGGCGCCTGTAGTGCCATTTACCCCCATTCACTGCCAGAGCCGTGAGCGCAGCGAACTGAATGTCACGAAAAAGACAGCGACTCAGGTGCCT GATGGTCGGAGACAAAAGGAATATTCAGCGATTTGCCCGAGCTTGCGAGGGTGCTACTTAAGCCTTTAGGGTTTTAAGGTCTGTTTTGTAGAGGAGCAAACAGCGTTTG CGACATCCTTTTGTAATACTGCGGAACTGACTAAAGTAGTGAGTTATACACAGGGCTGGGATCTATTCTTTTTATCTTTTTTTATTCTTTCTTTATTCTATAAATTATA ACCACTTGAATATAAACAAAAAAAACACACAAAGGTCTAGCGGAATTTACAGAGGGTCTAGCAGAATTTACAAGTTTTCCAGCAAAGGTCTAGCAGAATTTACAGATAC CCACAACTCAAAGGAAAAGGACTAGTAATTATCATTGACTAGCCCATCTCAATTGGTATAGTGATTAAAATCACCTAGACCAATTGAGATGTATGTCTGAATTAGTTGT TTTCAAAGCAAATGAACTAGCGATTAGTCGCTATGACTTAACGGAGCATGAAACCAAGCTAATTTTATGCTGTGTGGCACTACTCAACCCCACGATTGAAAACCCTACA AGGAAAGAACGGACGGTATCGTTCACTTATAACCAATACGCTCAGATGATGAACATCAGTAGGGAAAATGCTTATGGTGTATTAGCTAAAGCAACCAGAGAGCTGATGA CGAGAACTGTGGAAATCAGGAATCCTTTGGTTAAAGGCTTCGAGATTTTCCAGTGGACAGACTATGCCAAGTTCTCAAGCGAAAAATTAGAATTAGTTTTTAGTGAAGA GATATTGCCCTATCTTTTCCAGTTAAAAAAATTCATAAAATATAATCTGGAACATGTTAAGTCTTTTGAAAACAAATACTCTATGAGGATTTATGAGTGGTTATTAAAA GAACTAACACAAAAGAAAACTCACAAGGCAAATATAGAGATTAGCCTTGATGAATTTAAGTTCATGTTAATGCTTGGAAATAACTACCATGAGTTTAAAAGGCTTAACC AATGGGTTTTGAAACCAATAAGTAAAGATTTAAACACTTACAGCAATATGAAATTGGTGGTTGATAAGCGAGGCCGCCCGACTGATACGTTGATTTTCCAAGTTGAACT AGATAGACAAATGGATCTCGTAACCGAACTTGAGAACAACCAGATAAAAATGAATGGTGACAAAATACCAACAACCATTACATCAGATTCCTACCTACATAACGGACTA AGAAAAACACTACACGATGCTTTAACTGCAAAAATTCAGCTCACCAGTTTTGAGGCAAAATTTTTGAGTGACATGCAAAGTAAGTATGATCTCAATGGTTCGTTCTCAT GGCTCACGCAAAAACAACGAACCACACTAGAGAACATACTGGCTAAATACGGAAGGATCTGAGGTTCTTATGGCTCTTGTATCTATCAGTGAAGCATCAAGACTAACAA ACAAAAGTAGAACAACTGTTCACCGTTACATATCAAAGGGAAAACTGTCCATATGCACAGATGAAAACGGTGTAAAAAAGATAGATACATCAGAGCTTTTACGAGTTTT TGGTGCATTCAAAGCTGTTCACCATGAACAGATCGACAATGTAACAGATGAACAGCATGTAACACCTAATAGAACAGGTGAAACCAGTAAAACAAAGCAACTAGAACAT GAAATTGAACACCTGAGACAACTTGTTACAGCTCAACAGTCACACATAGACAGCCTGAAACAGGCGATGCTGCTTATCGAATCAAAGCTGCCGACAACACGGGAGCCAG TGACGCCTCCCGTGGGGAAAAAATCATGGCAATTCTGGAAGAAATAGCGCTTTCAGCCGGCAAACCGGCTGAAGCCGGATCTGCGATTCTGATAACAAACTAGCAACAC CAGAACAGCCCGTTTGCGGGCAGCAAAACCCGTAC

incW	CAAACCAACGTTTTATCTATACCGCTACAGGGTATTTAATTCCTATTTAATCTGCGCTAGAATGAGGCATGTTTAACCGAATCTGACGTTTTCCCTGCAAATGCCAAAA TACTATGCCTATCTCCGGGTTTCGCGTGACGGCCAAGACCCGGAAAACCAAAAATACGGTTTGCTCGAATACGCGAACGCCAAAGGCTTCGCGCCGCTACAGATCGAGG AAGAAATTGCCAGCAGAGCAAAGGACTGGCGCAAGCGCAAGCTCGGAGCAATCATCGAAAAGGCCGAGCGTGGCGACGTGCTACTGACGCCGGAGATTACGCGCATTGC CGGTTCCGCCCTCGCCGCCTTGGAAATTCTCAAAGCGGCGAGCGAGCGCGGCCTAATCGTCCATGTGACCAAACAGAAGATCATCATGGACGGCAGCCTACAAAGCGAC ATCATGGCAACCGTGCTTGGCTTGGCTGCACAGATCGAGCGGCATTTCATTCAGGCACGTACCACCGAGGCGCTACAAGTCGCCAGAGAGCGCGGCAAGACGCTCGGGC