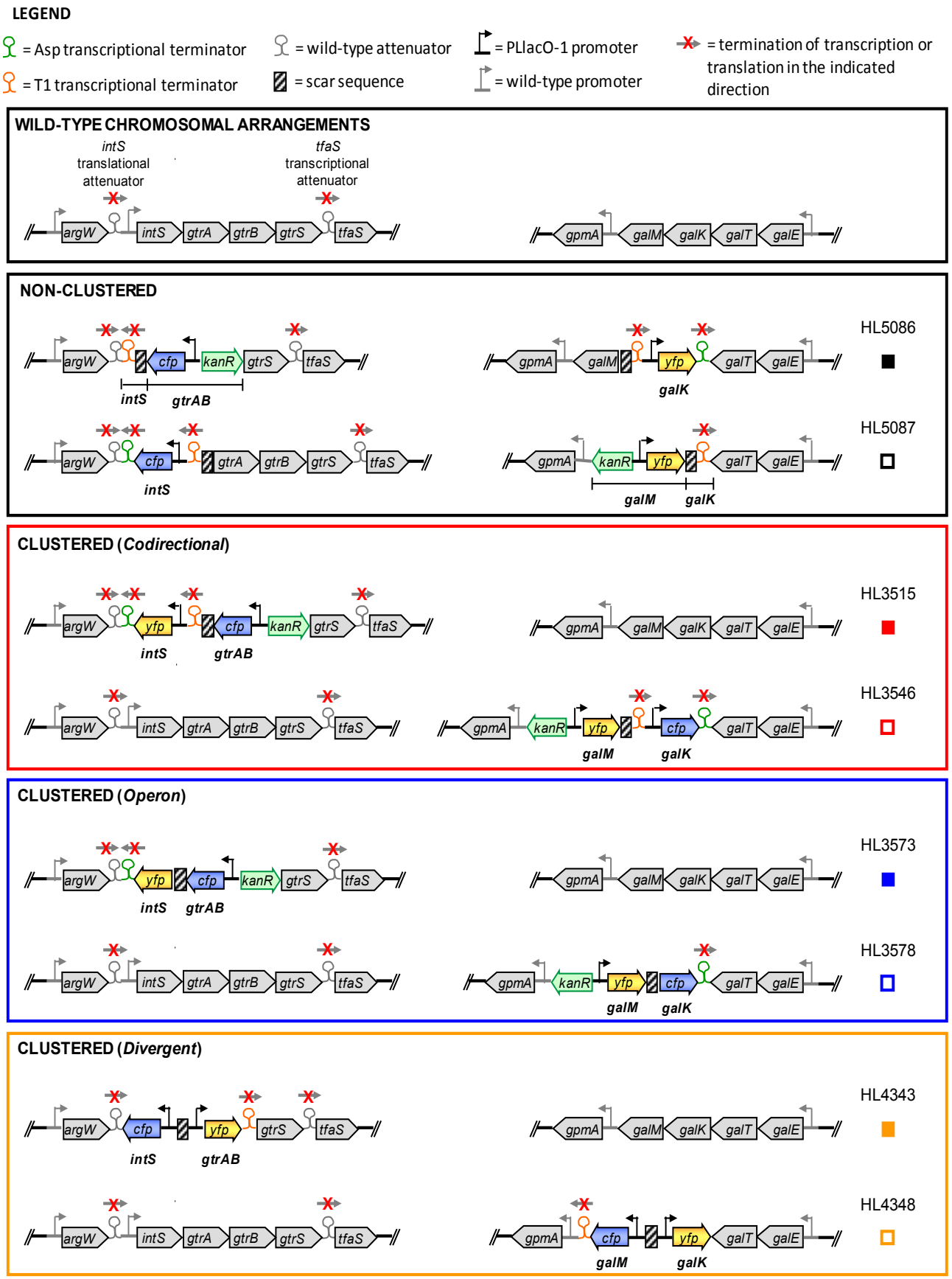

Figure S1 Details of the non-clustered and clustered gene arrangements shown in **Figure 1A**.

