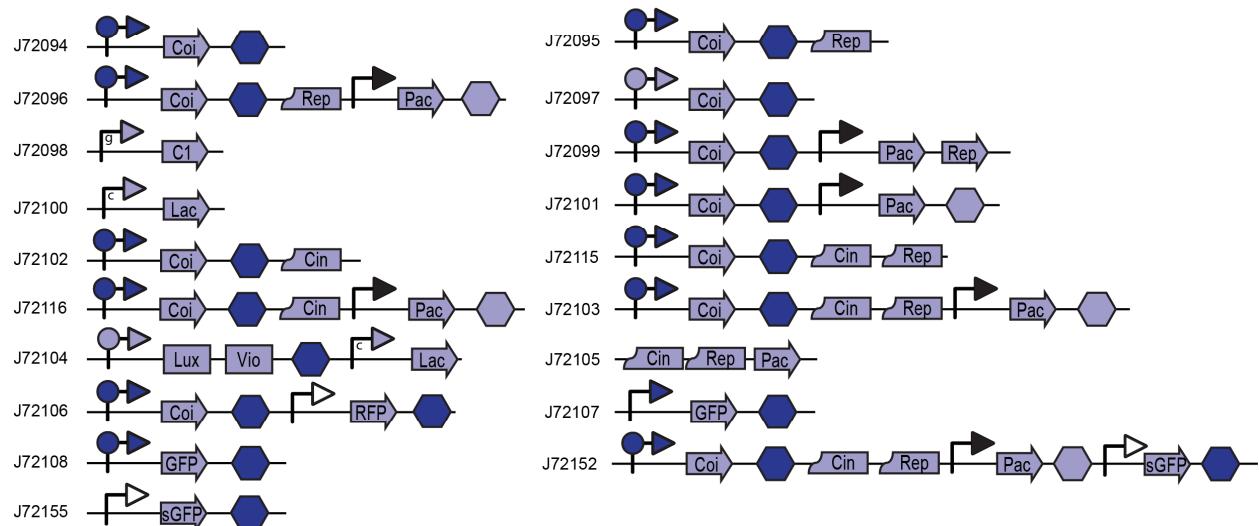
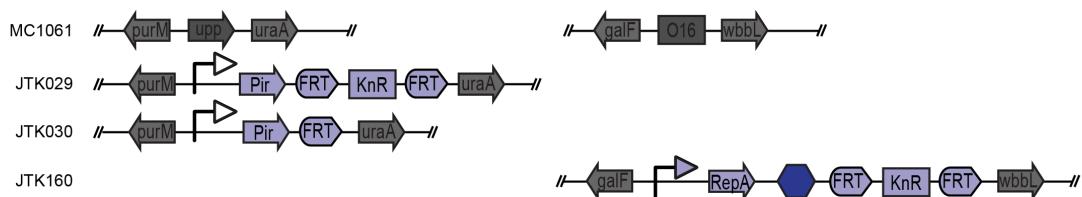


**a Basic parts**

J72071	AraC + P <sub>BAD</sub> promoter	J72082	Coi	rbs. <i>coi</i> cds
J72072	RhaRS + P <sub>RHA</sub> promoter	J72083	Pac	rbs. <i>pac</i> cds
J72047	constitutive promoter P <sub>CON</sub> J23101	J72084	RFP	rbs.RFP cds
J72073	constitutive promoter P <sub>CON</sub>	J72085	Rep	rbs. <i>repL</i> cds
J72074	constitutive promoter P <sub>CON</sub> c5	J72046	GFP	GFP cds with b0015 terminator
J72075	constitutive promoter P <sub>CON</sub> g6	J72086	Pir	rbs. <i>pir</i> cds
J72005	TetR respressible promoter, P <sub>TET</sub>	J72087	Lac	rbs. <i>lacZ</i> cds
J72076	late promoter (LP <sub>sr</sub> )	J72088	RepA	rbs. <i>repA</i> cds of ColE2
J72077	<i>trnpB</i> terminator	J72089	C1	rbs. <i>c1</i> cds
J72049	b0015 double terminator	J72048	sGFP	rbs.sGFP cds
J72079	Cin	J72090	Vio	violacein synthesis operon (VioABCDE), no promoter
J72080	repL	J72091	Lux	luciferin/luciferase synthesis operon (LuxCDABE), no promoter
J72001	FRT	J72092	KnR	kanamycin resistance cassette

