

Cell 156/6

Supplemental Information

The Transcription Factor Titration Effect Dictates Level of Gene Expression

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Table S1. Primers Used in this Study to Create Strains, Related to Figure 1

The names of the chromosomal integration primers are formatted with the gene location followed by the side of the plasmid it binds to (the resistance or the FP reporter; see Figure S6) with <> between. The red bases bind to the plasmid to amplify, and the black bases are homologous to the integration site on the chromosome.

Name	Sequence
Chromosomal integrations	
<i>galK</i> <>res	TTCATATTGTTTCAGCGACAGCTTGCTGTACGGCAGGCACCAGCTCTTCCGGCTAATGCACCCAGTAAGG
<i>galK</i> <>YFP	GTTTGGCGCAGTCAGCGATATCCATTTTCGCGAATCCGGAGTGAAGAACTAGCAACACCAGAACAGCC
<i>gspI</i> <>res	TGCCAGAACTGGAGCTGTTTTCTCGCCGAATGAATCTTGTGACTGAAGCGGCTAATGCACCCAGTAAGG
<i>gspI</i> <>YFP	TCAAACGCTCGCCAGAGATACCCGCCCATGAACAAACAATCAGGGATGACACTAGCAACACCAGAACAGCC
<i>intS</i> <>res	ATAGTTGTTAAGTTCGCTCCTCACCTTCTCATCAAGCCAGTCCGCCAGGCTAATGCACCCAGTAAGG
<i>intS</i> <>YFP	CCGTAGATTTACAGTTCGTCATGTTTCGCTTCAGATCGTTGACAGCCGCACTAGCAACACCAGAACAGCC
<i>yffO</i> <>res	TTTCAAACCTATTTCAGCTTGGCTGCTGCCAGTAGTGCCTTTGCCCTTTGCTTGGCTAATGCACCCAGTAAGG
<i>yffO</i> <>YFP	GGTGAATCATGAAACACGTTTTTAAATATCTTGATTTGCGAAGACCCACTAGCAACACCAGAACAGCC
<i>intB</i> <>res	ACGCATATTTACCGTATTCCTACTCATGGGTTTGTGCGAATCGTGATCAAGCTAATGCACCCAGTAAGG
<i>intB</i> <>YFP	TGTCCATCCAAATGGTTCTAAGTACTGGCGTTGCGAGTACCGTTATGAGGACTAGCAACACCAGAACAGCC
<i>intE</i> <>res	CAAGCGATCCAGGATGACAGGCTTAAAAGTGGTGATATAAGACTCAACACGGCTAATGCACCCAGTAAGG
<i>intE</i> <>YFP	TCACAACGCTACTTTGCTGCATCCTTACCTCGATCATCATGATAACGATACTAGCAACACCAGAACAGCC
<i>essQ</i> <>res	TAAGGCTACAGTTACCGTAACTTTATTCTCAAAATTACGGACTCCTTCAAGCTAATGCACCCAGTAAGG
<i>essQ</i> <>YFP	TAAAGGTCCTGCGAGCAGCAATGTCATCATTACTGATTAAAATTATCGCAGCTAGCAACACCAGAACAGCC
Mutagenesis	
15.15	GGAGTCCAAGCGAGCTCAGTTCCTATTCCGAAGTTCCTATT CTCTAGAAAAGTATAGGAACTTCGATATCCGTCGGCTTGAACG
15.16	GGTTCGTGCTTCATCGAAGTTCCTATTCCGAAGTTCCTATT CTCTAGAAAAGTATAGGAACTTCATATCGACGCTCAAGAAAAC
21.3	CCGGCTCGTATAATGTGTGGGATTGTTAGC GGAGAAGAATTGAATTCATTAAGAGGAG
Lacl-mCherry fusion	
13.12	ATTATTGGTACCGCATGGTTTCCAAGGGCGAGGAGG
13.13	ATATCTAAAGCTTATTTGTACAGCTCATCCATGCCACC
13.28	ATTATAGGTACCATATGGTGAATGTGAAACCAAGTAAC
13.30	CTCGCCCTGGAAACCATCACCAGTCCAGGCCGCCAGCTGCATTAATGAATCGGCCAA
CFP amplification	
15.14	ATTATTGGTACCGCATGACTAGCAAAAAGCAAGGTTG
15.14R	ATAATATAAGCTTTATACAGTTCATCCATGCCAG
TetR cloning	
13.6	ATACAAAAGCTTAAGACCCACTTTCACATTTAAGTTGTT
13.7v2	ATACAACCTCGAGGCGCAACGCAATTAATGTAAGTTAGC
Competitor plasmid	
O1Add-F	CTAGACTCAGCTAATTAAGAATTGTTATCCGCTCACAATTATAATGGTTCTTAGACGT
O1Add-R	CTAAGAAAACCATTAATAATTGTGAGCGGATAACAATTCCTTAATTAGCTGAGT
O2Add-F	CTAGACTCAGCTAATTAAGGGTTGTTACTCGCTCACATTTATAATGGTTCTTAGACGT
O2Add-R	CTAAGAAAACCATTAATAATGTGAGCGAGTAACAACCCCTTAATTAGCTGAGT
OidAdd-F	CTAGACTCAGCTAATTAAGAATTGTGAGCGCTCACAATTATAATGGTTCTTAGACGT
OidAdd-R	CTAAGAAAACCATTAATAATTGTGAGCGCTCACAATTCCTTAATTAGCTGAGT
qPCR primers	
forward	CAGTGGAGAGGGTGAAGGTG
reverse	GTGTCTTGTAGTCCCGTCAT
probe	/56-FAM/TCAAGAGTG/ZEN/CCATGCCCGAAGGT/3IAbkFQ/

Extended Experimental Procedures

Genetic elements and details of the dilution method

Dilution circuit

The dilution method is used in this work to measure the transcription factor (TF) titration curve for the expression of a gene under the control of that TF (Rosenfeld et al., 2005). The required genetic elements of the dilution circuit are: the target gene with a fluorescent protein product (YFP) whose promoter has the regulatory architecture to be queried, a transcription factor-fluorescent protein fusion (LacI-mCherry) whose production is tightly regulated and shut off by another repressor (TetR) that can be inactivated by a small molecule inducer (aTc). Finally, a volumetric marker (CFP) is used to easily segment cells in microscopy images. A schematic of the dilution circuit used here is shown in Figure S1A. To measure fold-change, we also measure the rate of expression of a strain which does not contain the LacI-mCherry fusion gene.

