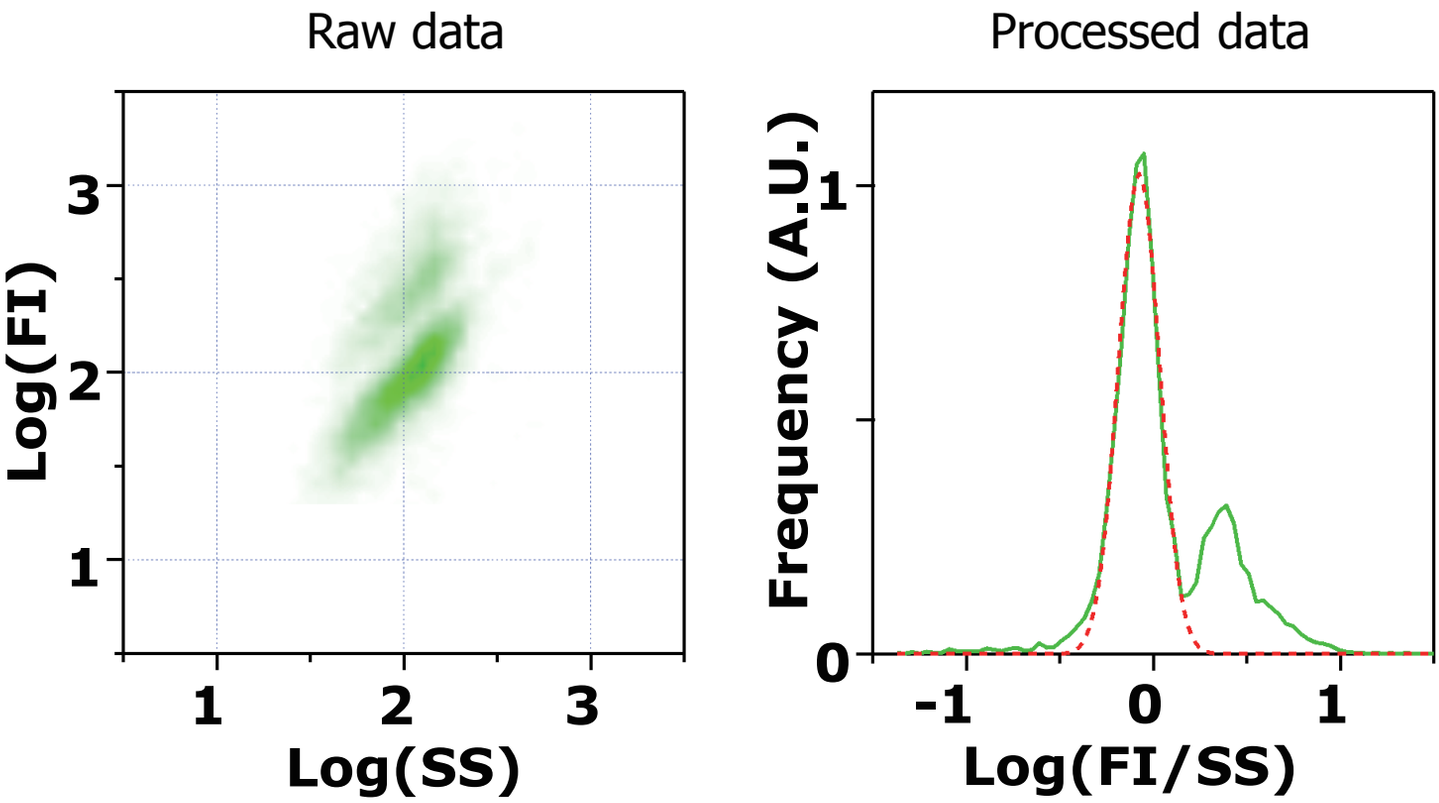

Figure S1

Representative GFP fluorescence data from flow cytometry of a sample culture expressing the CEN/ARS plasmid. A typical distribution pattern of GFP fluorescence intensity (FI) values showed two groups: a major group with low values and a minor group with high values (left panel). The distribution of the side-way scattering (SS) values was similar between the two groups. Each FI value was divided by the respective SS value, and these processed FI values showed two peaks, each with a log-normal distribution (right panel). The means of the divided FI values in each group were calculated by curve fitting with a Gaussian function. The mean value of the minor group was about twice that of the major group. Taken together, the two groups might reflect the copy number of the CEN/ARS plasmid. In this study, the average value of the major group was classed as the FI average of the corresponding terminator strain.

