

Supplementary Information

Iterative plug-and-play methodology for constructing and modifying synthetic gene networks

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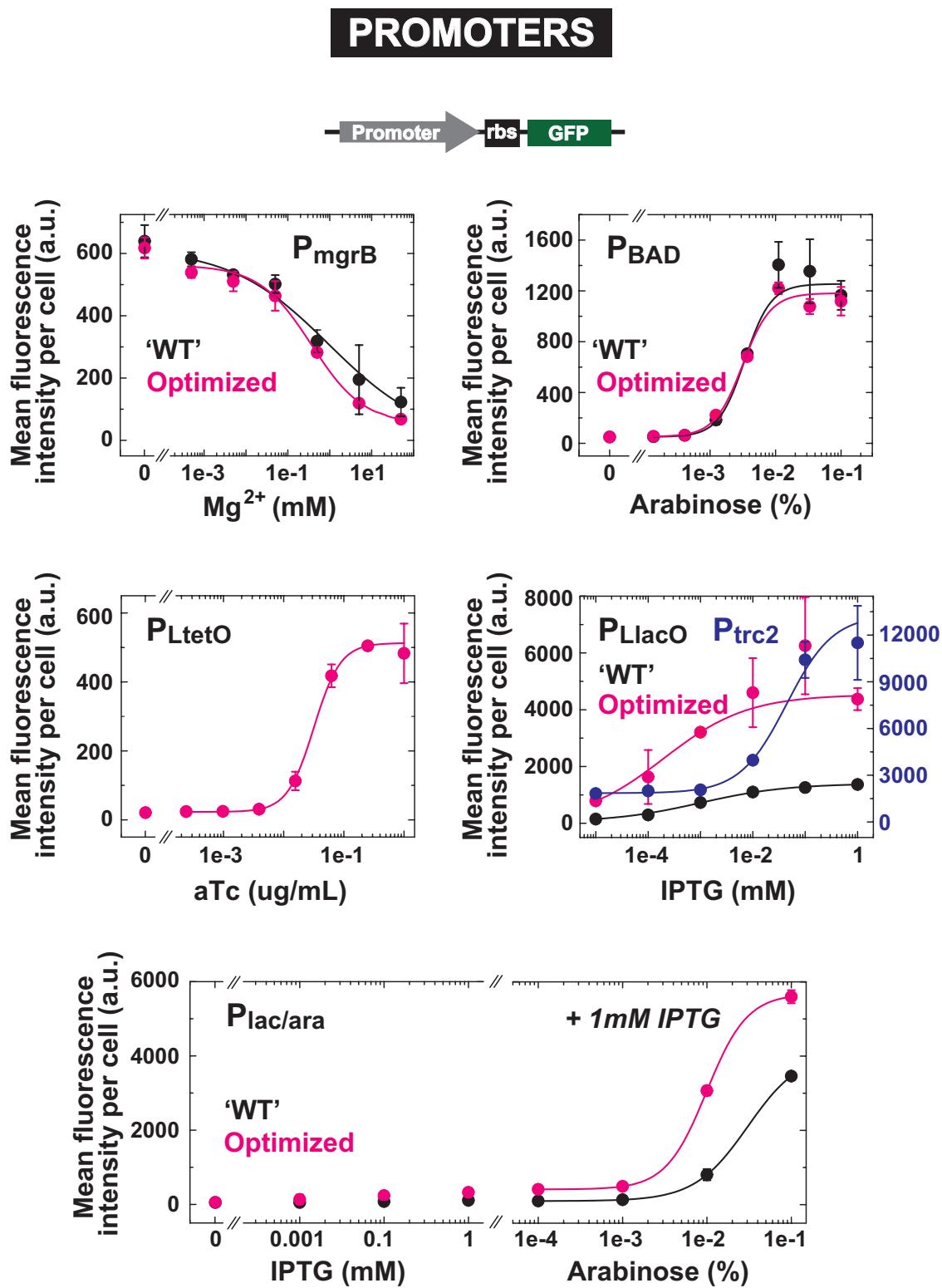
**These authors contributed equally to this work.*

§Co-senior authors.

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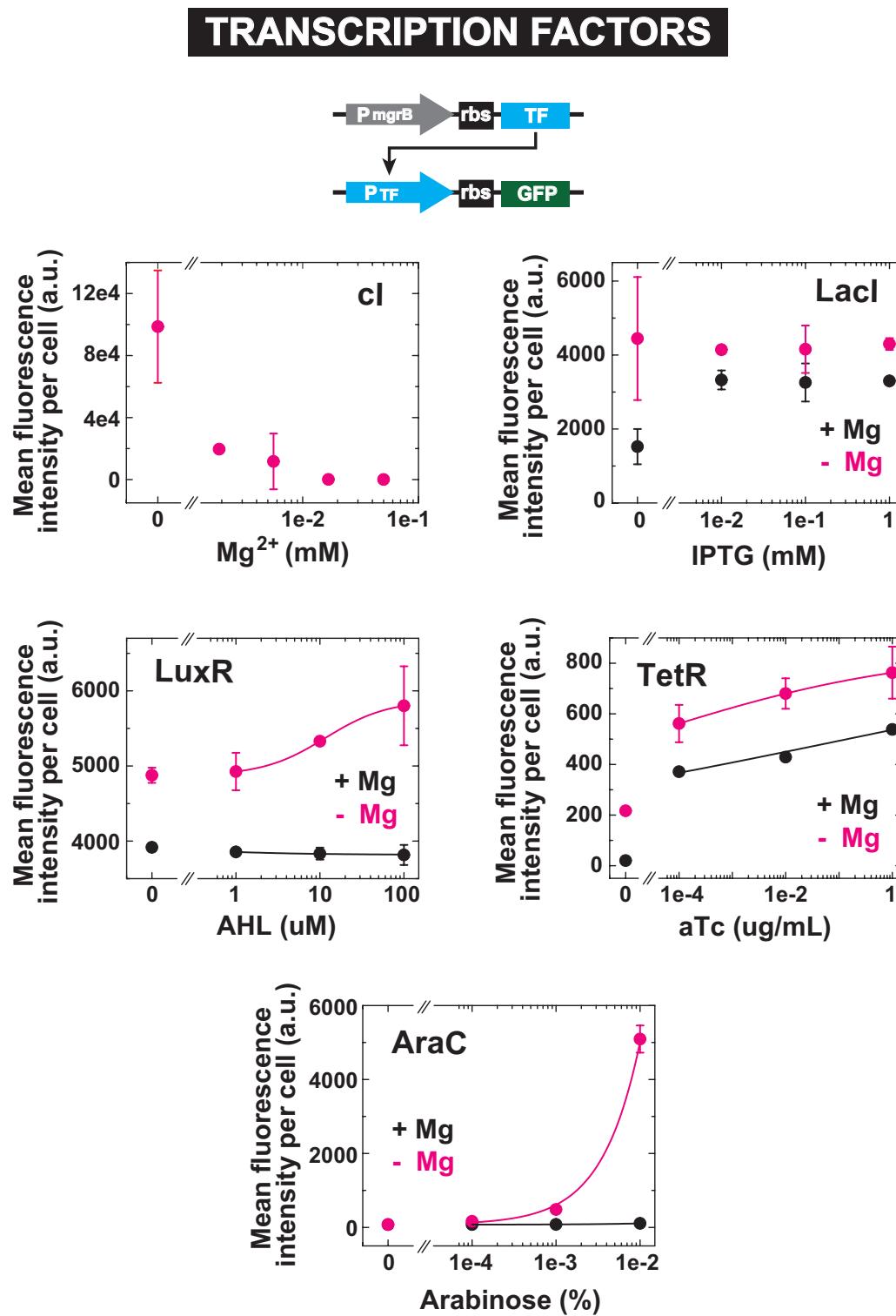
Supplementary Figure 1	Input/output functions for optimized promoters
Supplementary Figure 2	Input/output functions for optimized transcription factors
Supplementary Figure 3	Input/output functions for optimized terminator and reporters
Supplementary Figure 4	aTc-induced switching and subsequent maintenance of the genetic toggle switch
Supplementary Table 1	Restriction enzymes and recognition sites
Supplementary Table 2	Component library
Supplementary Note	DNA sequence and annotation of optimized components and circuit constructs

Supplementary Figure 1. Input/output functions for optimized promoters.



