Supplementary Information

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

Kevin D. Litcofsky[1,*], Raffi B. Afeyan[1,*], Russell J. Krom[1],
Ahmad S. Khalil[1,§] and James J. Collins[1,2,§]

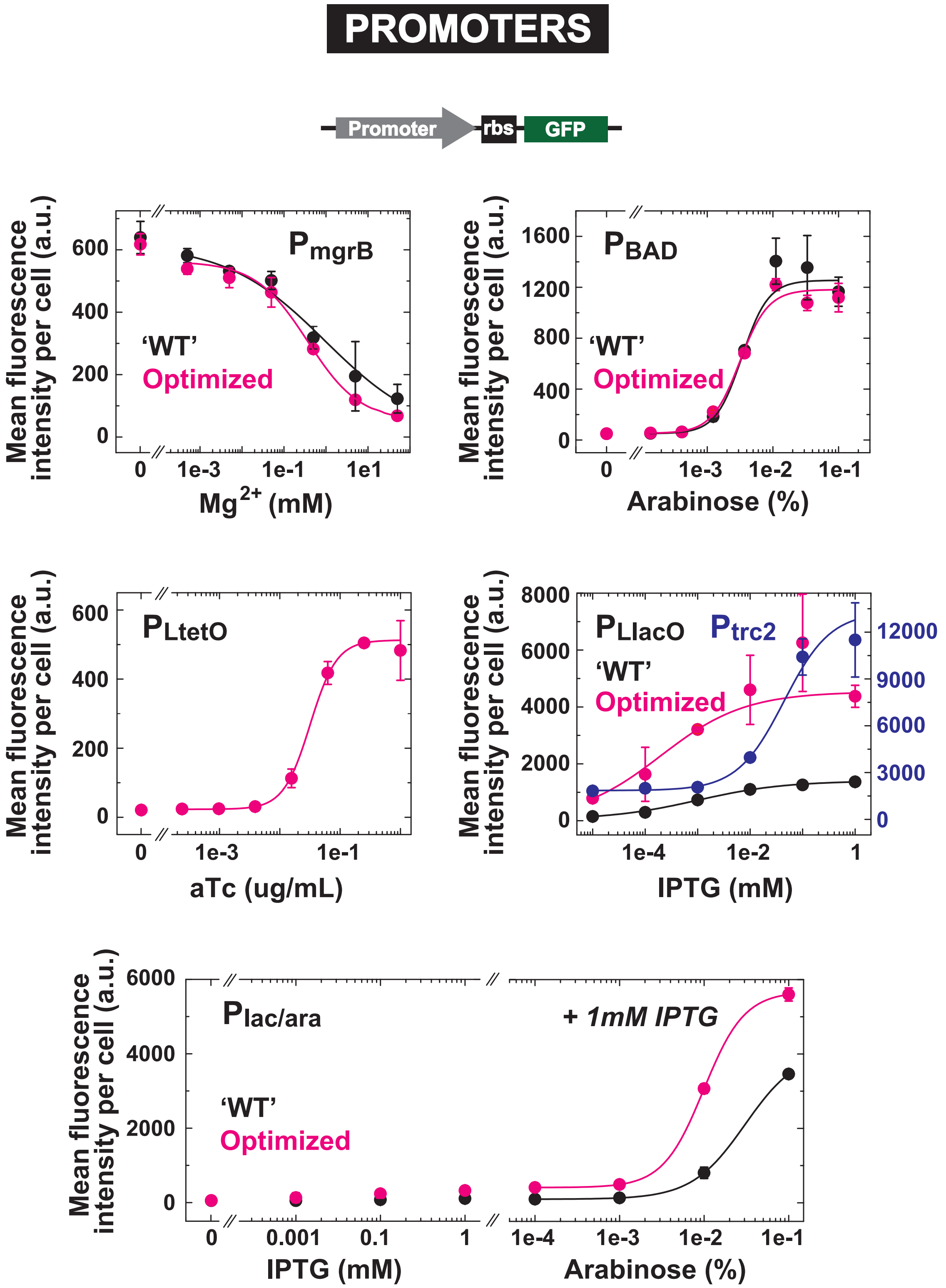
[1]*Howard Hughes Medical Institute, Department of Biomedical Engineering, and Center for BioDynamics, Boston University, Boston, MA 02215, USA.*