**b Composite parts****c Strains****Figure S1 - Illustration of parts and strains used in the study**

Sequences for the basic parts, composite parts, vectors, and strains are listed in tables S1-S4, and sequences are available in the Registry of Standard Biological Parts (<http://partsregistry.org>).

**Table S1 – Basic Parts used in the study**

Registry #	Description	Description/Source
bba_J72071	<i>araC</i> -P <sub>BAD</sub>	Arabinose responsive regulator and corresponding promoter from <i>E. Coli</i> (30)
bba_J72072	<i>rhaRS</i> -P <sub>RHA</sub>	Rhamnose responsive regulators and corresponding promoter from <i>E. Coli</i> (31)
bba_J72047	P <sub>CON</sub> J23101	BglBrick standard version of Bba_J23101, synthetic constitutive promoter (see <a href="http://partsregistry.org/wiki/index.php?title=Part:BBa_J23101">http://partsregistry.org/wiki/index.php?title=Part:BBa_J23101</a> )
bba_J72073	P <sub>CON</sub>	Synthetic constitutive promoter
bba_J72074	P <sub>CON-c5</sub>	Synthetic constitutive promoter
bba_J72075	P <sub>CON-g6</sub>	Synthetic constitutive promoter
bba_J72005	P <sub>TET</sub>	BglBrick standard version of BBa_R0040, TetR repressible promoter (see <a href="http://partsregistry.org/Part:BBa_R0040">http://partsregistry.org/Part:BBa_R0040</a> )
bba_J72076	LP <sub>sit</sub>	Late promoter, Bacteriophage P1 (6)
bba_J72077	<i>trnpB</i> terminator	Terminator from <i>E. coli</i> DH1
bba_J72049	double terminator	Tandem terminators; BglBrick standard version of Bba_B0015 (see <a href="http://partsregistry.org/Part:BBa_B0015">http://partsregistry.org/Part:BBa_B0015</a> )
bba_J72079	<i>cin</i> , no start, frameshift	Derived from Bacteriophage P1 (6)
bba_J72080	<i>repL</i> , no start	Lytic replication origin, Bacteriophage P1 (6)
bba_J72001	FRT	FLP recombinase recognition site, <i>Saccharomyces cerevisiae</i> (32)
bba_J72082	rbs. <i>coi</i>	Repressor inactivator, Bacteriophage P1 (6)
bba_J72083	rbs. <i>pacA</i>	DNA packaging enzyme subunit and DNA packaging sites, Bacteriophage P1 (6)
bba_J72084	rbs.mRFP1	Monomeric red fluorescent protein derivative (33)
bba_J72085	rbs. <i>repL</i>	Initiates lytic replication, Bacteriophage P1 (6)
bba_J72046	rbs.GFP.double terminator	BglBrick standard version of (BBa_E0040 joined with BBa_B0015)
bba_J72086	rbs. <i>pir</i>	Factor required for replication of gamma origin of <i>E. coli</i> plasmid R6K (34)
bba_J72087	rbs. <i>lacZ</i>	Beta-galactosidase from <i>E. coli</i> MG1655
bba_J72088	rbsC.ColE2 <i>repA</i>	Replication initiator of <i>E. coli/Shigella</i> plasmid ColE2-P9 (35), used to support replication of vector J72111 (referred to as pBjk2741 in (36))
bba_J72089	rbs. <i>c1</i>	Master repressor, Bacteriophage P1 (6)
bba_J72090	<i>vioABCDE</i>	Synthetic (recoded) violacein synthesis operon from <i>Chromobacterium violaceum</i> . BglBrick standard version of BBa_K274002 (see <a href="http://partsregistry.org/Part:BBa_K274002">http://partsregistry.org/Part:BBa_K274002</a> )
bba_J72091	<i>luxCDABE</i>	Luciferase and substrate synthesis operon from <i>Photinus luciferus</i> (ATCC 29999) (37)
bba_J72092	kanamycin resistance cassette	
bba_J72048	rbs.sfGFP	Superfolder GFP mutant (38). BglBrick standard version of BBa_I746916 (see <a href="http://partsregistry.org/Part:BBa_I746916">http://partsregistry.org/Part:BBa_I746916</a> )