The one step dilution method

As originally outlined (Rosenfeld et al., 2005), the dilution method consists of fully inducing a culture to the maximal level of a TF concentration before shutting off production of the TF and observing under a microscope as individual cells grow to form colonies. During this growth process, the quantity of transcription factor in each cell drops and the response of a gene product which is regulated by that TF is measured in successive generations, with each generation diluting the TF by roughly a factor of two. A powerful aspect of this method is that by observing how the TF partitions to the daughters, one can arrive at a “calibration” relating the fluorescence of the TF-fluorescence protein fusion to the actual number of TF molecules present.

In the experiments reported here we alter the strategy slightly. Instead of fully inducing the culture and taking a long dilution movie, we variably induce with 6 to 10 distinct concentrations of inducer such that the entire range of starting TF concentration is covered. Individual cells are followed over one full division cycle with only one mCherry fluorescence measurement (which measures relative LacI-mCherry concentrations) followed by 75 minutes of gene production measurements (10 exposures, once every 7.5 minutes). We argue this method has several advantages which improve the measurement over its original version:

Data points are acquired in a uniform fashion over the whole range of induction. In the long growth method, the data is exponentially distributed towards lower TF concentrations (for every one cell with N repressors, there are 2 with $N/2$, 4 with $N/4$, etc.).

The accuracy of the measurement is uniform over the range of repressor concentrations. In the long growth method, measurements in the high TF copy number regime are less photobleached as compared to cells in the low TF copy number regime, which occurs towards the end of the movie. A bleaching correction needs to be done for the entire movie’s worth of exposures that were previously taken. This results in a statistical averaging of our partitioning events during cell division and amplifies noise which is the dominant source of error, particularly late in the movie, when the signal is low.

By using one, long exposure in the repressor measurement we get an extremely accurate measurement since we don’t need to limit the exposure times in order to minimize photobleaching.

Colony size is small and independent of TF copy number. In the long growth method, low TF numbers are always correlated with larger colony sizes which can make *very* significant contributions to the background fluorescence from neighboring cells. In particular, once multiple layers of cells begin to grow in the middle of big colonies, we find that the contributions from out of plane fluorescence can be as big as the signal itself.

Physiological effect of repressor induction

To demonstrate that the induction of repressor does not introduce a global physiological change to the cell as a function of induction, in Figure S2 we show that the relative expression of the volume marker from a constitutive *lacUV5* promoter integrated on the chromosome (solid circles) remains unaffected as we change the repressor copy number by almost three orders of magnitude. This promoter is identical to the one responsible for the expression of our reporter gene (data also shown in corresponding color and open circles), except that its repressor binding site has been mutated away (see SI section “Constructs and strains”). We conclude that the fold-change of our reporter gene does not change significantly as a result of physiological changes in the cell resulting from varying the intracellular repressor load.

Cell growth and detailed experimental procedure

Three distinct strains are grown for each experiment. First, the rate of YFP expression from the construct of interest is measured in a strain background bearing *LacI-mCherry*. Second, the rate of expression of the same construct is measured in a strain lacking *Lac* repressor. Finally, the autofluorescence is determined using a strain which does not have the *LacI-mCherry* construct or a reporter YFP construct.

Overnight cultures are grown in 2 ml of LB in the presence of the appropriate antibiotic (chloramphenicol is always present for the TetR plasmid and kanamycin for the both *ColE1* based plasmids or ampicillin for the “competitor” plasmids) at 37°C. They are then diluted ~ 1: 10,000 in M9 + 0.5% glucose minimal media with antibiotics and anhydrotetracycline (aTc; Acros Organics cat. num. 233131000) at several different concentrations (1ng/ml, 2ng/ml, 3ng/ml, 4ng/ml, 6ng/ml, 8ng/ml or 100ng/ml) to induce the production of various levels of *LacI-mCherry*. The induction curve for *LacI-mCherry*, shown in Figure S1B, is used as a guide for choosing aTc concentrations that cover the full repressor range is shown. These minimal media cultures are grown at 37°C until they reached an OD600 \approx 0.2 – 0.4 and then they are washed twice with fresh, M9 media (without aTc) to remove the inducer.

The resuspended cultures are then diluted (typically 1: 10 in fresh M9 + 0.5% glucose minimal media) to give several cells per field of view when 2 μ l are placed on a 2% low melting point M9 + 0.5% glucose agar pad (NuSieve GTG Agarose, Lonza cat. no. 50081). An automated Nikon fluorescent microscope (Nikon Eclipse TI) is controlled by the software Micro-Manager (Edelstein et al., 2010), and multiple fields of view (totaling roughly 35 individual fields per experiment) are recorded simultaneously for each concentration of aTc. In addition, one pad contains cells without the repressor construct whose expression measurements serve as the denominator of our fold-change measurements (*i.e.* expression for $R = 0$). Before the growth movie is started, the autofluorescence signal in YFP and mCherry is measured from the autofluorescence strain with 10 positions accounting for roughly 500 individual cells.

Growth of the *lacI-mcherry* and Δ *lacI-mcherry* strains is observed by fluorescence microscopy at 37°C over 2.5 hours with CFP exposures every 7.5 minutes for the first 9 frames of growth. This initial period of exposures is used to record the lineage of all cells and identify daughter pairs. In the

10th frame, the CFP exposure is taken along with a single, long exposure of mCherry to determine the LacI concentration in every cell. The last 10 frames consist of both CFP and YFP exposures every 7.5 minutes. The difference in corrected fluorescence of consecutive YFP images (corrections explained below) makes one measurement of expression. By examining only the first division we eliminate colony size as a source of error in our fluorescence measurements; cells in large colonies can have non-trivial contributions to their fluorescence signal from neighboring cells. In addition, only measuring the LacI-mCherry concentration once eliminates the necessity to correct for photobleaching which necessarily assumes that the bleached fluorophores have been proportionately distributed to the daughters. Furthermore it increases the sensitivity of the LacI-mCherry measurement by allowing for longer exposures without worrying about bleaching. Exposures are chosen to be as long as possible without impacting the growth rate, compared to a control with no fluorescence exposures.