GACCCAAGGGCAGCAAATCGAGCGCCTTGAAGCTGGACAGCCGTATTGATGAAGTACAGGCATACGTGAACCTTGGCTTGCCGCAAAGTCGCGCAGCCGAGTTGTTAGG CGTCAGCCCTCACACCTTGCGCCTGTTCATCAAACGCCGGAACATCAAACCCACAAACACTAGACCAACCATCACCATGCCGGGGAGGGAACAACATGCCTAAGAACAA CAAAGCCCCCGGCCATCGTATCAACGAGATCATCAAGACGAGCCTCGCGCTCGAAATGGAGGATGCCCGCGAAGCTGGCTTAGTCGGCTACATGGCCCGTTGCCTTGTG CAAGCGACCATGCCCCACACCGACCCCAAGACCAGCTACTTTGAGCGCCACCAATGGCATCGTCACCTTGTCGATCATGGGCAAGCCGAGCATCGGCCTGCCCTACGGTT CTATGCCGCGCACCTTGCTTGCTTGGATATGCACCGAGGCCGTGCGAACGAAAGACCCCGTGTTGAACCTTGGCCGGTCGCAATCGGAATTTCTACAAAGGCTCGGAAT GCACACCGATGGCCGTTACACGGCCACCCTTCGCAATCAGGCGCAACGCCTGTTTTCATCCATGATTTCGCTTGCCGGCGAGCAAGGCAATGACTTCGGCATTGAGAAC GTCGTCATTGCCAAGCGCGCTTTTCTATTCTGGAATCCCAAGCGGCCAGAAGATCGGGCGCTATGGGATAGCACCCTCACCCTCACAGGCGATTTCTTCGAGGAAGTCA CCCGCTCACCGGTTCCTATCCGAATCGACTACCTGCATGCCTTGCGGCAGTCTCCGCTTGCGATGGACATTTACACGTGGCTGACCTATCGCGTGTTCCTGTTGCGGGC CAAGGGCCGCCCCTTCGTGCAAATCCCTTGGGTCGCCCTGCAAGCGCAATTCGGCTCATCCTATGGCAGCCGCGCACGCAACTCGCCCGAACTGGACGATAAGGCCCGA GAGCGGGCAGAGCGGGCAGCACTCGCCAGCTTCAAATACAACTTCAAAAAGCGCCTACGCGAAGTGTTGATTGTCTATCCCGAGGCAAGCGACTGCATCGAAGATGACG GCGAATGCCTGCGCATCAAATCCACACGCCTGCATGTCACCCGCGCACCCGGCAAGGGCGCTCGCATCGGCCCCCCTCCGACTTGACCAGGCCAACGCTACGCTTGGCT TGGTCAAGCCTTCCCATCCAACAGCCCGCCGTCGAGCGGGCTTTTTTATCCCCGGAAGCCTGTGGATAGAGGGTAGTTATCCACGTGAAACCGCTAATGCCCCGCAAAG CCTTGATTCACGGGGCTTTCCGGCCCGCTCCAAAAACTATCCACGTGAAATCGCTAATCAGGGTACGTGAAATCGCTAATCGGAGTACGTGAAATCGCTAATAAGGTCA CGTGAAATCGCTAATCAAAAAGGCACGTGAGAACGCTAATAGCCCTTTCAGATCAACAGCTTGCAAACACCCCTCGCTCCGGCAAGTAGTTACAGCAAGTAGTATGTTC AATTAGCTTTTCAATTATGAATATATATATCAATTATTGGTCGCCCTTGGCTTGTGGACAATGCGCTACGCGCACCGGCTCCGCCCGTGGACAACCGCAAGCGGTTGCC CACCGTCGAGCGCCAGCGCCTTTGCCCACAACCCGGCGGCCGCAACAGATCGTTTTATAAATTTTTTTTTTTGAAAAAGAAAAAGCCCGAAAGGCGGCAACCTCTCGGG CTTCTGGATTTCCGATCAACGCAGGAGTCGTTCGGAAAGTAGCTGTTCCAGAATTATAGGCGCAGAGACACCAGATTCCAAGATGGCTCTGTTAAATTGTTGTAGTATG TAGTATCATACAACATACTACAGTACAGAGGCCCGCAAGAATGGCAATCACTAAACAAGACATTTGGCGAGCAGCCGACGAACTGGACGCCGAAGGCATCCGGCCCACT TTGGCCGCCGTGCGCAAGAAACTCGGAAGCGGTAGCTTCACAACCATTTCCGATGCAATGGCTGAATGGAAAAACCGCAAGACCGCCACCCTGCCCTCATCAGACCCAT TGCCGGTTGCAGTCAACGAGCATCTTGCCGAGCTTGGCAATGCGCTATGGGCTATCGCCCTGGCGCACGCCAACGCCCGGTTTGACGAAGATCGGAAACAGATCGAGGC CGACAAAGCGGCCATCAGCCAGCAGCTTGCCGAAGCAATCGAACTAGCCGACACCTTCACCCGCGAAAACGACCAGCTCCGCGAACGAGTGAATCAGCTCGAACCTATG GAACGCGAGCGCGACAAGCTGGCCGACCAGCTTGCCGAAGTGAAACGCCGCAGCGGCGAAGAACTAAACCGCTGCATGGAAAAGCTCACCCAACGCGATAACGAGGCTA