**Table S2 – Composite Parts used in the study**

Registry #	Description	Composition
bba_J72094	P <sub>BAD</sub> driven, <i>coi</i> only phagemid	bba_J72071.bba_J72082.bba_J72049
bba_J72095	P <sub>BAD</sub> driven, <i>coi</i> + <i>repL</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72080
bba_J72096	P <sub>BAD</sub> driven, <i>coi</i> + <i>repL</i> + <i>pacA</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72080.bba_J72076.bba_J72083.bba_J72085
bba_J72097	P <sub>RHA</sub> driven, <i>coi</i> only phagemid	bba_J72072.bba_J72082.bba_J72049
bba_J72098	Constitutively expressed <i>c1</i>	bba_J72075.bba_J72089
bba_J72099	P <sub>BAD</sub> driven, <i>coi</i> + <i>repL</i> + <i>pacA</i> phagemid for library fragment insertion	bba_J72071.bba_J72082.bba_J72049.bba_J72076.bba_J72083.bba_J72085
bba_J72100	Constitutively expressed <i>lacZ</i>	bba_J72074.bba_J72087

bba_J72101	P <sub>BAD</sub> driven, <i>coi + pacA</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72076.bba_J72083.bba_J72077
bba_J72102	P <sub>BAD</sub> driven, <i>coi + cin</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72079
bba_J72115	P <sub>BAD</sub> driven, <i>coi + cin + repL</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72079.bba_J72080
bba_J72116	P <sub>BAD</sub> driven, <i>coi + cin + pacA</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72079.bba_J72076.bba_J72083. bba_J72077
bba_J72103	P <sub>BAD</sub> driven, <i>coi + repL + cin + pacA</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72079.bba_J72080.bba_J72076. bba_J72083.bba_J72077
bba_J72104	P <sub>RHA</sub> driven <i>lux + vio</i> operons, constitutive <i>lacZ</i> expression	bba_J72072.bba_J72091.bba_J72090.bba_J72049.bba_J72074.bba_J72087
bba_J72105	Competitor phagemid; <i>cin + repL + pacA</i>	bba_J72079.bba_J72080.bba_J72083
bba_J72106	P <sub>BAD</sub> driven, <i>coi + RFP</i> phagemid	bba_J72071.bba_J72082.bba_J72049.bba_J72005.bba_J72084.bba_J72049
bba_J72107	Reference promoter driving expression of GFP	bba_J72047.bba_J72046
bba_J72108	P <sub>BAD</sub> driven GFP	bba_J72071.bba_J72046
bba_J72152	P <sub>BAD</sub> driven, <i>coi + repL + cin + pacA</i> phagemid expressing GFP	bba_J72071.bba_J72082.bba_J72049.bba_J72079.bba_J72080.bba_J72076. bba_J72083.bba_J72077.bba_J72005.bba_J72048.bba_J72049
bba_J72155	GFP expression cassette	bba_J72005.bba_J72048.bba_J72049