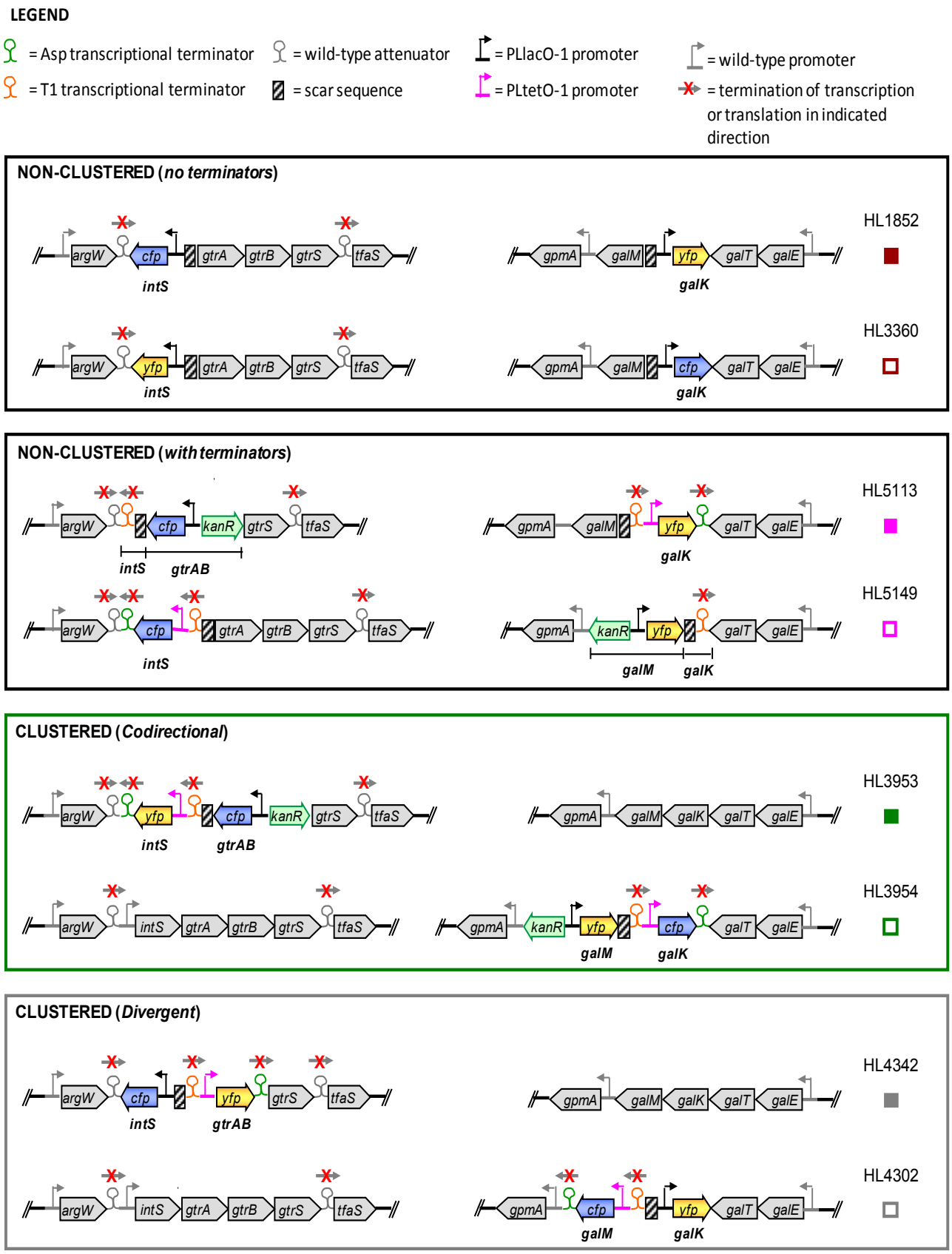

Figure S2 Details of the non-clustered and clustered gene arrangements shown in **Figure 3A** and non-clustered genes without terminators (filled and unfilled maroon symbols).

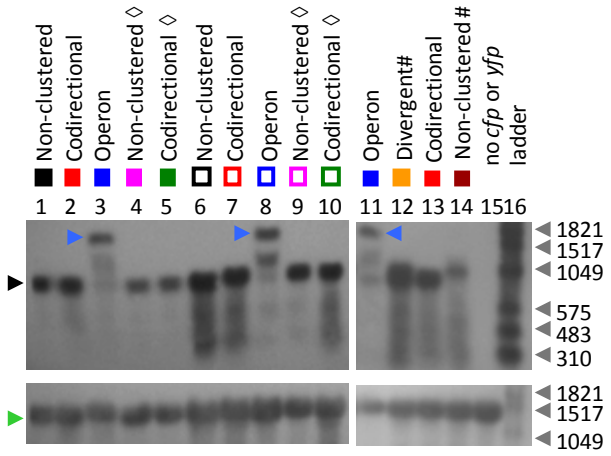

Figure S3 RNA measurements by Northern blotting. Error bars indicate the s.e.m. of duplicate measurements. Symbols indicate the gene arrangements in **Figure 1A** and **Figure 3A**. Representative Northern blot showing hybridization of a *cfp* probe which binds both *cfp* and *yfp* mRNAs (upper) and a 16S RNA probe (lower). Measurements were performed on duplicate sets of samples. # indicates at least one of the mRNA transcripts does not have a terminator. The maroon symbol represents a non-clustered gene arrangement where *cfp* at *intS* and *yfp* at *galK* do not have terminators (HL1852). Blue arrowheads indicate the full length mRNA for operons. Black arrowhead indicates the single gene mRNA. ◊ indicates one of the promoters is PLtetO-1. The green arrowhead indicates the 16S RNA. The grey arrowheads indicate mRNAs in the size ladder. Contrast and brightness were adjusted solely to enhance visualization of the printed figure; no bands were obscured or selectively enhanced.

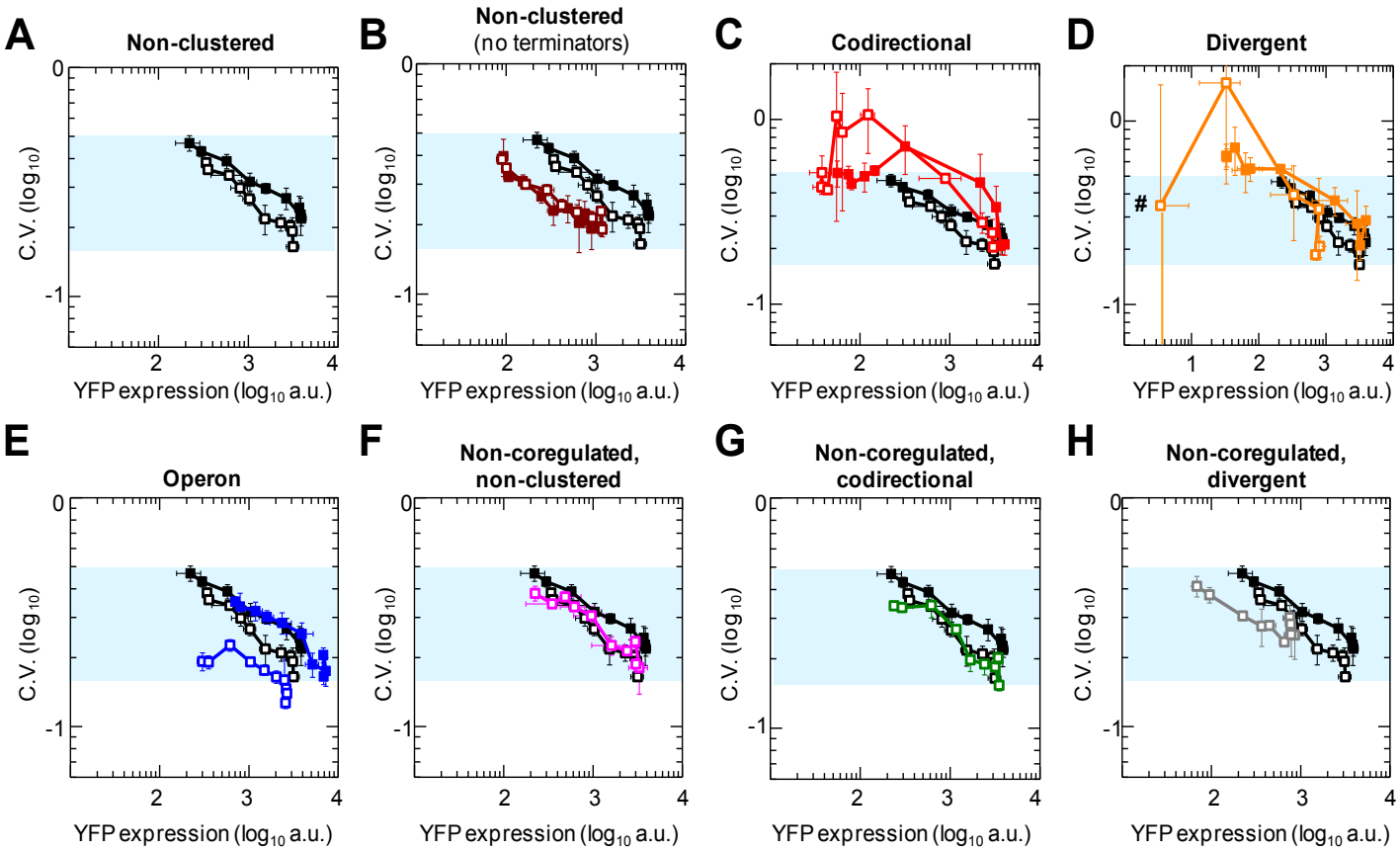

Figure S4 Gene expression noise for YFP in clustered and non-clustered gene arrangements. (**A-H**) Each panel shows the coefficient of variation (C.V.) for a different gene arrangement. Data symbols indicate the gene arrangements shown in **Figure 1A** and **Figure 3A**. In addition we include non-clustered genes without terminators (maroon symbols, **panel B**). All cells are included in the analysis. Error bars indicate the s.e.m. (number of replicates is stated in the legends of **Figures 1** and **3**). The non-clustered gene arrangement with terminators (and the blue shading which indicates the range of the C.V. in this arrangement) serves as a reference in all plots. # HL4348 had YFP expression values less than zero after background autofluorescence subtraction at 0, 1, 5 and 10 µM IPTG; these values are therefore not shown on the plot.