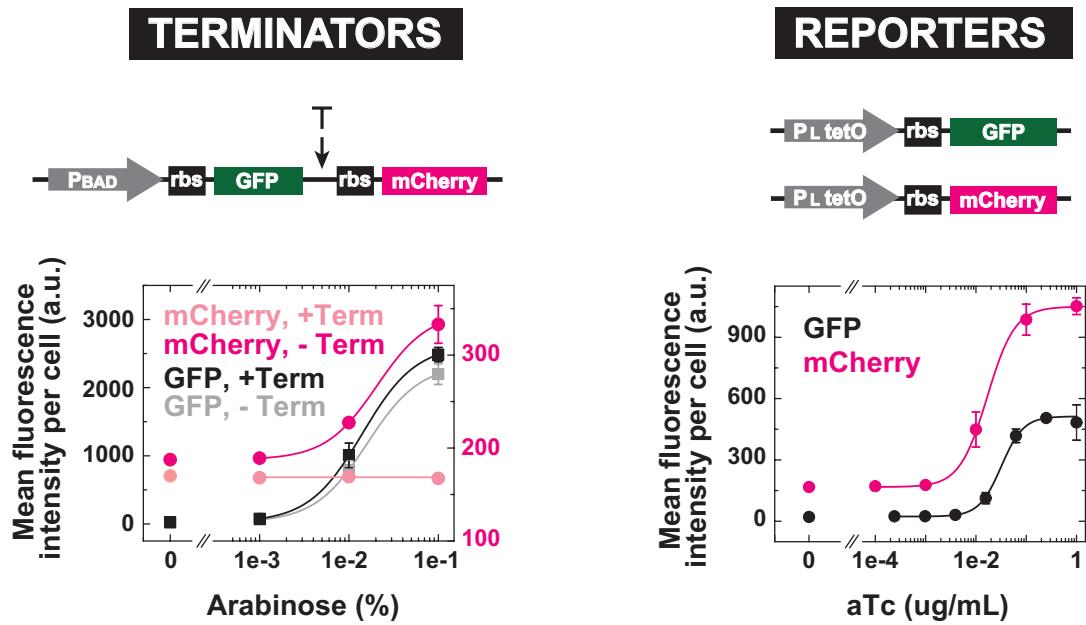
Cells were grown overnight in LB medium, then diluted 1:1000 into LB and induced for 5 hours prior to measurement. Fluorescence was measured via flow cytometry by collecting 10,000 events per replicate. P_{mgrB} was induced with 0–50 mM MgCl₂. P_{BAD} was induced with 0–0.01 % w/v arabinose. P_{LtetO} was induced with 0–1 µg/ml aTc. P_{LlacO} and P_{trc2} were induced with 0–1 mM IPTG. $P_{lac/ara}$ was induced with 0–1 mM IPTG (no arabinose) and with 0–0.01 % w/v arabinose (with 1 mM IPTG). Points represent mean values for three experiments ± SD. $n = 10,000$ events per experiment. Lines represent sigmoidal fits.

Supplementary Figure 2. Input/output functions for optimized transcription factors.



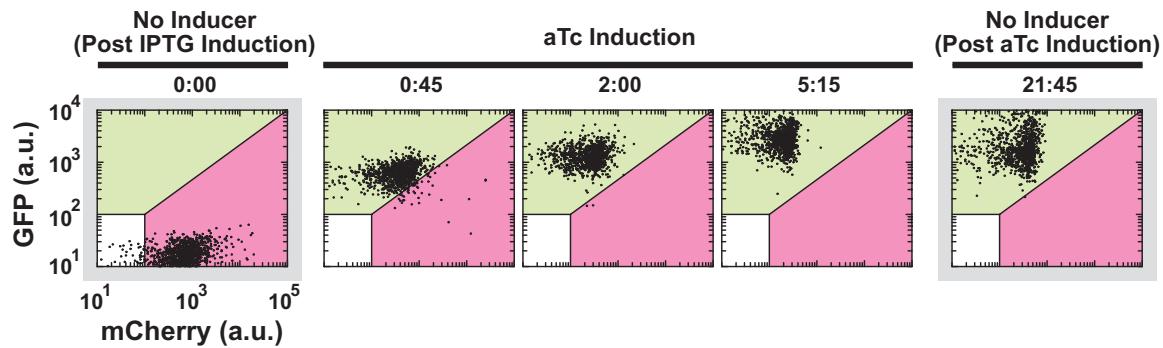
Cells were grown overnight in LB medium, then diluted 1:1000 into LB and induced for 8 hours prior to measurement. Fluorescence was measured via flow cytometry by collecting 10,000 events per replicate. All experiments were conducted without TF (black, 50 mM MgCl₂) or with TF (red, 0 mM MgCl₂), and dose responses were performed with the TF-specific inducer chemicals, except for cI (0–50 mM MgCl₂). Induction conditions: LacI / P_{trc2}, 0–1 mM IPTG; LuxR / P_{lux}, 0–100 μM N-acyl homoserine lactone (AHL); TetR / P_{LtetO}, 0–1 μg/ml aTc; AraC / P_{BAD}, 0–0.01% w/v arabinose. Points represent mean values for three experiments ± SD. n = 10,000 events per experiment. Lines represent sigmoidal fits.

Supplementary Figure 3. Input/output functions for the optimized terminator and reporters.



Cells were grown overnight in LB medium, then diluted 1:1000 into LB and induced for 8 hours prior to measurement. Fluorescence was measured via flow cytometry by collecting 10,000 events per replicate. The terminator was tested in a bicistronic reporter circuit, with or without a terminator between reporters. P_{BAD} was induced with 0–0.01% w/v arabinose. Reporters were expressed with P_{LtetO} (0–1 μ g/ml aTc). Points represent mean values for three experiments \pm SD. $n = 10,000$ events per experiment. Lines represent sigmoidal fits.

Supplementary Figure 4. aTc-induced switching and subsequent maintenance of the genetic toggle switch.



A time-course of cells harboring the toggle construct switching from the mCherry state (0 hrs) to the GFP state (0–5:15 hrs) through aTc induction, and then maintained in the GFP state when diluted into the no-inducer condition and grown overnight (21:45 hrs). Data were obtained by flow cytometry at the indicated times.

Supplementary Table 1. Restriction enzymes and recognition sites. The cleavage site within each recognition site is indicated by “|”.

Enzyme	Recognition Site
AatII	GACGT C
AclII	AA CGTT
AflII	C TTAAG
ApaLI	G TGCAC
AvrII	C CTAGG
BamHI-HF	G GATCC
BsrGI	T GTACA
EagI-HF	C GGCCG
EcoRI-HF	G AATTC
EcoRV-HF	GAT ATC
HindIII-HF	A AGCTT
KasI	G GCGCC
KpnI-HF	GGTAC C
MfeI-HF	C AATTG
MluI	A CGCGT
NcoI-HF	C CATGG
NdeI	CA TATG
NheI-HF	G CTAGC
PciI	A CATGT
PstI-HF	CTGCA G
PvuII-HF	CAG CTG
SacI-HF	GAGCT C
SacII	CCGC GG
SalI-HF	G TCGAC
ScalI-HF	AGT ACT
SpeI	A CTAGT
SphI-HF	GCATG C
SspI-HF	AAT ATT
XbaI	T CTAGA
XhoI	C TCGAG
XmaI	C CCGGG

Supplementary Table 2. Component library. List of optimized components.