Image segmentation and analysis

For cell segmentation and lineage identification, we use a modified version of Schnitzcells (Young et al., 2012) (kindly provided by the lab of Michael Elowitz, Caltech) designed to segment on a fluorescence marker. We have altered the program slightly such that segmentation and tracking is automated with error checks based on lineage verification (every cell either has a mother or was alive in frame 1, every cell has two daughters or was alive in frame 20) and growth verification (to check that cells do not grow (or shrink) too fast, this usually indicates a tracking error). Failing either of these error checks requires manual intervention, however, most movie positions do not require any intervention. Once all errors are resolved, the program provides a list of all cells, their lineages and the total fluorescence intensity (pixel intensities summed over the segmented pixels of a cell) for every channel and every frame for each cell.

There are essentially two separate data collections going on in the same experiment. One data collection corresponds to gathering pairs of daughter cells whose lineage is known (*i.e.* their common mother cell is known) and have an mCherry measurement (they had divided from their mother already by frame 10). The second data collection corresponds to expression measurements where a cell must have an mCherry measurement to quantify the LacI-mCherry number (*i.e.* the division event that produced the cell must have occurred before frame 10), and must have both been “born” during the movie and divided again sometime later in the movie (it must have an identified mother and daughter set). This prerequisite of two division events allows us to categorize where in the cell cycle the expression measurement occurs. Knowing the location in the cell cycle is important since the copy number of plasmids and chromosomal integrations changes over time. Fluorescence values are corrected for field non-uniformities, chromatic aberration, autofluorescence, photobleaching and crosstalk as described in the following sections. Autofluorescence values for YFP and mCherry are determined as the fluorescence value per pixel from the snapshots of the autofluorescence strain taken immediately preceding each movie.

Flattening fluorescence images

Our illumination is not spatially uniform over an entire field of view. We correct for this fact by taking fluorescence images in the YFP channel of a plastic slide with uniform but bright autofluorescence intensity (Autofluorescent Plastic Slides, Chroma cat. no. 92001) and averaging over 10 – 20 of these images, the resulting image is a map of illumination intensity at any given pixel I_{flat} . The raw images, I , are then renormalized such that for pixel i, j with raw intensity $I^{(i,j)}$,

$$I_{\text{corrected}}^{(i,j)} = \frac{I^{(i,j)} - I_{\text{dark}}^{(i,j)}}{I_{\text{flat}}^{(i,j)} - I_{\text{dark}}^{(i,j)}} \times \text{mean} \left(I_{\text{flat}}^{(i,j)} - I_{\text{dark}}^{(i,j)} \right), \quad (\text{S1})$$

where I_{dark} corresponds to an image taken with no illumination (mostly these counts are from the camera offset).

Chromatic aberration correction

Due to chromatic aberrations in the microscope, the various fluorescence channels are slightly offset from each other. We measure this offset by imaging microspheres (Invitrogen Tetraspeck microspheres, cat. no. T-7281) which fluoresce in all three channels we use (CFP, YFP and mCherry) and rapidly image in all three channels. We then measure the center to center distance of the identified sphere in the three images. We find that the YFP image is translated in the x -direction by 2 pixels and the mCherry is translated by 3 pixels in the same direction with respect to the CFP image, we find there is no offset in the y -direction. To account for this we translate all YFP images and all mCherry images by the measured offset and trim the edges such that we only look at pixels where there is a measurement in all three channels.

Autofluorescence correction

To calculate the autofluorescence stemming from cells in the YFP and the mCherry channel, we take 8 – 10 snapshots of a strain which is Δyfp and $\Delta lacI\text{-}mcherry$ and measure the average per pixel intensity of the identified cells in both the YFP channel and the mCherry channel. This average is then subtracted from each pixel of any YFP or mCherry measurement that is made.

Correcting for crosstalk and cross bleaching

We measure the crosstalk between any two channels used in our experiment by determining the difference between the autofluorescence of a strain without a given fluorophore in the presence of the other fluorophore fully induced. So, for instance, we can find the crosstalk of YFP into the mCherry channel by taking exposures of our $\Delta lacI\text{-}mcherry$ strain with the appropriate YFP construct (depending on the experiment in question). The ratio of the average per pixel mCherry fluorescence signal to the average per pixel YFP signal (corrected for all the above factors) is the crosstalk. Therefore we correct mCherry measurements for this factor, γ_{cross} by subtracting from the cells summed mCherry fluorescence, the summed YFP fluorescence times this crosstalk factor. We do not have to worry about normalizing exposure times because the crosstalk factor between any two channels is measured with the exposure time used in the experiment. We find this crosstalk factor is only relevant in the case of the ColE1 and ColE1 Δrom plasmids which express YFP. In this case we measure $\gamma_{\text{cross}} = 0.006$ or 0.6% of the YFP signal can be seen in the mCherry channel. For our other constructs there is 10 – 50 times less YFP (corresponding to 1 – 10 copies of the YFP gene compared to 50 – 70 plasmids) and so we can expect the effect would be correspondingly smaller though for our experiments it is too small to measure and this correction is not included in those cases.

Conveniently, we do not have to worry about CFP crosstalk into YFP channel. The first point is that all cells have the same expression of CFP (same constitutively expressed construct), so any potential crosstalk shows up as autofluorescence when we measure YFP; even our autofluorescence strain expresses CFP with the proper construct. Additionally, because our YFP measurement is always measured as a rate of production, which is the difference in production from consecutive frames, most of the autofluorescence and crosstalk corrections cancel out since the correction term is proportional to the size of the cell on both measurements.

We also check for cross bleaching between the fluorescence channels. It is possible that one of our exposures, shaped to excite a particular fluorophore, excites and bleaches a different fluorescent protein species (for instance if the CFP exposure excites and bleaches YFP). Bleaching in the CFP channel does not change our measurements and the mCherry exposure occurs only once before we begin to measure YFP and because we are only concerned with the rate of YFP production, bleaching YFP molecules before we begin measurements does not change the measured rate of production. Therefore, we only need to worry about how the CFP exposures bleach the YFP or mCherry signal. To account for the CFP exposure, all YFP bleaching curves are measured accounting for both the YFP and CFP exposure, therefore the bleaching from the CFP exposure is rolled into our measurement of the YFP bleaching rate. To check the cross bleach rate of CFP on mCherry, we take an mCherry exposure followed by a long CFP exposure 600× longer than that used in experiment followed by a last mCherry exposure. As a control we also measure the bleach rate of the mCherry exposures alone, without the CFP exposure. We find that the bleaching for this extremely long exposure is roughly 25% implying that the bleaching from a single exposure is less than 1/10th of a percent.