TCGAGGCCCGCAAACAGGCCAAGGAGGCCATCGAGCGCGCCGCCAGTCTGCAAGGTCAGGTGGAAGCCCTCAAAGAGCAGGTCGCCAATCTCACAGCCGTCTTGAAAAC AGGAGGCAAACAATGAAAAGCGCCCTTGCCGCCCTTCGCGCGGTCGCGGCCGCTGTCGTCCTAATCGTCAGTGTGCCCGCTTGGGCCGACTTCCGGGGTGAAGTCGTCC GAATCCTTGACGGTGACACTATCGACGTTTTGGTGAACCGTCAGACCATCCGCGTGAGATTGGCCGATATTGACGCACCGGAAAGCGGCCAAGCCTTCGGCTCCCGTGC TCGCCAACGGCTCGCCGACTTGACCTTTCGCCAAGAGGTTCAAGTGACCGAAAAAGAGGTTGATCGGTATGGCCGCACTCTTGGGGTCGTTTACGCGCCGTTGCAATAC CCCGGCGGCCAAACACAACTCACCAACATCAATGCGATCATGGTTCAAGAAGGCATGGCCTGGGCTTACCGTTATTACGGCAAACCAACCGACGCGCAGATGTACGAGT ATGAAAAAGAGGCCCGCCGCCAACGGCTCGGCCTTTGGTCAGACCCGAATGCTCAGGAGCCTTGGAAATGGCGTCGCGCCTCGAAAAATGCCACGAACTGACACCGGGC ACGCCCCTTGTTCGACGCGCCGCAGGCACGTCGAATTTACCGCCGGGACGCCCCTCGTCCCGACACTTCCAGATCGCCATAGCGCACAGCGC
p15A	TTAATAAGATGATCTTCTTGAGATCGTTTTGGTCTGCGCGTAATCTCTTGCTCTGAAAACGAAAAAACCGCCTTGCAGGGCGGTTTTTCGAAGGTTCTCTGAGCTACCA ACTCTTTGAACCGAGGTAACTGGCTTGGAGGAGCGCAGTCACCAAAACTTGTCCTTTCAGTTTAGCCTTAACCGGCGCATGACTTCAAGACTAACTCCTCTAAATCAAT TACCAGTGGCTGCTGCCAGTGGTGCTTTTGCATGTCTTTCCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGACTGAACGGGGGGTTCGTGCAT ACAGTCCAGCTTGGAGCGAACTGCCTACCCGGAACTGAGTGTCAGGCGTGGAATGAGACAAACGCGGCCATAACAGCGGAATGACACCGGTAAACCGAAAGGCAGGAAC AGGAGAGCGCACGAGGGAGCCGCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCACTGATTTGAGCGTCAGATTTCGTGATGCTTGTCAGGG GGGCGGAGCCTATGGAAAAACGGCTTTGCCGCGGCCCTCTCACTTCCCTGTTAAGTATCTTCCTGGCATCTTCCAGGAAATCTCCGCCCCGTTCGTAAGCCATTTCCGC TCGCCGCAGTCGAACGACCGAGCGTAGCGAGTCAGTGAGCGAGGAAGCGGAATATATCCTGTATCACATATTCTGCTGACGCACCGGTGCAGCCTTTTTTCTCCTGCCA CATGAAGCACTTCACTGACACCCTCATCAGTGCCAACATAGTAAGCCAGTATACACTCCGCTA
ColE1	TCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTG CTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAAT ACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCG ATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGAC CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAG CGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA GCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCG T
pUC	TCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTG CTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAAT ACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCG ATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGAC CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAG CGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA GCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCG T
ColE2	AGGGCGCTGTTATCTGATAAGGCTTATCTGGTCTCATTTT

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