Component	Length(bp)	Library Plasmid	Type
LuxR	756	pKLi049	Gene (Activator)
AraC_JK_LAA	879	pKLi051	Gene (Activator)
ECFP-LAA	759	pKLi015	Gene (Fluorescent Reporter)
EYFP-LAA	759	pKLi013	Gene (Fluorescent Reporter)
GFPmut3b-LAA	756	pKLi011	Gene (Fluorescent Reporter)
mCherry-LAA	750	pKLi037	Gene (Fluorescent Reporter)
mCherry-LCOpt-LAA	750	pKLi056	Gene (Fluorescent Reporter)
flpE-LAA	1305	pKLi025	Gene (Recombinase)
cI-LAA	753	pKLi021	Gene (Repressor)
lacI-LAA	1122	pKLi017	Gene (Repressor)
tetR-LAA	663	pKLi019	Gene (Repressor)
LuxI_RSR	618	pKLi047	Gene (Quorum Sensing)
PBAD	286	pKLi034	Promoter
Plac/ara	483	pKLi053	Promoter
PLs1con	558	pKLi031	Promoter
PLtetO	74	pKLi030	Promoter
Plux	219	pKLi055	Promoter
Plux/cI	97	pKLi048	Promoter
PmgrB	543	pKLi054	Promoter
Ptrc2	75	pKLi032	Promoter
T0	123	pKLi027	Transcriptional Terminator
T1T2	231	pKLi028	Transcriptional Terminator
Spacer-50bp	50	pKLi057	Spacer
Spacer-100bp	100	pKLi058	Spacer
Spacer-250bp	250	pKLi059	Spacer
Spacer-500bp	500	pKLi060	Spacer

Supplementary Note

DNA sequence and annotation of optimized components

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DEFINITION Carbenicillin-Resistant, ColE1 ori, Optimized Vector
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 541 cattatcaac aaaatactcc gattggcgat gcccctgtcc ttttaccaga caaccattac
 601 ctgtccacac aatctgccct ttgaaagat cccaacgaaa agagagacca catggcctt
 661 cttgagttt taaccgctgc tgggattaca catggcatgg atgaactata caaaacttagc
 721 gcagcgaacg acgaaaatta cgcccttgca gcgtga
 //
 LOCUS lacI-LAA 1122 bp DNA LINEAR SYN
 DEFINITION Gene (Repressor)
 ACCESSION pKLi017
 FEATURES Location/Qualifiers
 CDS 1..1122
 /note="lacI-LAA"
 primer_bind 1..20
 /note="Fw. Primer Homology"
 misc_feature 16..16
 /note="T->C AclI"
 misc_feature 18..18
 /note="A->G AclI"
 misc_feature 333..333

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        /note="G->T ApaLI"
misc_feature 351..351
        /note="A->G MluI"
misc_feature 801..801
        /note="T->C EcoRV"
misc_feature 993..993
        /note="G->T KasI"
misc_signal 1081..1122
        /note="LAA tag"
primer_bind complement (1103..1122)
        /note="Rev. Primer Homology"
BASE COUNT      253 a      313 c      318 g      238 t
ORIGIN
   1 atgaaaccag taacgctgta cgatgtcgca gaggatgccg gtgtctctta tcagaccgtt
  61 tccgcgtgg tgaaccaggc cagccacgtt tctgcgaaaa cgcggggaaaa agtggaagcg
 121 gcgtatggcg agctgaatta cattccaaac cgctggcac aacaactggc gggcaaacag
 181 tcgttgctga ttggcggttgc cacctccagt ctggccctgc acgcgcgc gcaaattgtc
 241 gcggcgatta aatctcgcgc cgatcaactg ggtgccagcg tgggtgttc gatggtagaa
 301 cgaagcggcg tcgaagcctg taaagcggcg gttcacaatc ttctcgcgca ggcgtcagt
 361 gggctgatca ttaactatcc gctggatgac caggatgcca ttgctgtgga agctgcctgc
 421 actaatgttc cggcggttatt tcttgatgtc tctgaccaga caccatcaa cagttattt
 481 ttctccatag aagacggtac gcgactggc gtggagcatc tggtcgcatt gggtcaccag
 541 caaatcgcgc tgtagcgaaa cccattaaatgt tctgtctcggt cgcgtcgctg tctggctggc
 601 tggcataaaat atctcactcg caatcaaattt cagccgatag cggAACGGGA aggccactgg
 661 agtccccatgt ccgggtttca acaaaccatg caaatgctga atgagggcat cgttcccact
 721 gcgtatggcgtt tgccaaacgta tcagatggcg ctggcgcaaa tgcgcgcatt taccgagtcc
 781 gggctgctggcgtt tggtgcggtt catctcggtt gtgggatacg acgataccga agacagctca
 841 tggttatatcc cggcggttac caccatcaaa caggattttc gcgtgtggg gcaaaccagc
 901 gtggaccgct tgctgcaact ctctcaggc caggcggtga agggcaatca actgttgccc
 961 gtctcactgg tgaaaagaaa aaccaccctg gctcccaata cgccaaaccgc ctctccccgc
1021 gcgttggccg attcattaaat gcaactggca cgacagggtt cccgactgga aagcgggcag
1081 actagcgcag cgaacgcacga aaattacgccc cttgcagcgtt ga
//
```

LOCUS tetR-LAA 663 bp DNA LINEAR SYN

DEFINITION Gene (Repressor)

ACCESSION pKLi019

FEATURES Location/Qualifiers

- CDS 1..663
 - /note="tetR-LAAP"
- primer_bind 1..20
 - /note="Fw. Primer Homology"
- misc_feature 7..7
 - /note="A->C XbaI"
- misc_feature 9..9
 - /note="A->T XbaI"
- misc_feature 582..582
 - /note="A->C NdeI"
- misc_signal 622..663
 - /note="LAA tag"
- primer_bind complement (644..663)
 - /note="Rev. Primer Homology"

BASE COUNT 211 a 128 c 148 g 176 t

ORIGIN

```

   1 atgtctcggtt tagataaaag taaagtgatt aacagcgcat tagagctgct taatgaggtc

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61 ggaatcgaag gtttacaac ccgtaaactc gcccagaagc taggtttaga gcagcctaca
121 ttgttattggc atgtaaaaaaaa taagcgggct ttgctcgacg ctttagccat tgagatgtta
181 gataggcacc atactcaatt ttgccctta gaaggggaaa gctggcaaga tttttacgt
241 aataacgcta aaagttttag atgtgttta ctaagtcatc gcgatggagc aaaagtacat
301 ttaggtacac ggcctacaga aaaacagtat gaaactctcg aaaatcaatt agcctttta
361 tgccaacaag gttttcaact agagaatgca ttatatgcac tcagcgtgt gggcatttt
421 actttagggt gcgttattgga agatcaagag catcaagtgc ctaaagaaga aaggaaaca
481 cctactactg atagtatgcc gccatttata cgacaagcta tcgaattatt tgatcaccaa
541 ggtgcagagc cagccttctt attcggcattt gaattgatca tctgcggatt agaaaaacaa
601 cttaaatgtg aaagtgggtc tactagcgca gcbaacgacg aaaattacgc cttgcagcg
661 tga