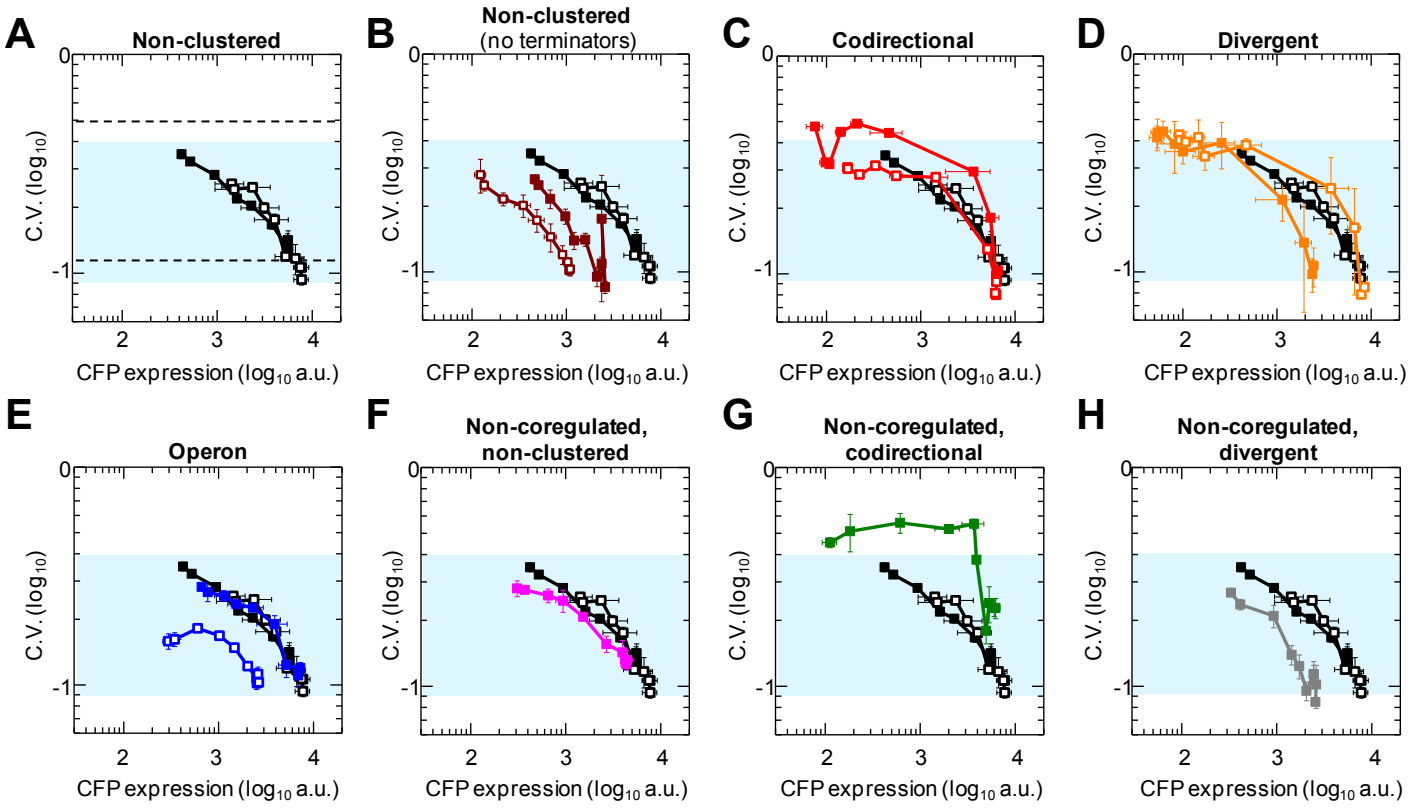

Figure S5 Gene expression noise for CFP in clustered and non-clustered gene arrangements with only cells with expression within 2 S.D. of the mean included in the analysis. (**A-H**) Each panel shows the coefficient of variation (C.V.) for a different gene arrangement. Data symbols indicate the gene arrangements shown in **Figures 1A** and **3A**. In addition we include non-clustered genes without terminators (maroon symbols, **panel B**). Error bars indicate the s.e.m. (number of replicates is stated in the legends of **Figures 1** and **3**). The non-clustered gene arrangement with terminators serves as a reference in all plots (blue shading indicates the range of the C.V. for this arrangement). Black dash lines indicate the upper and lower bounds of the C.V. in non-clustered gene arrangements from **Figure 4A**.