Correcting for photobleaching

The only channel which must be corrected for photobleaching is YFP. Due to the fact that only one mCherry image is taken per experiment, we do not need to correct it for photobleaching. Before each movie we measure a photobleaching curve of YFP using the highest expression strain available. The characteristic bleaching rate, τ , is extracted by fitting the autofluorescence subtracted bleaching curve to a single exponential decay. Then, all measurement of YFP production, ΔYFP , are corrected such that,

$$\Delta YFP = YFP_{i+1} - YFP_i (1 - \gamma), \quad (S2)$$

where $\gamma = e^{(-t_{\text{exp}}/\tau)}$ and t_{exp} is the exposure time for a YFP image.

Calibrating LacI-mCherry intensity to absolute copy number

The absolute number of TFs per cell is usually obtained by cross-calibrating to independent measurements such as immunostaining (Oehler et al., 1994; Martin et al., 2008; Garcia and Phillips, 2011). In our case, where our signal comes from the LacI-mCherry fusion, the total fluorescence intensity of a cell, I , can be related to the absolute number of TFs N through the calibration factor α such that,

$$I = \alpha N. \quad (S3)$$

The calibration factor is often determined by measuring the mean intensity of a single copy of the fluorescent molecule (Choi et al., 2008; Taniguchi et al., 2010; Garcia and Phillips, 2011) or of a bulk solution of purified fluorophore (Hirschberg et al., 1998; Piston et al., 1999; Sourjik and Berg, 2002; Gregor et al., 2007; Taniguchi et al., 2010). Here we determine α using a calibration method based on fluctuations in protein partitioning during cell division (Rosenfeld et al., 2005, 2006; Teng et al., 2010). By tracking fluorescence partitioning between two daughters after a division, the properties of binomial partitioning state that the average size of fluctuations in the signal of daughter 1 and 2 will be proportional to the total fluorescence signal partitioned.

This circumvents the need for a cross-calibration as it allows us to obtain α and simultaneously measure absolute TF copy number, R , in single cells. We expect the distribution of our LacI-mCherry between the two daughter cells should obey the statistics of a fair binomial partitioning (Figure S3A and B, discussed below). This simple fact alone is enough to determine the calibration factor α . In

particular, by observing the fluorescence of the two daughters, captured in the quantities I_1 and I_2 , it can be shown that

$$\langle (I_1 - I_2)^2 \rangle = \alpha(I_1 + I_2), \quad (\text{S4})$$

where α is the desired calibration factor that links fluorescence intensity and number of fluorophores via $I = \alpha N$. This relation follows from the properties of the binomial distribution as shown in the following subsection. In Figure S3C, we show an example of the calibration data from the experiment in Figure 3. The exact value for the calibration factor is specific for a given acquisition and the current settings of the microscope and is determined for each experiments unique imaging conditions (exposure times, illumination intensity, etc.).

Fairness of repressor partitioning

The fluctuation-based counting method employed here relies on measuring the asymmetries in partitioning of a transcription factor-fluorescent protein fusion during the cell division process. In this scenario of DNA bound transcription factors, it is *assumed* that the partitioning between daughter cells is random, mediated by the segregation of the chromosomal DNA (to which the LacI-mCherry are bound) to the daughter cells. This corresponds effectively to each molecule making a coin flip. In Figure S3A and B, we show the partitioning error of fluorescence with area. On the y -axis the percent of the difference in partitioned area of each daughter at division, normalized by the total area of the two daughters is shown. The x -axis shows the percent of the total difference in mCherry fluorescence between the two daughters divided by the total. If the protein was more likely to partition into bigger cells (because it has more volume), larger cells would have an increased probability of obtaining more protein and the cloud of points would tilt towards the upper right and lower left quadrants of Figure S3. This behavior is seen in Figure S3B for the partitioning of a cytoplasmic protein (CFP). The CFP results are consistent with previous reports where it was shown that for cytoplasmic proteins the error in volume partitioning on division can influence the “fairness” of the distribution (Teng et al., 2010). However, the correlation we see for LacI-mCherry is very weak, indicating that volume partitioning fluctuations do not have a strong effect on the fluctuations in the partitioning of LacI-mCherry.

Derivation of calibration factor

It is of interest to have a simple derivation of the relation between fluorescence intensity and repressor number. To do this we exploit a convenient statistical property of binomial partitioning. That is that if a mother cell had N_{tot} repressors and divided them randomly between two daughter cells which now have N_1 and N_2 repressors, respectively, then the variance in the total number of repressors in one daughter, N_1 , is $\sigma^2 = N_{\text{tot}}/4$. However, the variance can also be written,

$$\sigma^2 = \langle (N_1 - \langle N_1 \rangle)^2 \rangle, \quad (\text{S5})$$

$$= \left\langle \left(\frac{N_1 - N_2}{2} \right)^2 \right\rangle, \quad (\text{S6})$$

using $\langle N_1 \rangle = (N_1 + N_2)/2$. By combining these two expressions for the variance, we arrive at the final expression relating the total number of repressors in the daughters to the difference in that number,

$$\langle (N_1 - N_2)^2 \rangle = N_1 + N_2. \quad (\text{S7})$$

By assuming that the measured intensity in a cell I can be written as $I = \alpha N$, where α is some calibration factor that converts from number of proteins to intensity we now find,

$$\langle (N_1 - N_2)^2 \rangle = N_1 + N_2 \Rightarrow \left\langle \left(\frac{I_1}{\alpha} - \frac{I_2}{\alpha} \right)^2 \right\rangle = \frac{I_1 + I_2}{\alpha}, \quad (\text{S8})$$

$$\frac{1}{\alpha^2} \langle (I_1 - I_2)^2 \rangle = \frac{I_1 + I_2}{\alpha}, \quad (\text{S9})$$

$$\Rightarrow \sqrt{\langle (I_1 - I_2)^2 \rangle} = \sqrt{\alpha(I_1 + I_2)}. \quad (\text{S10})$$

This gives us the relationship between the fluctuations in the difference between the intensities of two daughter cells and the total intensity present between the two daughters, $I_{\text{tot}} = I_1 + I_2$. We can determine the unknown calibration factor α by taking time-lapse movies of dividing bacteria, tracing lineages to determine which pairs of daughter cells came from which mother cells, and for each set of daughters plotting $\langle (I_1 - I_2)^2 \rangle$ versus $I_1 + I_2$. A more sophisticated treatment using information from tracking over multiple generations and the introduction of random errors can be found in reference (Rosenfeld et al., 2006).