//



LOCUS      cI-LAA 753 bp    DNA    LINEAR    SYN
DEFINITION Gene (Repressor)
ACCESSION  pKLi021
FEATURES          Location/Qualifiers
  CDS            1..753
                 /note="cI-LAA"
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  misc_feature  477..477
                 /note="A->G HindIII"
  misc_signal   712..753
                 /note="LAA tag"
  primer_bind   complement (724..753)
                 /note="Rev. Primer Homology"
BASE COUNT     221 a    157 c    189 g    186 t
ORIGIN
  1 atgagcacaa aaaagaaacc attaacacaa gagcagcttg aggacgcacg tcgccttaaa
  61 gcaatttatg aaaaaaaagaa aaatgaactt ggcttatccc aggaatctgt cgccagacaag
 121 atggggatgg ggcagtcagg cgttgggtct ttatTTATG gcatcaatgc attaaatgct
181 tataacgccc cattgcttc aaaaattctc aaagtttagcg ttgaagaatt tagcccttca
241 atcggcagag aaatctacga gatgtatgaa gcggttagta tgcagcgtc acttagaagt
301 gagtagatgat accctgtttt ttctcatgtt cagggcaggta tggcttcacc tgagctttaga
361 acctttacca aaggtgatgc ggagagatgg gtaagcacaa caaaaaaaaagc cagtgattct
421 gcattctggc ttgaggttga aggttaattcc atgaccgcac caacaggctc caagccgagc
481 tttcctgacg gaatgttaat tctcgttgac cctgagcagg ctgtttagcc aggtgatttc
541 tgcataccca gacttggggg tgatgagttt accttcaaga aactgatcag ggatagcggt
601 caggtgtttt tacaaccact aaacccacag tacccatga tcccatgcaa tgagagttgt
661 tccgttgtgg ggaaagttat cgctagtcg tggcctgaag agacgtttgg cactagcgca
721 gcgaacgacg aaaattacgc cttgcagcg tga

//



LOCUS      f1pE-LAA 1305 bp    DNA    LINEAR    SYN
DEFINITION Gene (Repressor)
ACCESSION  pKLi025
FEATURES          Location/Qualifiers
  CDS            1..1305
                 /note="f1pE-LAA "
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  misc_feature  345..345
                 /note="T->C EcoRV"
  misc_feature  507..507

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/note="G->T XhoI"
misc_feature 508..508
 /note="A->C XhoI"
misc_feature 510..510
 /note="A->C XhoI"
misc_feature 564..564
 /note="T->C MfeI"
misc_feature 594..594
 /note="T->G AclI"
misc_feature 597..597
 /note="T->C AclI"
misc_feature 1068..1068
 /note="A->G SpeI"
misc_feature 1089..1089
 /note="A->G NdeI"
misc_feature 1140..1140
 /note="A->G MfeI"
misc_signal 1264..1305
 /note="LAA tag (RI linker)"
primer_bind complement (1286..1305)
 /note="Rev. Primer Homology"
BASE COUNT 462 a 245 c 259 g 339 t
ORIGIN
1 atgagccaat ttgatatatt atgtaaaaca ccacctaagg tcctggttcg tcagtttg
61 gaaaggtttgc aaagacccatc agggaaaaaa atagcatcat gtgctgctga actaacctat
121 ttatgttggta tgattactca taacggaaaca gcaatcaaga gagccacatt catgagctat
181 aatactatca taagcaatttc gctgagtttc gatattgtca acaaatttactt ccagttttaaa
241 tacaagacgc aaaaagcaac aattctggaa gcctcattaa agaaaatttacat tcctgcttgg
301 gaatttacaa ttattccatca caatggacaa aacatcaat ctgacatcac tgatattgtat
361 agtagtttgc aattacagttt cgaatcatcg gaagaaggcataa tagccacagt
421 aaaaaaatgc ttaaaggact tctaagttagt ggtgaaagca tctggagat cactgagaaaa
481 atactaaattt cgttttagtta tacccctcgc ttatccaaaaa caaaaactttt ataccaattt
541 ctcttccttag ctactttcat caactgttggaa agattcagcg atattaagaa cgtggaccgg
601 aaatcattta aatttagtcca aaataagtat ctggagat taaatccatgt ttttagtgaca
661 gagacaaaga caagcgtagt taggcacata tacttcttta ggcacagggg taggatcgat
721 ccacttgtat attttggatgat atttttggagg aactctgaac cagtcctaaa acgagtttac
781 aggaccggca atttcttcaag caacaaacag gaataccat tattaaaaga taacttagtc
841 agatcgtaca acaaggctttt gaagaaaaat ggccttatac caatcttgc tataaagaat
901 ggccaaaaat ctcacattgg aagacatttgc atgacccat ttctgtcaat gaagggcccta
961 acggagggttgc ctaatgttggat gggaaatttgg agcgataagc gtgcttgc cgtggccagg
1021 acaacgtata ctcatcgat aacagcaata cctgatcact acttcgcgc agtttctcg
1081 tactatgcgt atgatccat atcaaaggaa atgatagcat tgaaggatga gactaatccg
1141 attgaggaggat ggcagcatat agaacagcta aagggttagtgc ctgaaggaaag catacgatac
1201 cccgcatttgc atgggataat atcacaggag gtacttagact acctttcatc ctacataaat
1261 agacgcatac cagcaaacgc cggaaaactac gcttttagcag cttaa
//

LOCUS T0 123 bp DNA LINEAR SYN
DEFINITION Transcriptional Terminator
ACCESSION pKLi027
FEATURES Location/Qualifiers
misc_feature 1..123
 /note="T0"
primer_bind 1..20
 /note="Fw. Primer Homology"

```

misc_feature    15..109
                /note="T0"
primer_bind     complement (104..123)
                /note="Rev. Primer Homology"
BASE COUNT      28 a      27 c      32 g      36 t
ORIGIN
        1 cgagaattgg ctggactcc tggataga tccagtaatg acctcagaac tccatctggaa
        61 tttgttcaga acgctcggtt gccgcgggc gtttttatt ggtgagaatc caaggcgttag
        121 tca
//  

LOCUS          T1T2 231 bp    DNA    LINEAR    SYN
DEFINITION    Transcriptional Terminator
ACCESSION     pKLi028
FEATURES       Location/Qualifiers
    misc_feature 1..231
                /note="T1T2"
    primer_bind   1..20
                /note="Fw. Primer Homology"
    misc_feature  15..58
                /note="T1"
    misc_feature  121..121
                /note="T->C Acli"
    misc_feature  190..217
                /note="T2"
    primer_bind   complement (212..231)
                /note="Rev. Primer Homology"
BASE COUNT      59 a      56 c      67 g      49 t
ORIGIN
        1 gcttagaggca tcaaataaaaaa cgaaaggctc agtcgaaaga ctgggccttt cgtttatct
        61 gttgtttgtc ggtgaacgct ctcctgagta ggacaaatcc gccgggagcg gatttgaacg
        121 ctgcgaagca acggccccgga gggtggcggg caggacgccc gccataaact gccaggcatc
        181 aaattaagca gaaggccatc ctgacggatg gccttttgc gtttctacaa a
//  

LOCUS          PLtetO         74 bp    DNA    LINEAR    SYN
DEFINITION    Promoter
ACCESSION     pKLi030
FEATURES       Location/Qualifiers
    promoter     1..74
                /note="PLtetO"
    protein_bind 1..19
                /note="Otet2"
                /bound_moiety="TetR"
    primer_bind   1..28
                /note="Fw. Primer Homology"
    -35_signal    20..25
                /note="-35 Box"
    protein_bind  26..44
                /note="Otet2"
                /bound_moiety="TetR"
    -10_signal    43..48
                /note="-10 box"
    misc_feature   55
                /note="Transcriptional Start"