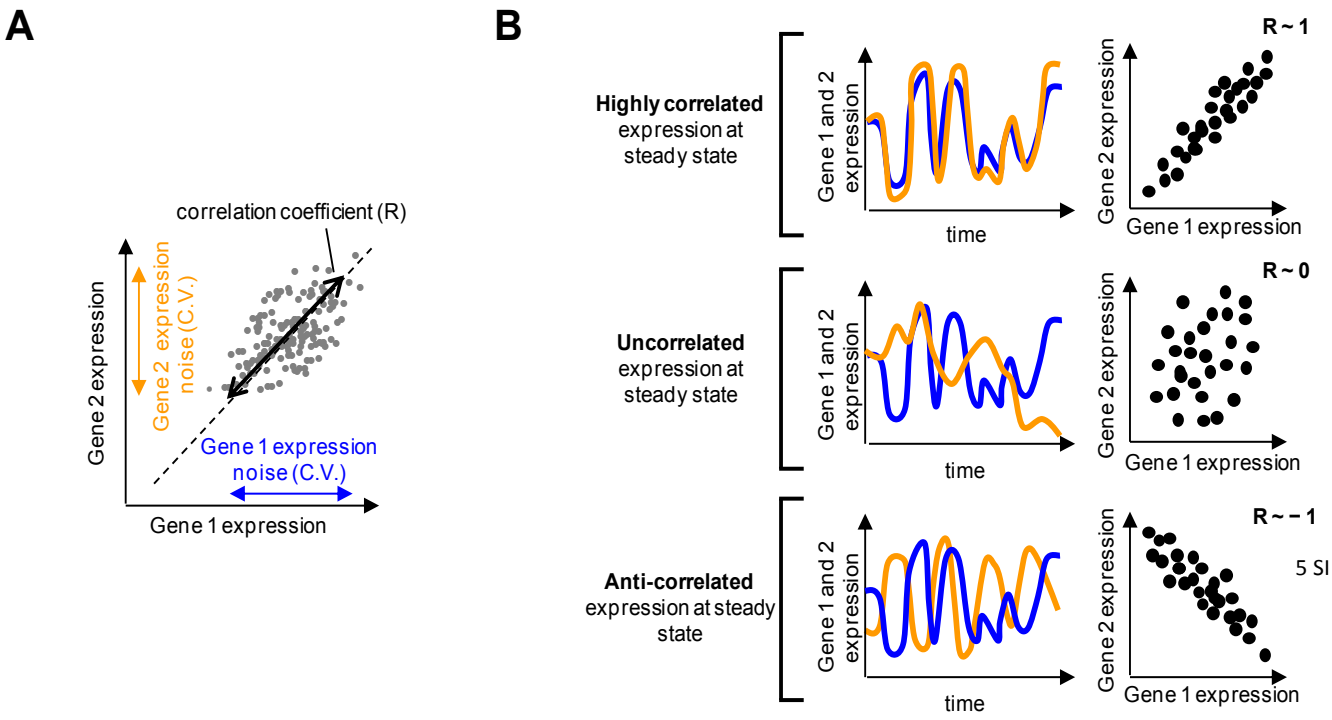

Figure S6 Description of the correlation coefficient. (**A**) Scatter plot of Gene 1 and Gene 2 expression showing the noise and the correlation coefficient (R). (**B**) Diagrams showing the steady state expression of Gene 1 (blue lines) and Gene 2 (gold lines) as a function of time and a corresponding scatter plot for highly correlated expression (top), uncorrelated expression (middle) and anti-correlated expression (bottom).

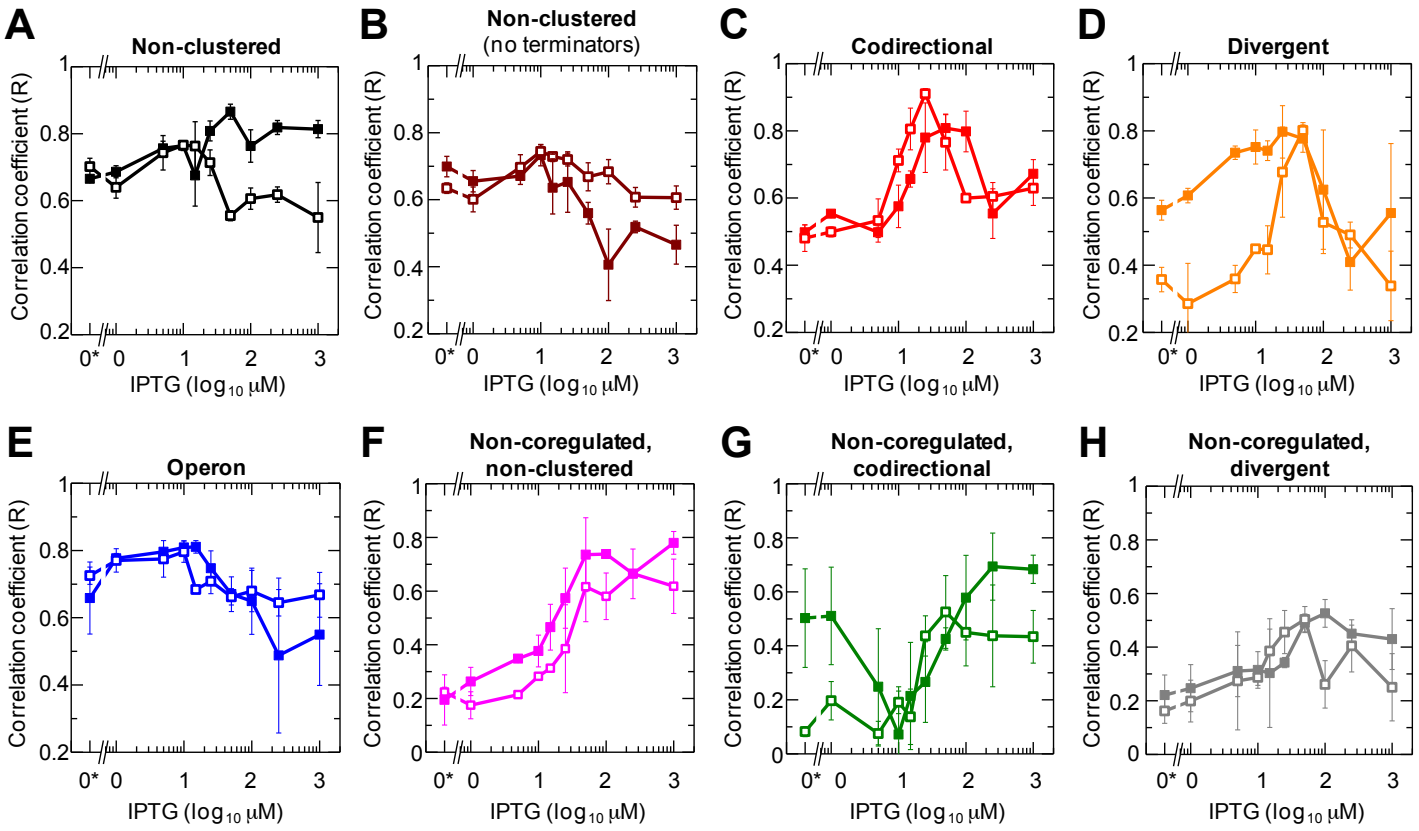

Figure S7 Correlation coefficient (R) as a function of the IPTG concentration for clustered and non-clustered gene arrangements with only cells within 2 S.D. of the mean included in the analysis. (**A-H**) Each panel shows the correlation coefficient for a different gene arrangement. Data symbols indicate the gene arrangements shown in **Figures 1A** and **3A**. In addition we include non-clustered genes without terminators (maroon symbols, **panel B**). Error bars indicate the s.e.m. (number of replicates is stated in the legends of **Figures 1** and **3**). 0* indicates an actual value of zero not 10^0.