Interpretation of $\langle (I_1 - I_2)^2 \rangle$

As noted above, the mathematical derivation for the error in partitioning is predicated on the idea that for a given value of $(I_1 + I_2)$, we have many division events to average over to arrive at a well averaged value for the partitioning error $\langle (I_1 - I_2)^2 \rangle$. However, the data itself in the experimental case does not come in this convenient format. This raises the concern of how data will be binned. In practice, data is binned by fracturing the data into bins of a set number of data points. The data point corresponding to the bin is placed in the geometric center of the data comprising that bin such that data points in the bin fall with equal weight to the left and right of the bin center in log space.

In Figure S3D we show the effect of choice in bin size on the calibration factor by plotting the calibration factor as a function of the number of points in each bin. Over the majority of the range of bin sizes the calibration factor is relatively insensitive to the bin size. However, when bins have few points the calibration factor is strongly affected by the presence of data points where, by chance, $\langle (I_1 - I_2)^2 \rangle \approx 0$ which weighs heavily on a log-log fit. It is interesting that the fit is not changed by making the size of the bins very large (thus averaging data over a larger range of $(I_1 + I_2)$). In this case, where the data should fit a straight line in log-log space, a point located a distance ϵ away from the bin center on either side is expected to contribute equal and opposite weight to the function value and thus should not change the fit.

Photon counting noise

One possible additional source of noise might be simply the Poisson noise corresponding to counting photons. Our camera is a Photometrics CoolSNAP ES² which has a linear full-well count of 13,500 electrons. This means that for this 12-bit output, a count on the camera corresponds to roughly 3.3 photons detected. As seen in Figure 3 of the main text, a LacI typically corresponds to roughly 100 counts on the camera which means 330 photons counted per LacI. The Poissonian standard deviation for a single LacI is then 18 photons or 6% of a LacI. This is small even in the single LacI limit. When we have 10 LacI-mCherry molecules or more, the noise is lower than 2%.

This error is smaller than any of the errors related to quantifying our fluorescence levels. As a result, Poisson statistics are not expected to influence the partitioning error significantly.

Limits in LacI-mCherry detection

In order to check our ability to distinguish low repressor copy numbers from cellular autofluorescence fluctuations, we examine the mCherry fluorescence signal of a collection of cells from our *ΔlacI-mcherry* control strain. On average these cells will have zero signal, once they are corrected for autofluorescence (each pixel has the average signal per pixel of the *ΔlacI-mcherry* strain subtracted) the remaining signal is, on average, 0. However, due to fluctuations the signal is typically not exactly 0 and instead has a distribution. This can be seen for an example experiment in Figure S3E, when we histogram the mCherry signal from a collection of our *ΔlacI-mcherry* cells. As can be seen, the average is indeed 0, but the distribution has a standard deviation of 3 LacI-mCherry repressors. Therefore, we set our confidence regime for measuring LacI-mCherry signal in this experiment at 3 LacI-mCherry and do not consider cells which are measured as having less signal than this since our measurements show we cannot resolve the difference between 0 and 3. This detection limit is calculated in every experiment and that value is used as a threshold for all data from that experiment; we do not accept cells with an mCherry signal lower than our detection threshold.

Limits in YFP production detection

In a similar fashion to the LacI-mCherry detection threshold, the production measurements also have a lower limit of detection. We account for this in the fold-change vs. LacI number measurements by rejecting binned data points where the standard error is larger than the value of the point itself. In almost all cases, this threshold occurs at a fold-change in the range of 10^{-3} and 10^{-2} . Intuitively, this is the range where the fluctuations of the autofluorescence in YFP become significant. For instance, taking our autofluorescence measurements of YFP (static snapshots), normalized by the YFP of the *ΔlacI-mcherry* strain, we find that the standard deviation of the fold-change in YFP of these cells is between 10^{-2} to 10^{-3} . This choice is designed to remove points without significant information and does not affect the quality of the data. This limitation can be seen in Figures 3-6 where the points typically cut off around a fold-change of 10^{-3} .

qPCR measurement of average plasmid copy number

To measure the average copy number of the ColE1 and ColE1 *Δrom* plasmids we performed qPCR measurements. The primers we used target part of the YFP gene and the sequences for these primers are given in table S1. The probe primer is ordered from Integrated DNA Technologies and the /56-FAM/, /ZEN/ and /3IABkFQ/ tags refer to modifications from parts of the ZEN internal quencher system.

A DNA sample to be used as a standard is obtained by Maxiprep (Qiagen Hi Speed Plasmid Maxi Kit) of the ColE1 *Δrom* plasmid which was further concentrated in a PCR purification column (QIAquick PCR Purification Kit). The final concentration of the stock plasmid is ≈ 600 ng/ μ l as measured by a Qubit fluorimeter (Invitrogen Qubit dsDNA HS Assay Kit). As a control to determine the purity of our purified plasmid stock from chromosomal DNA contamination, we also perform the same Maxiprep on a culture without the plasmid and find a final concentration of less than 5% of the measured plasmid concentration. Then, starting with a 16x dilution of the stock, we step down by factors of 4 to generate a standard dilution series; meaning we have 8 standard concentrations

ranging from a $16\times$ dilution of the stock down to a 10^6 dilution of the stock separated by factors of 4 in concentration.

For the qPCR measurement we start by growing the ColE1 Δrom and ColE1 cells in the same conditions as our cells used for microscopy measurements. However, we chose an aTc concentration ($4 \text{ ng}/\mu\text{l}$) which corresponds to a LacI concentration close to the transitional region of the fold-change curve (Figure S1B). We also grow a strain with no plasmid or YFP genes which will act as a background for the standard and make 8 samples out of this strain, one for each standard. When the cells are at the proper OD they are spun down, washed twice (exactly as described in section “Cell growth and detailed experimental procedure”) and finally resuspended in $200 \mu\text{l}$ of Qiagen P1 lysis buffer without LyseBlue or RNaseA. We then add $1 \mu\text{l}$ of the prepared pre-diluted standards to each control tube, such that the standards will undergo the exact same process as our samples to be measured. The cell mixtures are then set on ice while the cellular density in each sample is measured by hemocytometer chips (InCyto DHC-S01) under $10\times$ phase magnification.