```

```

primer_bind      complement (55..74)
                  /note="Rev. Primer Homology"
BASE COUNT       23 a      19 c      16 g      16 t
ORIGIN
1 tcccttatcag tgatagagat tgacatccct atcagtgata gagatactga gcacatcagc
61 aggacgcact gacc
//  

LOCUS          PLS1con    558 bp     DNA    LINEAR    SYN
DEFINITION    Promoter
ACCESSION     pKLi031
FEATURES        Location/Qualifiers
  promoter      1..558
                 /note="PL(s1con)"
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  protein_bind  7..23
                 /note="OL3"
  protein_bind  27..43
                 /note="OL2"
  -35_signal    41..46
                 /note="-35 box"
  protein_bind  51..67
                 /note="OL1"
  -10_signal    65..69
                 /note="-10 box"
  misc_feature  75
                 /note="Transcription Start"
  primer_bind   complement (539..558)
                 /note="Rev. Primer Homology"
BASE COUNT       181 a      133 c      134 g      110 t
ORIGIN
1 acagataaacc atctgcgggtg ataaattatc tctggcggtg ttgacataaa taccactggc
61 ggtgatactg agcacatcag caggacgcac tgaccacat gaagggtgacg ctctaaaaaa
121 ttaagccctg aagaagggca gcattcaaag cagaaggctt tgggtgtgt gatacgaac
181 gaagcattgg ccgttaagtgc gattccggat tagctgccaa tgtgccaatc gcggggggtt
241 ttcgttctagg actacaactg ccacacacca ccaaagctaa ctgacaggag aatccagatg
301 gatgcacaaa cacgcccggc cgaacgtcgc gcagagaaac aggctcaatg gaaagcagca
361 aatcccctgt tggttgggtt aagcgcaaaa ccagttccga aagattttt taactataaa
421 cgctgtatgga agcgtttatg cggaaagaggt aaagcccttc ccgagtaaca aaaaaacaac
481 agcataaaata accccgctct tacacattcc agccctgaaa aagggcatca aattaaacca
541 cacctatggt gtatgcaa
//  

LOCUS          PtRC2 75 bp     DNA    LINEAR    SYN
DEFINITION    Promoter
ACCESSION     pKLi032
FEATURES        Location/Qualifiers
  promoter      1..75
                 /note="PtRC-2"
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  -35_signal    14..19
                 /note="-35 Box"
  -10_signal    37..42

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```

        /note="-10 box"
misc_feature    49
                /note="Transcription Start"
protein_bind   49..69
                /note="Olac"
                /bound_moiety="LacI"
primer_bind    complement (56..75)
                /note="Rev. Primer Homology"
BASE COUNT      23 a      13 c      17 g      22 t
ORIGIN
1 ctgaaatgag ctgttgacaa ttaatcatcc ggctcgtata atgtgtggaa ttgtgagcgg
61 ataacaattt cacac
//


LOCUS      PBAD 286 bp     DNA   LINEAR   SYN
DEFINITION Promoter
ACCESSION  pKLi034
FEATURES          Location/Qualifiers
promoter         1..286
                /note="pBAD "
primer_bind     1..20
                /note="Fw. Primer Homology"
protein_bind    4..20
                /note="AraO2"
misc_feature    12..12
                /note="T->A MfeI"
misc_feature    135..135
                /note="A->G MluI"
protein_bind    140..157
                /note="AraC"
protein_bind    160..177
                /note="AraC"
protein_bind    182..203
                /note="CRP-cAMP"
protein_bind    214..230
                /note="AraI1"
protein_bind    235..252
                /note="AraI2"
misc_feature    244..244
                /note="C->A BamHI"
-35_signal      245..253
                /note="-35 box"
-10_signal       270..277
                /note="-10 box"
primer_bind     complement (267..286)
                /note="Rev. Primer Homology"
BASE COUNT      86 a      74 c      47 g      79 t
ORIGIN
1 aagaaaccaa tagtccatat tgcacatcagac attgccgtca ctgcgtcttt tactggctct
61 tctcgctaac caaacccggtt accccgctta ttaaaaggat tctgtaaacaa agcgggaccca
121 aagccatgac aaaaggcgcgt aacaaaaggat tctataatca cggcagaaaa gtcccacattt
181 attatttgca cggcgtcaca ctttgcgtatg ccatacgattttatccata agatttagcgg
241 atcatacctg acgttttta tcgcaactct ctactgtttc tccata
//
```

LOCUS mCherry-LAA 750 bp DNA LINEAR SYN
 DEFINITION Gene (Fluorescent Reporter)
 ACCESSION pKLi037
 FEATURES Location/Qualifiers
 primer_bind 1..20
 /note="Fw. Primer Homology"
 CDS 1..750
 /note="mCherry-LAA"
 misc_feature 63..63
 /note="G->T ApaLI"
 misc_feature 357..357
 /note="G->A PstI"
 misc_feature 435..435
 /note="C->T NcoI"
 misc_feature 483..483
 /note="C->G KasI"
 misc_feature 579..579
 /note="G->A PvuII"
 misc_feature 591..591
 /note="C->G KasI"
 misc_feature 705..705
 /note="C->T BsrGI"
 misc_feature 709..750
 /note="LAA tag"
 primer_bind complement (731..750)
 /note="Rev. Primer Homology"

BASE COUNT 178 a 233 c 230 g 109 t
 ORIGIN

```

  1 atggtgagca agggcgagga ggataacatg gccatcatca aggagttcat ggcgttcaag
  61 gttcacatgg agggctccgt gaacggccac gagttcgaga tcgagggcga gggcgagggc
 121 cgccttacg agggcaccca gaccgcaag ctgaaggtga ccaagggtgg cccctgccc
 181 ttgcctggg acatcctgtc ccctcagttc atgtacggct ccaaggccta cgtgaagcac
 241 cccggcaca tccccgacta cttgaagctg tccttcccg agggctcaa gtgggagcgc
 301 gtgtatgaaact tcgaggacgg cgccgtggtg accgtgaccc aggactcctc cctgcaagac
 361 ggcgagttca tctacaaggt gaagctgcgc ggcaccaact tccccccga cggcccccgt
 421 atgcagaaga agactatggg ctgggaggcc tcctccgagc ggatgtaccc cgaggacggc
 481 ggcgtgaagg gcgagatcaa gcagaggctg aagctgaagg acggcggcca ctacgacgct
 541 gaggtcaaga ccacctacaa ggccaagaag cccgtgcaac tgcccggcgc gtacaacgtc
 601 aacatcaagt tggacatcac ctcccacaac gaggactaca ccatcgtgga acagtacgaa
 661 cgcggcggagg gcccgcactc caccggcggc attggacgagc tgtataagac tagcgcagcg
 721 aacgacgaaa attacgccc tgcagcgtga
  //
```