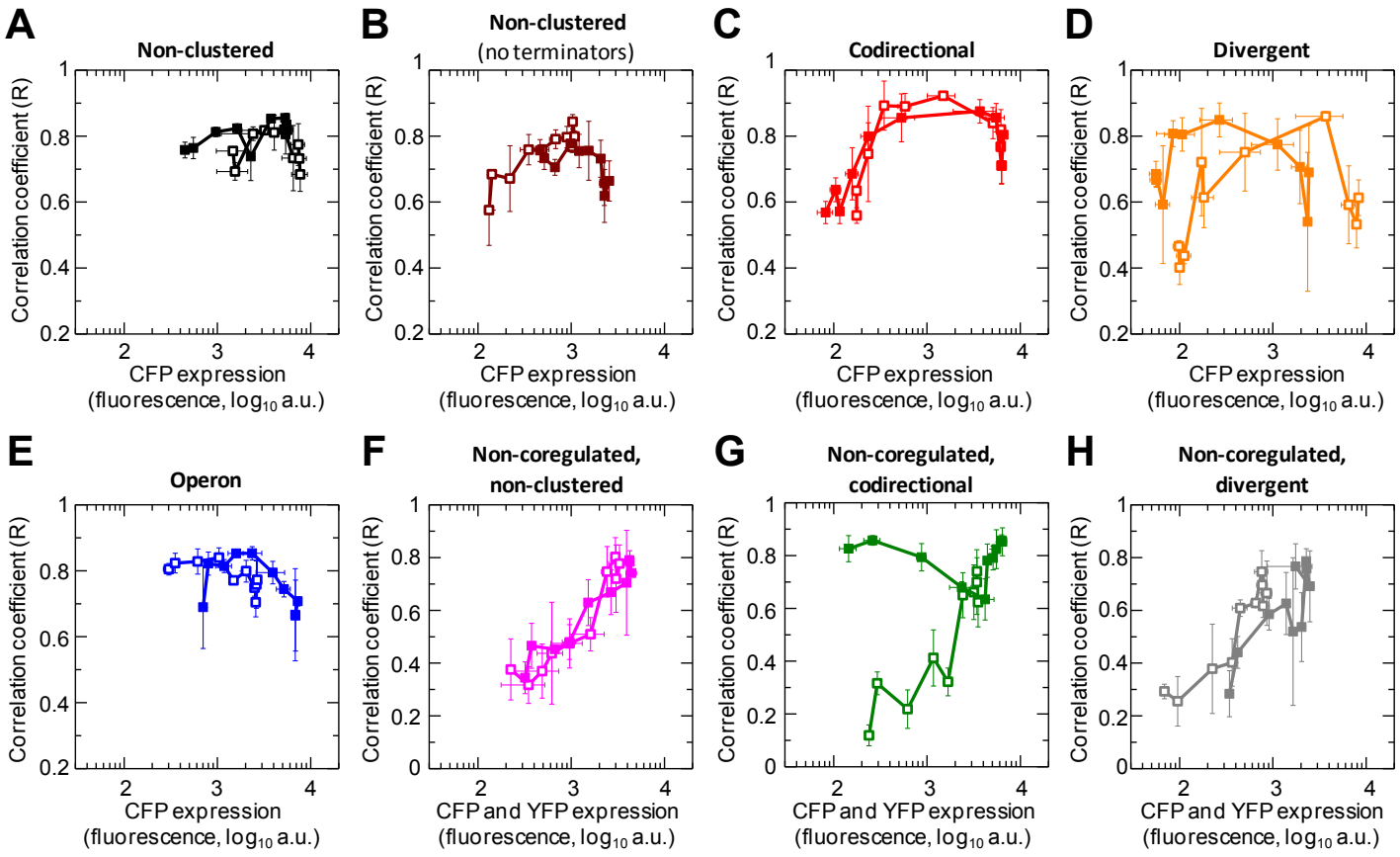

Figure S8 Correlation coefficient as a function of mean expression for clustered and non-clustered gene arrangements. (**A-H**) Each panel shows the correlation coefficient for a different gene arrangement. Data symbols indicate the gene arrangements shown in **Figures 1A** and **3A**. In addition we include non-clustered genes without terminators (maroon symbols, **panel B**). All cells are included in the analysis. Error bars indicate the s.e.m. (number of replicates is stated in the legends of **Figures 1A** and **3A**).

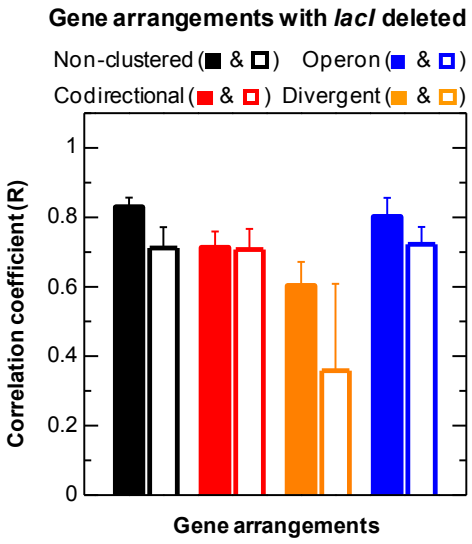

Figure S9 Correlation coefficient (R) for different gene arrangements calculated without outliers (cells more than 2 S.D. from the mean). Data symbols indicate the gene arrangements shown in **Figure 1A**. Error bars indicate the s.e.m. of 5-7 replicate measurements.