**Table S3 – Vectors used in the study**

Registry #	Description
bba_J72109	ampicillin resistant pUC vector
bba_J72110	ampicillin + chloramphenicol resistant p15A vector
bba_J72153	ampicillin + kanamycin resistant p15A vector
bba_J72154	kanamycin + chloramphenicol resistant p15A vector
bba_J72111	spectinomycin resistant split ColE2 vector
bba_J72112	spectinomycin resistant split R6K vector
bba_J72113	chloramphenicol resistant p15A vector
bba_J72114	complete, arabinose inducible phagemid, chloramphenicol resistant, p15a vector

**Table S4 – Strains used in the study**

Registry #	Description	Composition	Name in text
BBa_J72136	MC1061 <i>upp::pir</i> +FRT-kanR-FRT Cassette encoding constitutive expression of <i>pir</i> and kanamycin resistance inserted between <i>purM</i> and <i>uraA</i>	<i>purM</i> - bba_J72005.bba_J72086. bba_J72001.bba_J72092. bba_J72001 - <i>uraA</i>	JTK029
BBa_J72137	MC1061 <i>upp::pir</i> +FRT Cassette encoding constitutive expression of <i>pir</i> inserted between <i>purM</i> and <i>uraA</i>	<i>purM</i> - bba_J72005.bba_J72086. bba_J72001 - <i>uraA</i>	JTK030
BBa_J72138	MC1061 O16:: <i>repA</i> +FRT Cassette encoding constitutive expression of <i>repA</i> (from ColE2) and kanamycin resistance inserted between <i>galF</i> and <i>wbbL</i>	<i>galF</i> - bba_J72073.bba_J72088. bba_J72049.bba_J72001. bba_J72092.bba_J72001 - <i>wbbL</i>	JTK160C

## Supplemental References

30. Guzman, L. M., Belin, D., Carson, M. J., and Beckwith, J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter, *J Bacteriol* 177, 4121-4130.
31. Tobin, J. F., and Schleif, R. F. (1987) Positive regulation of the Escherichia coli L-rhamnose operon is mediated by the products of tandemly repeated regulatory genes, *J Mol Biol* 196, 789-799.
32. Babineau, D., Vetter, D., Andrews, B. J., Gronostajski, R. M., Proteau, G. A., Beatty, L. G., and Sadowski, P. D. (1985) The FLP protein of the 2-micron plasmid of yeast. Purification of the protein from Escherichia coli cells expressing the cloned FLP gene, *J Biol Chem* 260, 12313-12319.
33. Shaner, N. C., Campbell, R. E., Steinbach, P. A., Giepmans, B. N., Palmer, A. E., and Tsien, R. Y. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein, *Nat Biotechnol* 22, 1567-1572.
34. Metcalf, W. W., Jiang, W., and Wanner, B. L. (1994) Use of the rep technique for allele replacement to construct new Escherichia coli hosts for maintenance of R6K gamma origin plasmids at different copy numbers, *Gene* 138, 1-7.
35. Hiraga, S., Sugiyama, T., and Itoh, T. (1994) Comparative analysis of the replicon regions of eleven ColE2-related plasmids, *J Bacteriol* 176, 7233-7243.
36. Kittleson, J. T., Cheung, S., and Anderson, J. C. (2011) Rapid optimization of gene dosage in E. coli using DIAL strains, *Journal of biological engineering* 5, 10.
37. Winson, M. K., Swift, S., Hill, P. J., Sims, C. M., Griesmayr, G., Bycroft, B. W., Williams, P., and Stewart, G. S. (1998) Engineering the luxCDABE genes from Photorhabdus luminescens to provide a bioluminescent reporter for constitutive and promoter probe plasmids and mini-Tn5 constructs, *FEMS Microbiol Lett* 163, 193-202.
38. Pedelacq, J. D., Cabantous, S., Tran, T., Terwilliger, T. C., and Waldo, G. S. (2006) Engineering and characterization of a superfolder green fluorescent protein, *Nat Biotechnol* 24, 79-88.