LOCUS luxI-LAA 618 bp DNA LINEAR SYN
 DEFINITION Gene (Quorum Sensing)
 ACCESSION pKLi047
 FEATURES Location/Qualifiers
 primer_bind 1..20
 /note="Fw. Primer Homology"
 CDS 1..618
 /note="LuxI-LAA"
 misc_feature 577..618
 /note="LAA tag"
 primer_bind complement (599..618)

```

                                /note="Rev. Primer Homology"
BASE COUNT      221 a      84 c     119 g     194 t
ORIGIN
  1 atgactataa tgataaaaaaa atcggatttt ttggcaattc catcgagga gtataaagggt
  61 attctaagtc ttcttgcattca agtgtttaag caaagacttg agtggactt agttgttagaa
 121 aataaccttgc aatcagatga gatatgataac tcaaattgcag aatataatttgc tgcttgcgt
181 gatactgaaa atgtaagtgg atgctggcgt ttattaccta caacagggtga ttatatgcgt
241 aaaagtgttt ttccctgaattt gcttggtcaa cagagtgcgc ccaaagatcc taatatagtc
301 gaattaagtc gttttgcgt agttaaaaat agctcaaaaga taaataactc tgcttagtga
361 attacaatga aactatttgc agcttatataa aacacgcgtc ttatgtcaagg tattacagaa
421 tatgtaacag taacatcaac agcaatagag cgatttttaa agcgtattaa agtccttgcgt
481 catcgatattt gagacaaga aattcatgtt ttaggtgata ctaaatcggt tgtattgtct
541 atgccttattt atgaacagtt taaaaaaagca gtcttaatgc ctgcaaacga cgaaaactac
601 gctttagtag cttataaa

//


LOCUS      Plux-cI      97 bp      DNA      LINEAR      SYN
DEFINITION Hybrid Promoter
ACCESSION  pKLi048
FEATURES
  primer_bind          Location/Qualifiers
    1..20
    /note="Fw. Primer Homology"
  promoter              1..97
    /note="Plux/cI"
  protein_bind          6..25
    /note="LuxR_AHL binding site"
    /bound_moiety="LuxR"
  protein_bind          57..74
    /note="cI binding site 1"
    /bound_moiety="cI"
  protein_bind          81..97
    /note="cI binding site 2"
    /bound_moiety="cI"
  primer_bind           complement (78..97)
    /note="Rev. Primer Homology"
BASE COUNT      26 a      17 c     24 g     30 t
ORIGIN
  1 taagcacctg taggatcgta caggtttacg caagaaaatg gtttgttata gtcgaataac
  61 accgtgcgtg ttgactattt tacctctggc ggtgata

//


LOCUS      luxR 756 bp      DNA      LINEAR      SYN
DEFINITION Gene (Activator)
ACCESSION  pKLi049
FEATURES
  CDS                  Location/Qualifiers
    1..756
    /note="LuxR"
  primer_bind           1..20
    /note="Fw. Primer Homology"
  misc_feature          54..54
    /note="T->G HindIII"
  misc_feature          120..120
    /note="T->C SspI"
  misc_feature          204..204
    /note="A->G SspI"

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    misc_feature      582..582
                      /note="A->G SphI"
    misc_feature      618..618
                      /note="A->C SspI"
    misc_feature      729..729
                      /note="T->C MfeI"
    primer_bind      complement (737..756)
                      /note="Rev. Primer Homology"
BASE COUNT      295 a      121 c      111 g      229 t
ORIGIN
   1 atgaaaaaca taaatgccga cgacacatac agaataatta ataaaattaa agcgtgtaga
   61 agcaataatg atattaatca atgccttatct gatatgacta aaatggtaca ttgtgaatac
  121 tatttactcg cgatcattta tcctcattct atggtaaat ctgatatttc aatccttagat
  181 aattacccta aaaaatggag gcagtattat gatgacgcta atttaataaa atatgatcct
  241 atatggattt attctaactc caatcattca ccaattaatt ggaatatatt tgaaaacaat
  301 gctgtaaata aaaaatctcc aaatgttaatt aaagaagcga aaacatcagg tcttatact
  361 gggtttagtt tccctattca tacggctaac aatggcttcg gaatgcttag ttttgacat
  421 tcagaaaaag acaactataat agatagttt tttttacatg cgtgtatgaa cataccatta
  481 attttccctt ctctagttga taattatcga aaaataaaata tagcaaataa taaatcaaac
  541 aacgatttaa caaaaagaga aaaagaatgt ttagcgtggg cgtgcgaagg aaaaagctct
  601 tgggatattt caaaaatctt aggttgcagt gagcgtactg tcactttcca tttaaccaat
  661 gcgcaaatga aactcaatac aacaaaccgc tgccaaagta tttctaaagc aattttaaaca
  721 ggagcaatcg attgcccata ctttaaaaat taataa
//


LOCUS      araC_JK-LAA 879 bp      DNA      LINEAR      SYN      02-JUN-2011
DEFINITION Gene (Activator)
ACCESSION  pKLi046
FEATURES          Location/Qualifiers
  CDS            1..879
                  /note="AraC-LAA_Keasling "
  primer_bind    1..20
                  /note="Fw. Primer Homology"
  misc_feature   16..18
                  /note="N6I JK"
  misc_feature   193..195
                  /note="V65G JK"
  misc_feature   339..339
                  /note="A->C SspI"
  misc_feature   475..475
                  /note="T->C MfeI"
  misc_feature   493..495
                  /note="E165G JK"
  misc_feature   505..507
                  /note="E169V JK"
  misc_feature   588..588
                  /note="T->C EcoRV"
  misc_feature   670..670
                  /note="T->C AflII"
  misc_feature   672..672
                  /note="A->G AflII"
  misc_feature   877..879
                  /note="C280* JK"
  primer_bind    complement (860..879)
                  /note="Rev. Primer Homology"

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BASE COUNT 186 a 231 c 246 g 216 t
 ORIGIN

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  1 atggctgaag cgcaaatcg a tcccctgctg ccgggataact cgtttaacgc ccatctggtg
  61 gcgggttaa cgcgcattga ggcacaacgg tatctcgatt ttttatcga cgcaccgctg
  121 ggaatgaaag gttatattct caatctcacc attcgcggtc aggggggttgt gaaaaatcag
  181 ggacgagaat ttgggtgccg accgggtgat atttgctgt tccgcagg agagattcat
  241 cactacggtc gtcatccgga ggctcgcaa tggatcacc agtgggtta ctgcgtccg
  301 cgcgcctact ggcatgaatg gcttaactgg ccgtcaatct ttgccaatac gggtttctt
  361 cgcgcggatg aagcgcacca gccgcatttc akgacactgt ttggcggaaat cattaacgcc
  421 gggcaagggg aaggcgcta ttgggagctg ctggcgataa atctgcttga gcaactgtta
  481 ctgcggcgca tgggtgcgat taacgtgtcg ctccatccac cgatggataa tcgggtacgc
  541 gaggcttgc agtacatcgat cgatcacctg gcagacagca attttgacat cgccagcgctc
  601 gcacagcatg ttgcgttgc gccgtcgct ctgtcacatc ttttccgcca gcagtttaggg
  661 attagcgtcc tgagctggcg cgaggacaa cgcattagtc aggcaagct gctttgagc
  721 actacccgga tgcctatcgcc accgtcggt cgcaatgtt gttttgacga tcaactctat
  781 ttctcgcgag tatttaaaaaa atgcacccggg gccagccccga gcgagttcg tgccggtaact
  841 agcgcagcga acgacgaaaaa ttacccctt gcagcgtga
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LOCUS Plac/ara 483 bp DNA LINEAR SYN
 DEFINITION Hybrid Promoter
 ACCESSION pKLi053
 FEATURES Location/Qualifiers

protein_bind	1..28
	/note="Olac1"
primer_bind	1..48
	/note="Fw. Primer Homology"
promoter	1..483
	/note="Plac/ara "
misc_feature	376..376
	/note="C->T SalI, XhoI"
protein_bind	382..401
	/note="araII"
misc_feature	414..414
	/note="C->A BamHI"
misc_feature	418..418
	/note="G->T HindIII"