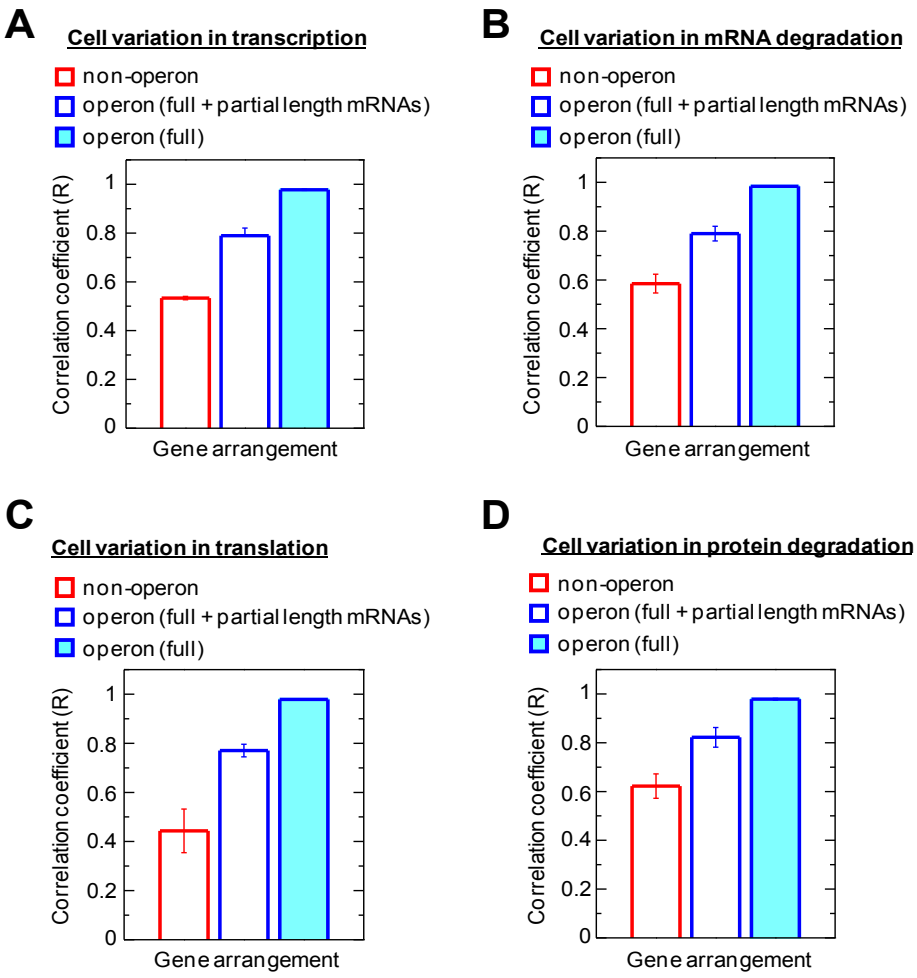

Figure S10 Correlation coefficients generated by stochastic simulations that include fluctuations in global factors. Cell-to-cell variation in global factors such as the concentrations and activity of RNA polymerases, RNA degradosomes and ribosomes, and growth rates are included in the stochastic simulations. Variation in these global factors effectively alters the rate constants for transcription, mRNA degradation, translation, and protein clearance (see **Materials and Methods**). Error bars indicate s.e.m. of quintuplicate simulations. Results in **panels A-D** should be compared to **Figure 7B** (stochastic simulation without any variation in global factors). (**A**) Cell variation in the transcription rate (k_m) from 0.15 to 0.45 mRNA/min. (**B**) Cell variation in the mRNA degradation rate constant (k_{-m}) from 0.075 to 0.225 per min. (**C**) Cell variation in the translation rate (k_p) from 2 to 6 proteins/mRNA/min. (**D**) Cell variation in the protein clearance rate (k_d) from 0.015 to 0.045 per min.

Table S1 Strains

Strain	Description	*cfp* position	*yfp* position	Structure
MG1655	Yale *E. coli* genetic stock center (CGSC#7740)	N/A	N/A	N/A
HL716*	MG1655 + *lacIq* at *intS* site	N/A	N/A	N/A
HL1745§	MG1655 + PLlacO-1::T710::*cfp* at *intS*	*intS*	N/A	Single color
HL1852§	HL1745 + PLlacO-1::T710::*yfp* at *galK*	*intS*	*galK*	Non-clustered
HL1951§	MG1655 + PLlacO-1::T710::*yfp* at *galK*	N/A	*galK*	Single color
HL2028*	HL1852 + Δ*lacI*	*intS*	*galK*	Non-clustered
HL2960	MG1655 + PLlacO-1::T710::*cfp* at *galK*	N/A	*galK*	Single color
HL3355*	MG1655 + KanR::PLlacO-1::T710::*cfp* at *gtrAB*	*gtrAB*	N/A	Single color
HL3360	HL2960 + PLlacO-1::T710::*yfp* at *intS*	*galK*	*intS*	Non-clustered
HL3368*	MG1655 + KanR::PLlacO-1::T710::*yfp* at *galM*	N/A	*galM*	Single color
HL3515*	HL3355 + T1 terminator::PLlacO-1::T710::*yfp*::Asp terminator at *intS*	*gtrAB*	*intS*	Codirectional: *cfp* upstream of *yfp*
HL3546*	HL3368 + T1 terminator::PLlacO-1::T710::*cfp*::Asp terminator at *galK*	*galK*	*galM*	Codirectional: *yfp* upstream of *cfp*
HL3573	HL3515 + (ΔT1 terminator::PLlacO-1)	*gtrAB*	*intS*	Operon: *cfp* upstream of *yfp*
HL3578	HL3546 + (ΔT1 terminator::PLlacO-1)	*galK*	*galM*	Operon: *yfp* upstream of *cfp*
HL3640	HL3515 + Δ*lacI*	*gtrAB*	*intS*	Codirectional: *cfp* upstream of *yfp*
HL3641	HL3546 + Δ*lacI*	*galK*	*galM*	Codirectional: *yfp* upstream of *cfp*
HL3642	HL3573 + Δ*lacI*	*gtrAB*	*intS*	Operon: *cfp* upstream of *yfp*
HL3643	HL3578 + Δ*lacI*	*galK*	*galM*	Operon: *yfp* upstream of *cfp*
HL3953	HL3355 + T1 terminator::PLtetO-1::T710::*yfp*::Asp terminator at *intS*	*gtrAB*	*intS*	Non-coregulated, codirectional: *cfp* upstream of *yfp*
HL3954	HL3368 + T1 terminator::PLtetO-1::T710::*cfp*::Asp terminator at *galK*	*galK*	*galM*	Non-coregulated, codirectional: *yfp* upstream of *cfp*
HL4302	HL1951 + T1 terminator::PLtetO-1::T710::*cfp*::Asp terminator inserted at *galM*	*galM*	*galK*	Non-coregulated, divergent

Previously reported strains in Lim *et al.* 2011 (*) and Block *et al.* 2012 (§).

Table S1 continued Strains

Strain	Description	*cfp* position	*yfp* position	Structure
HL4342	HL1745 + T1 terminator::PLtetO-1::T710::*yfp*::Asp terminator inserted at *gtrAB*	*intS*	*gtrAB*	Non-coregulated, divergent
HL4343	HL1745 + PLlacO-1::T710::*yfp*::T7 terminator::T1 terminator inserted at *gtrAB*	*intS*	*gtrAB*	Divergent
HL4348	HL1951 + PLlacO-1::T710::*cfp*::T7 terminator::T1 terminator inserted at *galM*	*galM*	*galK*	Divergent
HL5056	HL3355 + T1 terminator at *intS*	*gtrAB*	N/A	Single color
HL5057	HL3368 + T1 terminator at *galK*	N/A	*galM*	Single color
HL5086	HL5056 + T1 terminator::PLlacO-1::T710::*yfp*::Asp terminator at *galK*	*gtrAB*	*galK*	Non-clustered
HL5087	HL5057 + T1 terminator::PLlacO-1::T710::*cfp*::Asp terminator at *intS*	*intS*	*galM*	Non-clustered
HL5113	HL5056 + T1 terminator::PLtetO-1::T710::*yfp*::Asp terminator at *galK*	*gtrAB*	*galK*	Non-coregulated, non-clustered
HL5149	HL5057 + T1 terminator::PLtetO-1::T710::*cfp*::Asp terminator at *intS*	*intS*	*galM*	Non-coregulated, non-clustered
HL5553	HL5086 + Δ*lacI*	*gtrAB*	*galK*	Non-clustered
HL5554	HL5087 + Δ*lacI*	*intS*	*galM*	Non-clustered
HL5555	HL4343 + Δ*lacI*	*intS*	*gtrAB*	Divergent
HL5556	HL4348 + Δ*lacI*	*galM*	*galK*	Divergent