Meanwhile, $25\mu\text{l}$ of cells is then added to $25\mu\text{l}$ of Qiagen buffer P2 to lyse the cells. The cells are allowed to sit for 5 minutes. The cells are then diluted 1:100 into $1\times$ NEB buffer 2 ($1 \mu\text{l} + 99 \mu\text{l}$) and $20 \mu\text{l}$ of that mixture is added to a thin walled PCR tube with $0.5 \mu\text{l}$ HindIII (NEB) restriction enzyme. The mixture digests at 37°C for 30 minutes followed by heat deactivation for 20 minutes. This mixture is then diluted 1:10 in water. The final $20 \mu\text{l}$ qPCR reaction consists of: $4.2 \mu\text{l}$ of template, $10 \mu\text{l}$ Supermix (PerfeCTa MultiPlex qPCR SuperMix, Quanta BioSciences Cat. no. 95063-200), $0.4 \mu\text{l}$ forward primer, $0.4 \mu\text{l}$ Rox, $5 \mu\text{l}$ water. The number of copies of the YFP gene are determined by comparing the measured CT of each sample and interpolating from the standard. Together with the knowledge of the number of cells in the sample (from the hemocytometer measurements) we arrive at an average copy number of the plasmid in our cells.

Additional theoretical details of the thermodynamic model

Equivalence of fold-change in steady-state measurements and video microscopy

In bulk, the fold-change is calculated by comparing the steady state fluorescence, P , of cells with repressor to the fluorescence of those without repressor. To determine this steady-state fluorescence, we consider the rate of production of the fluorescent reporter,

$$\frac{dP}{dt} = rp_{\text{bound}} - \gamma P, \quad (\text{S11})$$

where r is the rate of production, p_{bound} is the probability that the promoter is occupied by RNA polymerase and γ is the degradation rate. In steady-state, we find

$$P = \frac{rp_{\text{bound}}}{\gamma}, \quad (\text{S12})$$

which implies that the fold-change in steady-state experiments can be written as

$$\text{fold-change} = \frac{P(R \neq 0)}{P(R = 0)} = \frac{p_{\text{bound}}(R \neq 0)}{p_{\text{bound}}(R = 0)}. \quad (\text{S13})$$

The right hand side is a quantity that is directly calculable in the thermodynamic framework (Buchler et al., 2003; Vilar and Leibler, 2003; Bintu et al., 2005b,a). However, over the time scales of our experiments, YFP is stable (*i.e.* $rp_{\text{bound}} \gg \gamma$) (Andersen et al., 1998). As a result, the rate of fluorescent increase we measure in video microscopy is simply rp_{bound} . This implies that we can write the fold-change in our experiments as

$$\text{fold-change} = \frac{\frac{dP}{dt}(R \neq 0)}{\frac{dP}{dt}(R = 0)} = \frac{p_{\text{bound}}(R \neq 0)}{p_{\text{bound}}(R = 0)}, \quad (\text{S14})$$

and thus the comparison of fold-change as measured in steady-state experiments should be directly comparable to that measured as a production rate in video microscopy and to the theoretical predictions of the thermodynamic theory which calculated p_{bound} .

Thermodynamic model in the limit $R \gg N$ or $R \gg N_c$

Equations 4 and 5 from the main text predict the fold-change in expression as a function of the number of binding sites available to the repressor (N or N_c , respectively). However, when the number of repressors is much larger than the number of binding sites available, such that the approximation $R!/(R - N)! \approx R^N$ is valid, these equations immediately simplify to

$$\text{fold-change} = \frac{1}{1 + \frac{R}{N_{\text{NS}}} e^{-\Delta\epsilon/k_B T}}, \quad (\text{S15})$$

identical to the prediction for a single isolated copy of the gene in equation 3.

Accounting for chromosome replication in competitor theory

In the theoretical predictions of equation 5 it is assumed that the reporter gene integrated into the chromosome exists at only a single copy. This introduces an error in our calculation of N_c during the portion of the cell cycle where two copies of the reporter gene exist. This error does not come from the presence of an extra copy of the gene producing more of the reporter gene product; measuring fold-change ensures that we are normalizing by cells expressing with the same average copy number of the gene. However, the addition of a new operator site associated with the chromosomal gene copy will change the expression profile by contributing to the demand for repressor and this will be interpreted in our measurement as a larger value for of N_c . The general formula to derive this effect follows from the partition function,

$$Z = \sum_{r_c=0}^{\min(R, N_c)} \sum_{r_{\text{int}}=0}^{\min(R-r_c, N_r)} \frac{R!}{N_{\text{NS}}^{(r_c+r_{\text{int}})} (R-r_c-r_{\text{int}})!} Z_{r_c}^c Z_{r_{\text{int}}}^{\text{int}}, \quad (\text{S16})$$

with $Z_i^c = \binom{N_c}{i} \exp(-\beta i \Delta\epsilon_c)$ and $Z_i^{\text{int}} = \binom{N_{\text{int}}}{i} \exp(-\beta i \Delta\epsilon_{\text{int}}) (1+p)^{(N_{\text{int}}-i)}$ where N_{int} is the number of integrated copies that exist on the chromosome and N_c is the number of competitor plasmids, $\Delta\epsilon_c$ and $\Delta\epsilon_{\text{int}}$ are the repressor binding energies to the chromosomal operator and plasmid operator, respectively, and finally $p = (n_p/N_{\text{NS}}) \exp(-\Delta\epsilon_p/k_B T)$ where n_p is the number of RNA polymerase, and $\Delta\epsilon_p$ is the energy of polymerase binding to the promoter. The fold change is then,

$$\text{fold-change} = \frac{\partial_p \ln(Z)}{\partial_p \ln(Z_{R=0})}. \quad (\text{S17})$$

For our particular experiments, the integrated copy begins at a single copy and doubles over the course of the cell cycle. Figure S5 shows the predicted fold change for an integrated O1 promoter with $N_{\text{int}} = 1$ (solid lines) or 2 (dashed lines) and a competitor plasmid with $N_c = 64$ and an O1 operator site identical to the chromosomal operator, an O2 operator site weaker than the

chromosomal operator or an Oid operator site stronger than the chromosomal operator. In all cases the predicted change between one and two integrated gene copies is small.