protein_bind	425..444
	/note="O's"
misc_feature	429..429
	/note="G->T MfeI"
protein_bind	456..483
	/note="Olac1"
misc_feature	457..457
	/note="A->G MfeI"
primer_bind	complement (436..483)
	/note="Rev. Primer Homology"

BASE COUNT 162 a 104 c 111 g 106 t
 ORIGIN

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  1 aatttgtgagc ggataacaat ttcacacagg gccctcgac accgaggaga atgtcaagag
  61 gcgaacacac aacgtttgg agcgccagag gaggaacgag ctaaaacgga gctttttgc
  121 cctgcgtgac cagatccgg agttggaaaa caatggaaaag gcccccaagg tagttatcct
  181 taaaaaagcc acagcataca tcctgtccgt ccaaggcagag gagcaaaagc tcatttctga
  241 agaggacttg ttgcggaaac gacgagaaca gttgaaacac aaacttgaac agtacggaa
  301 ctcttgtgca taaggaaaag taaggaaaac gattccttct aacagaaaatg tcctgagtca
  
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361 cctatgaact gtcgattcga gcatagcatt tttatccata agattagcgg atcataatct
 421 ttacaatttt gagcgctcac aattatgata gattcagttg tgagcggata acaatttcac
 481 aca
//
LOCUS PmgrB 543 bp DNA LINEAR SYN
DEFINITION Promoter
ACCESSION pKLi054
FEATURES Location/Qualifiers
promoter 1..543
/note="PmgrB"
primer_bind 1..20
/note="Fw. Primer Homology"
-35_signal 480..485
/note="-35 box"
misc_feature 500..500
/note="G->C SphI"
-10_signal 509..514
/note="-10 box"
misc_feature 518..518
/note="TSS"
primer_bind complement (524..543)
/note="Rev. Primer Homology"
BASE COUNT 162 a 112 c 112 g 157 t
ORIGIN
1 gatgagagta agaacctgtc ggaatatcaa acagacaggt tctttatcta gcatgagaaa
61 aataaaagtgtt aagggtggcgt tatattaaac gcgcgttgcata taagagtatt ttactcagga
121 gtgagaatct gggttatattat tgcccttaac cattatcgac cacgatattg cttttgcgt
181 acagcgggca atctgttattc cccaaaaaaac cacttttagt gtgcaagtat tgtaccgtgc
241 tgggtgcctct ggcagtcaga taggtacatt gcaaaccctaa tcctgcggca ttctctttgc
301 ttccaatcaa aacgccatat ccgctgagta ataatcctat ccataaccagt gctatcagca
361 taactgtgcg aatgtatgaat cgcatcataa cctcttctct ttttatgttc gcttaatcgt
421 agcggcaata tgcgcgtgaag caagcgactc attccaaaaa agcacgaata tcgacatagt
481 taggcgctgt ttaactaacc catgctagtt taatgacata aggttaggtga aacggagatt
541 gga
//
LOCUS Plux 219 bp DNA LINEAR SYN
DEFINITION Promoter
ACCESSION pKLi055
FEATURES Location/Qualifiers
primer_bind 1..20
/note="Fw. Primer Homology"
promoter 1..219
/note="Plux"
primer_bind complement (524..543)
/note="Rev. Primer Homology"
BASE COUNT 64 a 39 c 40 g 76 t
ORIGIN
1 acccatctct ttatccttac ctattgtttg tcgcaagttt tgcgtttat atatcattaa
61 aacggtaatg gattgacatt tgattctaat aaattggatt tttgtcacac tattgtatcg
121 ctggaaatac aattacttaa cataagcacc tggatcg tacaggtta cgcaagaaaa
181 tggtttgtta tagtcgata tcagcaggac gcactgacc
//

LOCUS mCherry-LAA-LCOpt 750 bp DNA LINEAR SYN
 DEFINITION Gene (Fluorescent Reporter)
 ACCESSION pKLi056
 FEATURES Location/Qualifiers
 CDS 1..750 /note="mCherry-LAA-LCOpt"
 primer_bind 1..20 /note="Fw. Primer Homology"
 misc_feature 18..18 /note="G->A LC"
 misc_feature 21..21 /note="G->A LC"
 misc_feature 63..63 /note="G->T ApaLI"
 misc_feature 357..357 /note="G->A PstI"
 misc_feature 435..435 /note="C->T NcoI"
 misc_feature 483..483 /note="C->G KasI"
 misc_feature 579..579 /note="G->A PvuII"
 misc_feature 591..591 /note="C->G KasI"
 misc_feature 705..705 /note="C->T BsrGI"
 misc_feature 709..750 /note="LAA tag"
 primer_bind complement (731..750) /note="Rev. Primer Homology"
 BASE COUNT 180 a 233 c 228 g 109 t
 ORIGIN

 1 atggtgagca agggcgaaga agataacatg gccatcatca aggagttcat gcgcattcaag
 61 gttcacatgg agggctccgt gaacgccac gagttcgaga tcgagggcga gggcgagggc
 121 cgcgcctacg agggcaccca gaccgccaag ctgaagggtga ccaagggtgg cccctgccc
 181 ttgcgcctggg acatccgttc ccctcagtgc atgtacggct ccaaggccta cgtgaagcac
 241 cccggcaca tccccgacta cttgaagctg tccttccccg agggcttcaa gtgggagcgc
 301 gtgtatgaact tcgaggacgg cggcgtggtg accgtgaccc aggactcctc cctgcaagac
 361 ggcgagttca tctacaaggt gaagctgcgc ggcaccaact tccctccga cggcccccgt
 421 atgcagaaga agactatggg ctgggaggcc tcctccgagc ggatgtaccc cgaggacggc
 481 gcgcgtgaagg gcgagatcaa gcagaggctg aagctgaagg acggcggcca ctacgacgct
 541 gaggtcaaga ccacctacaa ggccaagaag cccgtgcaac tgcccggcgc gtacaacgtc
 601 aacatcaagt tggacatcac ctcccacaac gaggactaca ccatcgtgga acagtacgaa
 661 cgcggcgagg gcccgcactc caccggcggc atggacgagc tgtataagac tagcgcagcg
 721 aacgacgaaa attacgcctc tgcaaggta

//

LOCUS 50bp Spacer 50 bp DNA LINEAR SYN
 DEFINITION Spacer
 ACCESSION pKLi057
 FEATURES Location/Qualifiers
 misc_feature 1..50 /note="50bp Spacer"
 primer_bind 1..20 /note="Fw. Primer Homology"

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primer_bind      complement (31..50)
                  /note="Rev. Primer Homology"
BASE COUNT       22 a      7 c      10 g      11 t
ORIGIN
1 agcttgtatt gtgagaataa tgaaagcgaa aagaaatctg ccacatacaa
//  

LOCUS          100bp Spacer 100 bp    DNA   LINEAR   SYN
DEFINITION     Spacer
ACCESSION      pKLi058
FEATURES        Location/Qualifiers
  misc_feature  1..100
                 /note="100bp Spacer"
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  primer_bind   complement (81..100)
                 /note="Rev. Primer Homology"
BASE COUNT      40 a      19 c      20 g      21 t
ORIGIN
1 agcttgtatt gtgagaataa tgaaagcgaa aagaaatctg ccacatacaa cattggtaa
61 cgaatgcatt gcgcaatcac accaaagggtt caatgcaaag
//  

LOCUS          250bp Spacer 250 bp    DNA   LINEAR   SYN
DEFINITION     Spacer
ACCESSION      pKLi059
FEATURES        Location/Qualifiers
  misc_feature  1..250
                 /note="250bp Spacer "
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  primer_bind   complement (231..250)
                 /note="Rev. Primer Homology"
BASE COUNT      89 a      46 c      55 g      60 t
ORIGIN
1 agcttgtatt gtgagaataa tgaaagcgaa aagaaatctg ccacatacaa cattggtaa
61 cgaatgcatt gcgcaatcac accaaagggtt caatgcaaag gtttctatgg tcaagagagc
121 catcgatagc ttaatacAAA agggataacct acagagggga gacgatggtg aatcgatgc
181 ttacccgtt taatcatctt tgaaggctt tgctgtatcgac acgaagcaaa tcctacgagt
241 aaatacataaa
//  

LOCUS          500bp Spacer 500 bp    DNA   LINEAR   SYN
DEFINITION     Spacer
ACCESSION      pKLi060
FEATURES        Location/Qualifiers
  misc_feature  1..500
                 /note="500bp Spacer"
  primer_bind   1..20
                 /note="Fw. Primer Homology"
  primer_bind   complement (481..500)
                 /note="Rev. Primer Homology"
BASE COUNT      163 a      82 c      109 g      146 t
ORIGIN
1 agcttgtatt gtgagaataa tgaaagcgaa aagaaatctg ccacatacaa cattggtaa