Table S2 Plasmids used as templates for chromosomal integrations

Plasmid	Description
pHL471*	KanR with no FRT sites + PLlacO-1::T710::*cfp*::T7 terminator::T1 terminator + ColE1. Template for PCR amplification for *cfp* integrations.
pHL538*	KanR with FRT sites + PLlacO-1::T710::*cfp*::T7 terminator::T1 terminator + ColE1. Template for PCR amplification for *cfp* integrations.
pHL582*	KanR with FRT sites + PLlacO-1::T710::*yfp*:: T7 terminator::T1 terminator + ColE1. Template for PCR amplification for *yfp* integrations.
pHL1167*	CamR with FRT sites + T1 terminator::PLlacO-1::T710::*yfp*::Asp terminator + ColE1. Template for PCR amplification for *yfp* integrations.
pHL1168*	CamR with FRT sites + T1 terminator::PLlacO-1::T710::*cfp*::Asp terminator + ColE1. Template for PCR amplification for *cfp* integrations.
pHL1181*	KanR with no FRT sites + PLlacO-1::T710::*yfp*::T7 terminator::T1 terminator + ColE1. Template for PCR amplification for *yfp* integrations
pHL1257	CamR with FRT sites + T1 terminator:: PLtetO-1::T710::*cfp*::Asp terminator + ColE1. Template for PCR amplification for *cfp* integrations.
pHL1274	CamR with FRT sites + T1 terminator:: PLtetO-1::T710::*yfp*::Asp terminator + ColE1. Template for PCR amplification for *yfp* integrations.
pHL1580	CamR with FRT sites + T1 terminator + ColE1. Template for PCR amplification for integration of the T1 terminator at the end of *cfp* or *yfp*.

Previously reported plasmids in Lim *et al.*, 2011 (*).

Table S3 Oligonucleotides used for chromosomal integrations

Oligonucleotide	Description	Sequence (5' to 3')
CYFPendpKD1F*	integrate a second gene downstream of *cfp* or *yfp*	tcgtgaccgccgccgggatcactcacggcatggacgagctgtacaagtaagtgtaggctggagctgcttc
CYFPgalKR*	integrate *cfp* or *yfp* with no terminators at *galK*	gtttgcgcgcagtcagcgatatccattttcgcgaatccggagtgtaagaattacttgtacagctcgtccatgcc
CYFPintCR*	integrate *cfp* or *yfp* with no terminators at *intS*	ccgtagatttacagttcgtcatggttcgcttcagatcgttgacagccgcattacttgtacagctcgtccatgcc
GalKColER*	integrate *cfp* or *yfp* with terminators at *galK*	gtttgcgcgcagtcagcgatatccattttcgcgaatccggagtgtaagaaagctgataccgctcgccgcagccgaacg
GalKFRCCYFPR*	integrate *yfp* with no terminators at *galM*	cggaagagctggtgcctgccgtacagcaagctgtcgctgaacaatatgaattacttgtacagctcgtccatgcc
GalMFtermOext*	integrate *yfp* at *galM*	ctggtgatttgaacaatatgagataaagccctcatgacgagggcgtaacaatcaacaggagtccaagcgagctctcg
GtrBFtermOext*	integrate *cfp* at *gtrAB*	tcatttttttgactctcttgatgatgtatttcgggcgtttttttggtttcaaatcaacaggagtccaagcgagctctcg
IntCColER*	integrate *cfp* or *yfp* with terminators at *intS*	ccgtagatttacagttcgtcatggttcgcttcagatcgttgacagccgcaagctgataccgctcgccgcagccgaacg
IntCFRCCYFPR*	integrate *cfp* with no terminators at *gtrAB*	tgggcggactggcttgatgagaaggtggagtgagcgaccttaacaactatttacttgtacagctcgtccatgcc
PKD1FgalKF*	integrate *cfp* or *yfp* at *galK*	ttcatattgttcagcgacagcttgctgtacggcaggcaccagctcttccggtgtaggctggagctgcttc
PKD1FintCF*	integrate *cfp* or *yfp* at *intS*	atagttgttaaggtcgctcactccaccttctcatcaagccagtccgcccagtgtaggctggagctgcttc
RevgalMColER	integrate *cfp* with terminators at *galM*	ctggtgatttgaacaatatgagataaagccctcatgacgagggcgtaacaagctgataccgctcgccgcagccgaacg
RevgalMpKD1F§	integrate *cfp* at *galM*	ggtattaaagagacttttttacgtttgtaaaccatcacaaggagcaggacagtgtaggctggagctgcttc
RevgtrApKD1F§	integrate *yfp* at *gtrAB*	aagacttggatgatagacttcattccttttgattattagctgatagaagaagtgtaggctggagctgcttc
RevgtrBColER	integrate *yfp* with terminators at *gtrAB*	tcatttttttgactctcttgatgatgtatttcgggcgtttttttggtttcaaagctgatacgctcgccgcagccgaacg
T710RBSpKD4R	remove T1 terminator::PLlacO-1 from codirectional genes to create an operon	ctagccatatgtatatctccttcttaaagttaaacaaaattatttctagaattccgggatccgtcgacc

Previously reported oligonucleotides in Lim *et al.*, 2011 (*) and Block *et al.* 2012 (§).

Table S4 Maximum and minimum expression levels and parameters obtained from fits to the Hill function