Thermodynamic model with plasmid distribution

The fold change predictions in equations 4 and 5 of the main text are derived by assuming that any given cell has exactly N plasmids. However, our measurements are averaged over many different cells and thus we do not expect the copy number of the plasmid to be exactly the same in every cell. While this static single parameter characterization of the copy number is sufficient to predict the fold-change repression titration curve in most of the cases we examine, we wish to determine how the reality of the plasmid distribution changes our predictions. To begin, we rewrite the fold-change in terms of expression measurements for the case of a static number of plasmids, N ,

$$\text{fold-change} = \frac{\text{expression}(R, N)}{\text{expression}(R = 0, N)}. \quad (\text{S18})$$

However, if there is a distribution of plasmids $p(n)$ then the expression is the sum of the probability of finding a cell with N plasmids times the expression from a cell with N plasmids such that,

$$\text{fold-change}_{\text{dist}} = \frac{\sum_{n=1}^{\infty} p(n) \text{expression}(R, n)}{\sum_{n=1}^{\infty} p(n) \text{expression}(R = 0, n)}. \quad (\text{S19})$$

First let's examine this in terms of a single chromosomal copy expressing YFP in the presence of competitor plasmids which do not express the measured gene product (corresponding to equation 5). In this case when there is no repressor, the number of plasmids is irrelevant to the predicted expression. Now we can rewrite

$$\text{fold-change}_{\text{dist}} = \frac{\sum_{n=1}^{\infty} p(n) \text{expression}(R, n)}{\text{expression}(R = 0)}, \quad (\text{S20})$$

which can be rewritten as

$$\text{fold-change}_{\text{dist}} = \sum_{n=1}^{\infty} p(n) \text{fold-change}(R, n), \quad (\text{S21})$$

where $\text{fold-change}(R, n)$ is the expression for the fold-change of a static, fixed- N plasmid from equation 5 from the main text and the above equation is listed in the main text as equation 9. The situation differs slightly when one considers, instead, identical genes expressing the same measured gene product. Now the expression of the $R = 0$ strain (in the denominator of the fold-change) does depend on the number of plasmids, the expression of a cell with n plasmids is equivalent to n times the production of a cell with just one plasmid. As such we rewrite equation S19,

$$\text{fold-change}_{\text{dist}} = \frac{\sum_{n=1}^{\infty} p(n) \text{expression}(R, n)}{\langle n \rangle \text{expression}(R = 0, N = 1)}, \quad (\text{S22})$$

by breaking up the above sum term by term we see the same equivalence, $\text{expression}(R = 0, N = n) = n \times \text{expression}(R = 0, N = 1)$, allows us to arrive at equation 8 from the main text,

$$\text{fold-change}_{\text{dist}} = \sum_{n=1}^{\infty} p(n) \frac{n}{\langle n \rangle} \text{fold-change}(R, n), \quad (\text{S23})$$

where $\langle n \rangle = \sum_{n=1}^{\infty} np(n)$ and $\text{fold-change}(R, n)$ is the fold-change from a static fixed- N distribution from equation 4 of the main paper.

Determining errors in theoretical predictions

Figures of fold-change vs. repressor copy number often show the standard deviation in theoretical predictions stemming from uncertainty in the parameters of the model such as operator binding energies $\Delta\epsilon$, $\Delta\epsilon_c$, gene copy number N , or competitor binding site copy number N_c while assuming the repressor copy number is fixed. We estimate the standard deviation in fold-change by a first order Taylor expansion around the mean values of these parameters, $\overline{\Delta\epsilon}$, $\overline{\Delta\epsilon_c}$, \overline{N} , $\overline{N_c}$. For instance, calculating the error bars for Figure 5A where the uncertainty in $\Delta\epsilon$, $\Delta\epsilon_c$ and N are all included, the calculation goes as follows,

$$\begin{aligned} \text{fold-change}(R, \Delta\epsilon, \Delta\epsilon_c, N_c) &\approx (\Delta\epsilon - \overline{\Delta\epsilon}) \frac{\partial}{\partial \Delta\epsilon} \text{fold-change}(R, \overline{\Delta\epsilon}, \overline{\Delta\epsilon_c}) \\ &+ (\Delta\epsilon_c - \overline{\Delta\epsilon_c}) \frac{\partial}{\partial \Delta\epsilon_c} \text{fold-change}(R, \overline{\Delta\epsilon}, \overline{\Delta\epsilon_c}) \\ &+ (N_c - \overline{N_c}) \frac{\partial}{\partial N_c} \text{fold-change}(R, \overline{\Delta\epsilon}, \overline{\Delta\epsilon_c}, \overline{N_c}) \end{aligned} \quad (\text{S24})$$

which gives us the corresponding estimated variance in fold-change

$$\begin{aligned} V[\text{fold-change}(R, \Delta\epsilon, \Delta\epsilon_c, N_c)] &\approx V[\Delta\epsilon] \left(\frac{\partial}{\partial \Delta\epsilon} \text{fold-change}(R, \overline{\Delta\epsilon}, \overline{\Delta\epsilon_c}) \right)^2 \\ &+ V[\Delta\epsilon_c] \left(\frac{\partial}{\partial \Delta\epsilon_c} \text{fold-change}(R, \overline{\Delta\epsilon}, \overline{\Delta\epsilon_c}) \right)^2 \\ &+ V[N_c] \left(\frac{\partial}{\partial N_c} \text{fold-change}(R, \overline{\Delta\epsilon}, \overline{\Delta\epsilon_c}, \overline{N_c}) \right)^2, \end{aligned} \quad (\text{S25})$$

where we used the additional assumption of no correlation between any of the expanded parameters. The derivatives in equation S25 can be computed either numerically or analytically using standard mathematical software. To be explicit, here we list the relevant figures and which parameters contribute to the uncertainty. Figure 3 has uncertainty stemming only from uncertainty in the binding energy $\Delta\epsilon$. Figure 4A has uncertainty from both $\Delta\epsilon$ and the copy number of the reporter plasmid N while in part B of that figure, we use only the error from $\Delta\epsilon$. Figure 5A has uncertainty contributions from $\Delta\epsilon$, as well as the binding strength and number of competitor plasmids, $\Delta\epsilon_c$ and N_c . In Figure 5B, the distribution is initially fit to the Oid data and thus the only uncertainty shown there is due to $\Delta\epsilon$ and $\Delta\epsilon_c$.