```

61 cgaatgcatt gcgcaatcac accaaagggtt caatgcaaag gtttctatgg tcaagagagc
121 catcgatagc ttaatacataaa agggataacct acagagggga gacgatgggtg aatcgatgc
181 ttacccttgct taatcatctt tgaaggcttg tgctgatcga acgaagcaaa tcctacgagt
241 aaatacataaa gcgtatacat atatatatat atatatatat atatatatat atgtatatat
301 atatatgtgt gtgtgtgtaa ttgtgtgtat tcaactgaac tatgaagagt cttgaccc
361 ttgagaatct catagtatga agatatggca ctctctttc cggtgtaaca tccttaccg
421 ggcggctttt cggcctgctt gagaagagat caggctgaaa tgatgagact gacaacaagg
481 atcagtcagt ggcagagttg

//

DNA sequence and annotation of circuit constructs

LOCUS pKDL071 5797 bp DNA CIRCULAR SYN
DEFINITION Lac/Tet pTrc2 Monocistronic Toggle
ACCESSION pKDL071
FEATURES Location/Qualifiers
misc_feature complement (4849..1)
/note="KanR"
CDS complement (55..771)
/note="GFPmut3b"
misc_feature complement (772..822)
/note="rbsKDL025"
misc_feature complement (823..896)
/note="PLtetO"
CDS complement (939..2021)
/note="lacI"
misc_feature complement (2022..2051)
/note="rbsKDL026"
misc_feature complement (2058..2131)
/note="PLtetO"
misc_feature 2150..2224
/note="P_{trc}-2"
misc_feature 2231..2262
/note="rbsKDL028-SDM"
CDS 2263..2886
/note="tetR"
misc_feature 2929..3003
/note="P_{trc}-2"
misc_feature 3010..3045
/note="rbsKDL027"
CDS 3046..3756
/note="mCherry"
misc_feature 3811..3915
/note="T1"
/product="transcriptional terminator from rrnB operon"
misc_feature 3922..4729
/note="ColE1"
/product="High Copy Origin of Replication"
misc_feature 4736..4841
/note="T0"
/product="transcriptional terminator from phage lambda"
CDS complement (4867..5661)
/note="KanR"
BASE COUNT 1415 a 1530 c 1491 g 1361 t
ORIGIN
1 gacgtctgtg caagtactac tggctcgag tcacttgaat tcgataccca gctgttattt
61 gtatagttca tccatgccat gtgtaatccc agcagcgggtt acaaactcaa gaaggaccat
121 gtggctctc tttcggtgg gatcttcga aagggcagat tgggtggaca ggtaatgggtt
181 gtctggtaaa aggacagggc catcgccaaat cgaggtatTT tggtgataat ggctgcttag
241 ttgaacgctt ccatcttcaa tgggtgtct aatTTGAAG ttaacttga ttccattctt
301 ttgttgtct gccatgatgt atacattgtg ttagttatag ttgttattcca atttgtgtcc
361 aagaatgttt ccatcttcaa taaaatcaat accttttaac tcgatttat taacaagggt
421 atcaccttca aacttgactt cagcacgtgt ctgttagttc ccgtcatctt tgaaaaatat

481 agttcttcc tgtacgtaac cttcggcat ggcactttg aaaaagtcat gctgtttcat
541 gtgatctggg tatctcgaa acgattgaac accataaccg aaagtagtga caagtgtgg
601 ccacggaaca ggtagtttc cagtagtgca aataaaattta agggtaagtt ttccgtatgt
661 tgcacaccc tcaccccttc cactgacaga aaatttgc ccatatacat caccatctaa
721 ttcaacaaga attgggacaa ctccagtcaa aagttcttct cctttacgca tggactac
781 ctccttgggt gtctatgcta tgctgatcta caactggcat gcggtagtg cgccctgctg
841 atgtgctcag tatctctatc actgataggg atgtcaatct ctatcactga tagggaaacg
901 tttcgagaa gcttccgaa ggtaccatt tgccgcggc actgcccct ttccagtcgg
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1021 gtattgggag ccagggtggg ttttctttc accagtgaga cggcaacag ttgattgccc
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1861 tgtgccacgc gtttggaaat gtaattcagc tccgcacatcg ccgttccac ttttccgc
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1981 ccggcataact ctgcgcacatcg gtacagcgatc actgggttca ttgtacaccc tctccttata
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2161 tggatcaat taatcatccg gtcgtataa tggatggaaat tggatggcga taacaatttc
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2581 gaaaaacagt atgaaactct cggaaatcaa ttgcgtttt tgcgtatggc aagttttca
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2701 gaagatcaag agcatcaagt cgctaaagaa gaaaggaaa cacctactac tgatgtatgt
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3001 caccattgtt gatattacta tcgttcaact gatagggggg cggccgtatgtt gggcaaggggc
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3421 aagggttgcgac tcgcgcggc ccaacttccccc tccgacggcc cggtaatgca gaagaagact
3481 atgggctggg aggcccttc cggccgtatgtt tttttttttt acggccgcgtt gaaaggccgg
3541 atcaaggcaga ggctgaagctt gggacggc gggccactacg acgctggatgtt caagaccacc
3601 tacaaggcca agaaggccgtt gcaactgcggc ggcgcgtaca acgtcaacat caagttggac
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3721 cactccaccgc gggcatggc cgagctgtat aagtaagata tctatgcggc tagggaccgt

3781 ctcgagagaa tcaatattaa tccaaacgcgt ggcataaat aaaacgaaag gctcagtcga
3841 aagactggc ctttcgaaaa atctgttggtt tgctcggtgaa cgctctcctg agtaggacaa
3901 atccgcccgcctt ctagacttag gcgttcggct gccccgagcg gtatcagctc actcaaaggc
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4801 gttctgaggt cattactggta tctatcaaca ggagtccaag ccaattctcg aaccccgag
4861 tcccgctcag aagaactcgt caagaaggcg atagaaggcg atgcgcgtcg aatcgggagc
4921 ggcgataccg taaaggcacga ggaagggtc agccattcg ccgccaagct cttcagcaat
4981 atcacggta gccaacgccta tgcgtcgata ggggtccggc acacccagcc ggccacagtc
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5161 tggcgcgagc ccctgatgct cttcgatcgatccatccatcgatccatcgatccatcgatccat
5221 ccgagtagt gtcgctcgatccatcgatccatcgatccatcgatccatcgatccatcgatccat
5281 atcaagcgta tgcagccggc gcattgcattc agccatgatgatactttct cggcaggagc
5341 aaggtagat gacaggagat cctgccccgg cacttcgcccc aatagcagcc agtcccttcc
5401 cgcttcagtg acaacgtcgatccatcgatccatcgatccatcgatccatcgatccatcgatccat
5461 tagccgcgct gcctcgatccatcgatccatcgatccatcgatccatcgatccatcgatccat
5521 aagaaccggg cgaccctcgatccatcgatccatcgatccatcgatccatcgatccatcgatccat
5581 ctgttgtgcc cagtcatagc cgaatagcc ctccacccaa gccgcggag aacctgcgt
5641 caatccatct tggatcatcgatccatcgatccatcgatccatcgatccatcgatccatcgatccat
5701 tccctcgcc catcgatccatcgatccatcgatccatcgatccatcgatccatcgatccatcgatccat
5761 cccaacctta ccagagggcg gcccacttgcgatccatcgatccatcgatccatcgatccatcgatccat

//

LOCUS pKDL106 7687 bp DNA CIRCULAR SYN
DEFINITION 3FFL
ACCESSION pKDL106
FEATURES Location/Qualifiers
misc_feature complement (6799..1)
/note="KanR"
CDS complement (55..771)
/note="GFPmut3b"
misc_feature complement (772..822)
/note="rbsKDL025"
misc_feature complement (823..896)
/note="PLtetO"
CDS complement (939..2021)
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