Group	Fluorescent reporter, construction (strain) ‡	Max*	Min*	α	δ	K	n	Reduced χ^2 (R^2)
Non-clustered	**CFP**, PLlacO-1::*cfp* at *int*S & PLlacO-1::*yfp* at *galK* (HL1852)	2263 ± 253	463 ± 16	2145 ± 70	479 ± 9	24.9 ± 2.0	1.30 ± 0.09	1.34 (>0.99)
Non-clustered	**YFP**, PLlacO-1::*cfp* at *int*S & PLlacO-1::*yfp* at *galK* (HL1852)	824 ± 213	93 ± 10	771 ± 87	96 ± 8	24.8 ± 4.1	1.54 ± 0.19	0.13 (>0.99)
Non-clustered	**CFP**, PLlacO-1::*cfp* at *galK* & PLlacO-1::*yfp* at *intS* (HL3360)	1088 ± 19	132 ± 14	927 ± 47	135 ± 7	20.1 ± 2.5	1.71 ± 0.26	0.07 (>0.99)
Non-clustered	**YFP**, PLlacO-1::*cfp* at *galK* & PLlacO-1::*yfp* at *intS* (HL3360)	1180 ± 84	90 ± 12	1122 ± 89	92 ± 8	27.0 ± 3.0	1.58 ± 0.14	0.09 (>0.99)
Non-clustered	**CFP**, PLlacO-1::*cfp* at *int*S & PLlacO-1::*yfp* at *galK* (HL5086)	5622 ± 119	449 ± 23	5175 ± 88	524 ± 10	19.8 ± 0.5	1.91 ± 0.06	6.14 (>0.99)
Non-clustered	**YFP**, PLlacO-1::*cfp* at *int*S & PLlacO-1::*yfp* at *galK* (HL5086)	3901 ± 507	221 ± 66	3655 ± 86	282 ± 9	19.2 ± 0.6	2.04 ± 0.08	3.47 (>0.99)
Non-clustered	**CFP**, PLlacO-1::*cfp* at *int*S & PLlacO-1::*yfp* at *galK* (HL5087)	7721 ± 1482	1470 ± 499	6285 ± 292	1464 ± 27	18.1 ± 2.3	1.39 ± 0.12	0.19 (>0.99)
Non-clustered	**YFP**, PLlacO-1::*cfp* at *int*S & PLlacO-1::*yfp* at *galK* (HL5087)	3161 ± 512	340 ± 16	2975 ± 322	341 ± 15	31.7 ± 7.2	1.53 ± 0.32	0.10 (>0.99)
Non-coregulated, non-clustered #	**CFP**, PLlacO-1::*cfp* at *int*S & PLtetO-1::*yfp* at *galK* (HL5113)	4258 ± 223	320 ± 10	4075 ± 273	330 ± 26	19.9 ± 3.2	1.80 ± 0.27	0.25 (>0.99)
Non-coregulated, non-clustered #	**YFP**, PLlacO-1::*yfp* at *galK* & PLtetO-1::*cfp* at *intS* (HL5149)	3028 ± 230	225 ± 22	3165 ± 321	237 ± 15	31.0 ± 6.7	1.51 ± 0.30	1.11 (0.99)
Operon (1st gene)	**CFP**, PLlacO-1::*cfp-yfp* at *intS* (HL3573)	6881 ± 94	701 ± 56	6320 ± 300	713 ± 27	25.8 ± 3.0	1.51 ± 0.14	0.76 (>0.99)
Operon (1st gene)	**YFP**, PLlacO-1::*yfp-cfp* at *gal*K (HL3578)	2913 ± 241	120 ± 15	2847 ± 212	123 ± 15	17.6 ± 2.7	1.67 ± 0.36	0.13 (>0.99)

*Actual values obtained at 0 and 1 mM IPTG as opposed to fit values. ‡ *lacI* is in the native position in all strains. # Hill function was fitted only to the gene under the control of PLlacO-1.

Table S4 continued Maximum and minimum expression levels and parameters obtained from fits to the Hill function

Group	Fluorescent reporter, construction (strain) ‡	Max*	Min*	α	δ	K	n	Reduced χ^2 (R^2)
Operon (2nd gene)	**CFP**, PLlacO-1::*yfp-cfp* at *gal*K (HL3578)	2671 ± 198	302 ± 39	2395 ± 260	308 ± 27	14.6 ± 4.2	1.75 ± 0.44	0.06 (>0.99)
Operon (2nd gene)	**YFP**, PLlacO-1::*cfp-yfp* at *intS* (HL3573)	995 ± 48	162 ± 7	1080 ± 232	165 ± 15	29.1 ± 12.0	2.16 ± 1.26	0.19 (0.98)
Codirectional (1st gene)	**CFP**, PLlacO-1::*cfp*::T1 term::PLlacO-1::*yfp* at *intS* (HL3515)	6621 ± 326	82 ± 15	5934 ± 219	85 ± 15	45.4 ± 2.3	3.59 ± 0.54	0.74 (>0.99)
Codirectional (1st gene)	**YFP**, PLlacO-1::*yfp*::T1 term::PLlacO-1::*cfp* at *gal*K (HL3546)	2913 ± 241	38 ± 6	3171 ± 176	39 ± 15	36.3 ± 3.3	4.06 ± 0.92	0.03 (>0.99)
Codirectional (2nd gene)	**CFP**, PLlacO-1::*yfp*::T1 term::PLlacO-1::*cfp* at *galK* (HL3546)	6169 ± 222	177 ± 14	6034 ± 271	180 ± 25	34.4 ± 2.2	3.97 ± 0.64	0.07 (>0.99)
Codirectional (2nd gene)	**YFP**, PLlacO-1::*cfp*::T1 term::PLlacO-1::*yfp* at *intS* (HL3515)	3138 ± 244	56 ± 0	3864 ± 60	60 ± 6	47.3 ± 0.6	3.53 ± 0.27	4.35 (>0.99)
Non-coregulated, codirectional#	**CFP**, PLlacO-1::*cfp*::T1 term::PLtetO-1::*yfp* at *intS* (HL3953)	6298 ± 326	144 ± 29	5995 ± 271	154 ± 27	15.9 ± 1.9	1.63 ± 0.16	0.83 (>0.99)
Non-coregulated, codirectional#	**YFP**, PLlacO-1::*yfp*::T1 term::PLtetO-1::*cfp* at *galK* (HL3954)	3425 ± 119	239 ± 20	3397 ± 213	242 ± 15	17.7 ± 2.3	1.67 ± 0.30	0.15 (>0.99)
Divergent	**CFP**, PLlacO-1::*cfp* & PLlacO-1::*yfp* at *intS* (HL4343)	2429 ± 76	56 ± 8	2366 ± 62	62 ± 3	61.0 ± 3.5	2.64 ± 0.12	1.95 (>0.99)
Divergent	**YFP**, PLlacO-1::*cfp* & PLlacO-1::*yfp* at *intS* (HL4343)	3934 ± 58	34 ± 4	3385 ± 60	46 ± 2	61.7 ± 2.4	3.11 ± 0.12	9.20 (0.99)
Divergent	**CFP**, PLlacO-1::*cfp* & PLlacO-1::*yfp* at *galK* (HL4348)	8316 ± 633	98 ± 13	7973 ± 61	127 ± 2	64.0 ± 1.0	3.04 ± 0.05	39.73 (0.99)
Divergent	**YFP**, PLlacO-1::*cfp* & PLlacO-1::*yfp* at *galK* (HL4348)	811 ± 126	-4 ± 5	750 ± 49	5.0 ± 2	48.9 ± 9.1	4.47 ± 1.25	0.73 (0.99)
Non-coregulated, divergent#	**CFP**, PLlacO-1::*cfp* & PLtetO-1::*yfp* at *intS* (HL4342)	1751 ± 876	342 ± 17	2027 ± 35	341 ± 4	9.6 ± 0.2	1.42 ± 0.03	10.52 (>0.99)
Non-coregulated, divergent#	**YFP**, PLlacO-1::*yfp* & PLtetO-1::*cfp* at *galK* (HL4302)	786 ± 132	69 ± 7	797 ± 50	69 ± 4	15.3 ± 1.4	1.24 ± 0.07	3.83 (>0.99)

*Actual values obtained at 0 and 1 mM IPTG as opposed to fit values. ‡ *lacI* is in the native position in all strains. # Hill function was fitted only to the gene under the control of PLlacO-1.