The copy number of multiple chromosomal integrations strain

The genetic location (and position on the chromosome in minutes; where 1 minute = 1/100th of the *E. coli* chromosome and *oriC* is located at minute 85) of each specific integration is: *intS* (53 minutes), *yffO* (55 minutes), *intB* (97 minutes), *intE* (26 minutes), and *essQ* (35 minutes). There is some uncertainty in the number of copies of these genes at any given time in the cell cycle. We chose to make measurements at the end of the cell cycle because we know that there are two completed copies of the genome at that point in time (Bremer and Dennis, 1996). However, the gene copy at *essQ* is directly opposite of the origin of replication, *oriC*, on the chromosome (50 minutes away) and

is one of the last parts of the chromosome to be replicated during a round of replication. Therefore, although all of our measurements take place in the D period when the first round of chromosomal replication should be complete, fluorescent protein maturation times may make it such that the extra copy of *essQ* is not fully measurable yet. A second source of uncertainty comes from the fact that at 65 minutes division time, we expect that the next round of chromosome replication to have already begun by the end of the cell cycle. *intB* is a mere 12 minutes (or 600 kbp) away from *oriC*, the origin of chromosomal replication. Therefore it is possible that there are already 4 copies of the *intB* integration when we make our measurements. As a result, we estimate the range of chromosomal construct copy number during our measurements to be between 9 and 12 with 10 being most probable. As such, we expect that there is some cell-to-cell variation in copy number within our measurement. However this small, tight range would not cause a major correction to the predictions of the thermodynamic model. Figure S4 shows the difference in theoretical predictions between assuming exactly 10 copies (red line, as is reported in Figure 4B) and allowing a normal distribution centered on 10.5 copies with a standard deviation of 1.5 copies (black line). While the model of chromosome copy number as a normal distribution is not correct in detail, we intend to show an upper limit on the effect of copy number distribution on our predictions.

Constructs and strains

The base strain through this work is HG105, which is MG1655 with a *lacIZYA* deletion (Garcia and Phillips, 2011). A constitutive CFP marker has been integrated at the *gspI* chromosomal location (Posfai et al., 2006). The marker is expressed from a *lacUV5* promoter with no Lac repressor binding site. In addition, every strain contains a low copy number plasmid which expresses TetR pZS3P_{N25}-*tetR*. This is a plasmid with a SC101 origin and a P_{N25} promoter controlling the TetR gene and was obtained by PCR from the chromosome of DH5 α Z1 (Lutz and Bujard, 1997). Specific details of the construction of the individual strains used in each part of the experiment are now listed below:

Single copy chromosomal integration: This originates from plasmid pZS2501+11-YFP (map shown in Figure S6). From this plasmid, we have produced, by site directed mutagenesis the same plasmid with the Oid, O2 and O3 repressor binding sites in place of O1 (sequences listed in table S6) (Garcia and Phillips, 2011). These constructs consisting of the terminators, resistance marker and EYFP gene are integrated into the chromosomal location of *galk* using recombineering (Sharan et al., 2009; Garcia and Phillips, 2011) with primers listed in Table S1.

High copy number plasmids: The SC101 origin of plasmids pZS2501+11-YFP was removed by digestion with *SacI* and *AvrII* and ligated to a *ColE1 Δ rom* origin to make pZE2501+11-YFP (Lutz and Bujard, 1997). This procedure was repeated for plasmids with the binding sites Oid, O2 and O3. To create the *ColE1* origin, we have added the Rom protein near the origin of the pZE2501+11-YFP plasmid to make pRE2501+11-YFP. This is achieved by PCR of the Rom protein from plasmid pBR322 followed by Gibson assembly with plasmid pZE2501+11-YFP to make our *ColE1* plasmid.

Multiple chromosomal integrations: The plasmids pZS2*5Oid+11-YFP, pZS3*5Oid+11-YFP and pZS4*5Oid+11-YFP contain resistance gene for kanamycin, chloramphenicol and spectinomycin, respectively. These resistance genes are flanked by FLP recombinase sites. The kanamycin and chloramphenicol cassettes were obtained by PCR from plasmids pKD4 and pKD3, respectively (Datsenko and Wanner, 2000) and places between the *SacI* and *AatII* sites of pZS25Oid+11-YFP. FLP

recombinase sites were placed flanking the spectinomycin resistance gene in pZS4*5Oid+11-YFP by site directed mutagenesis on pZS45Oid+11-YFP using primers 15.15 and 15.16 (table S1). These constructs were integrated into the chromosomal locations of genes *intS*, *yffO*, *intB*, *intE*, and *essQ* (Posfai et al., 2006; Kuhlman and Cox, 2012) using recombineering (Sharan et al., 2009). The oligos used to amplify the pZS plasmid to integrate constructs at every chromosomal location are listed in table S1. All resistances are then flipped out by FLP recombinase transiently expressed from plasmid pCP20 (Datsenko and Wanner, 2000).

Competitor plasmids: These plasmids are made from the pZE25O1+11-YFP plasmid digested with AatII and XbaI. An insert containing Oid, O1, or O2 flanked with sticky ends for the same restriction sites restriction sites (sequence of inserts listed in Table S1) are ordered as annealed double stranded oligos (Integrated DNA Technologies) and then ligated into the pZE vector. The result is a plasmid with the ColE1 Δ *rom* origin of replication, a resistance marker and a LacI binding site without an active YFP gene or promoter.

Constitutive marker: The cerulean (CFP in this work) gene was obtained from (Dunlop et al., 2007), amplified using primers 15.14 and 15.14R (table S1), and ligated between the KpnI and HindIII sites of pZS4*5O1+11-YFP to create pZS4*5O1+11-CFP. The O1 binding site was deleted using mutagenesis primer 21.3 (table S1) (Oehler et al., 1994) in order to create pZS4*5NoO1-CFP. This construct was integrated into the *gspI* locus.

TetR plasmid: The *tetR* gene controlled by the P_{N25} promoter was amplified from the genome of DH5 α Z1 (Lutz and Bujard, 1997) using primers 13.6 and 13.7v2 (table S1). The PCR product was digested between the XhoI and HindIII of pZS3*1-LacI to create pZS3P_{N25}-tetR.

LacI-mCherry fusion: A construct bearing mCherry was obtained from (Eldar et al., 2009) and amplified using primers 13.12 and 13.13 (table S1). The *lacI* gene was amplified from pZS3*1-LacI (Garcia and Phillips, 2011) using primers 13.28 and 13.30. Both of these PCR products were combined and amplified once again using primers 13.28 and 13.13 (table S1). The resulting LacI-mCherry PCR product has a KpnI site on its 5' end and a HindIII site on its 3' end. This repressor cannot tetramerize due to the deletion of the last 11 amino acids of its sequence. The fusion was ligated between the KpnI and HindIII sites of pZS3*1-LacI to create pZS3*1-LacI-mCherry. Finally this construct was integrated into the chromosome at the *ybcN* chromosomal location with the *ybcN* primers listed below.

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