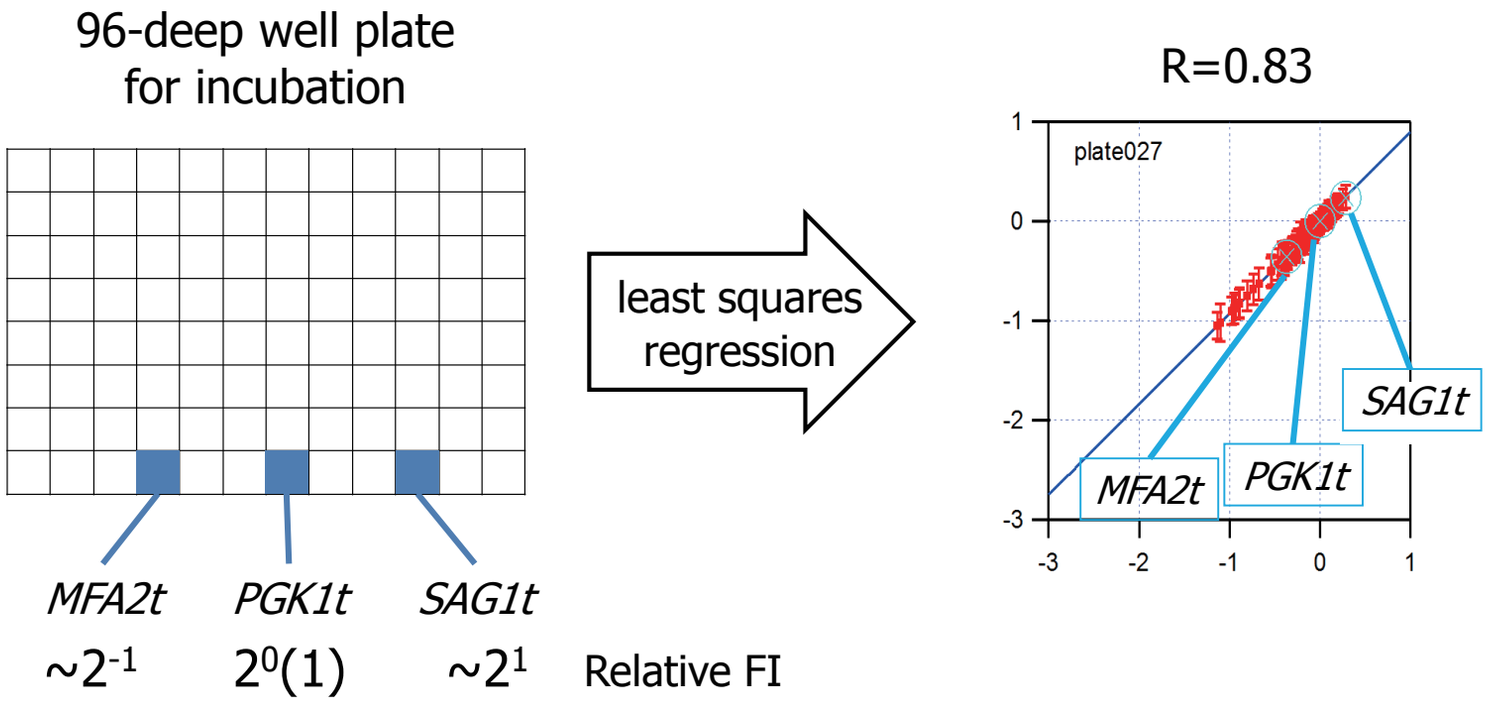

Figure S2.

Intra- and inter-plate normalization of FI values to produce relative FI values. The left panel is a schema of an incubation plate indicating the position of the three control strains that each harbour a different standard terminator. The right panel shows a representative graph of relative FI values normalized with the use of FI values for the three standard terminators; horizontal and vertical axes on the log2 scale represent the relative FI values of the genome-integrated (or stable) transformants and the relative FI values of transient transformants produced by the gap-repair cloning method, respectively. For each 96-well culture plate, a calibration line was established with the use of the three average values for the control stable transformants and those of the transient transformants produced by the gap-repair cloning method, respectively. Within each plate, FI values were fit to the calibration line by using linear regression. To perform inter-plate normalization, the FI value for each transformant was divided by the average value for the transient *PGK1t* transformant on the same plate; *i.e*, the log2 FI value for PGK1t on each plate was set to equal 0. The resultant normalized values are termed relative FI values. Error bars represent standard deviation. *R*, correlation coefficient.

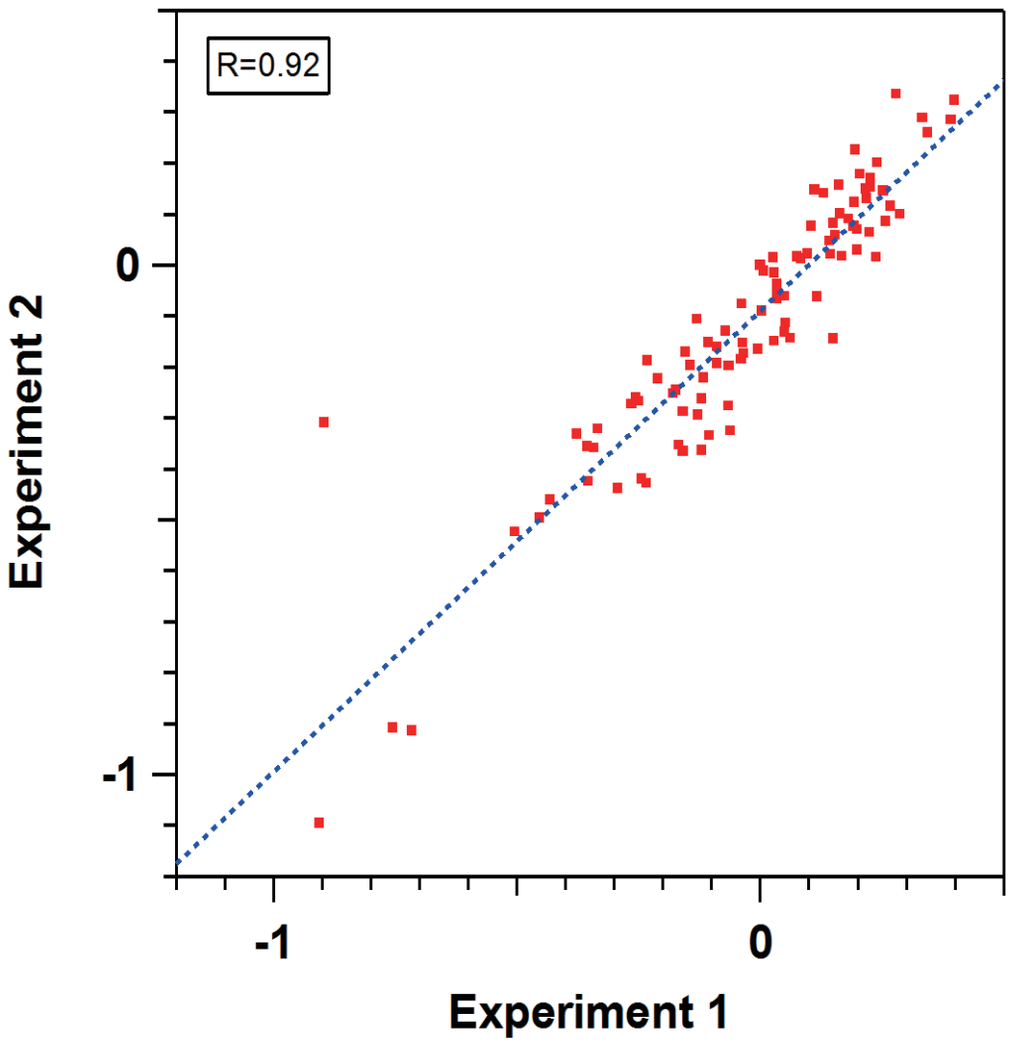

Figure S3.

Reproducibility of the experimental procedure. Each red dot shows the average of the relative fluorescence intensity (FI) values for a strain (n=3,000). The blue dotted line shows a regression line. *R,* correlation coefficient.

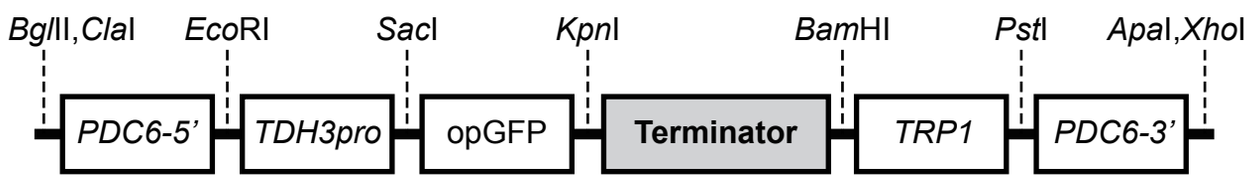

Figure S4.

Schematic diagram of the genome-integrated gene constructs. Each terminator was inserted as a module downstream of a codon-optimized GFP gene (opGFP). *PDC6-5′* and *PDC6-3′* denote the 5′- and 3′-regions of *PDC6*, respectively. *TRP1* was used as a selection marker.