[2]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA.*

[*]*These authors contributed equally to this work.*
[§]*Co-senior authors.*
Correspondence should be addressed to J.J. Collins (jcollins@bu.edu)

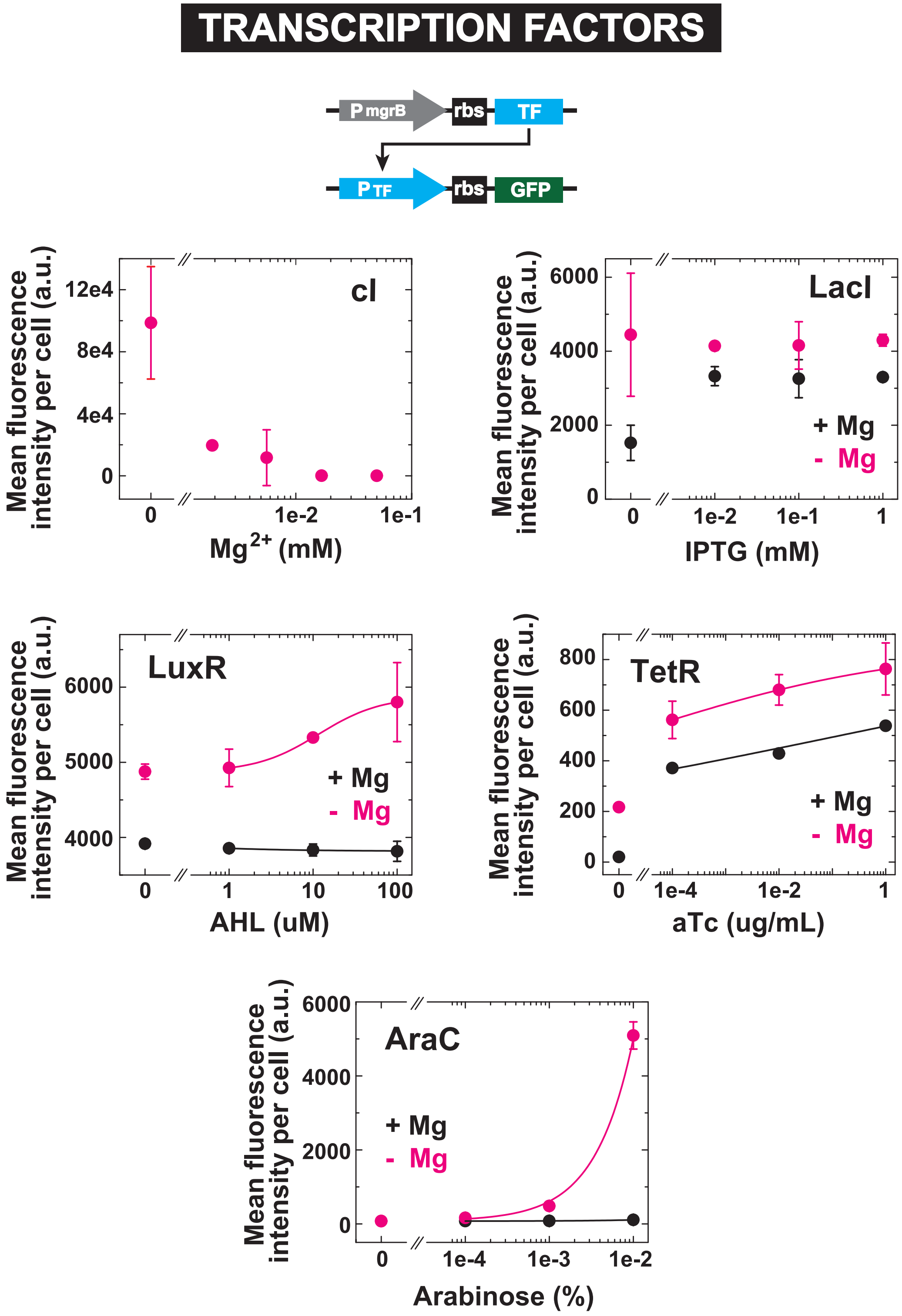
Supplementary Figure 1	Input/output functions for optimized promoters
Supplementary Figure 2	Input/output functions for optimized transcription factors
Supplementary Figure 3	Input/output functions for optimized terminator and reporters
Supplementary Figure 4	aTc-induced switching and subsequent maintenance 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.



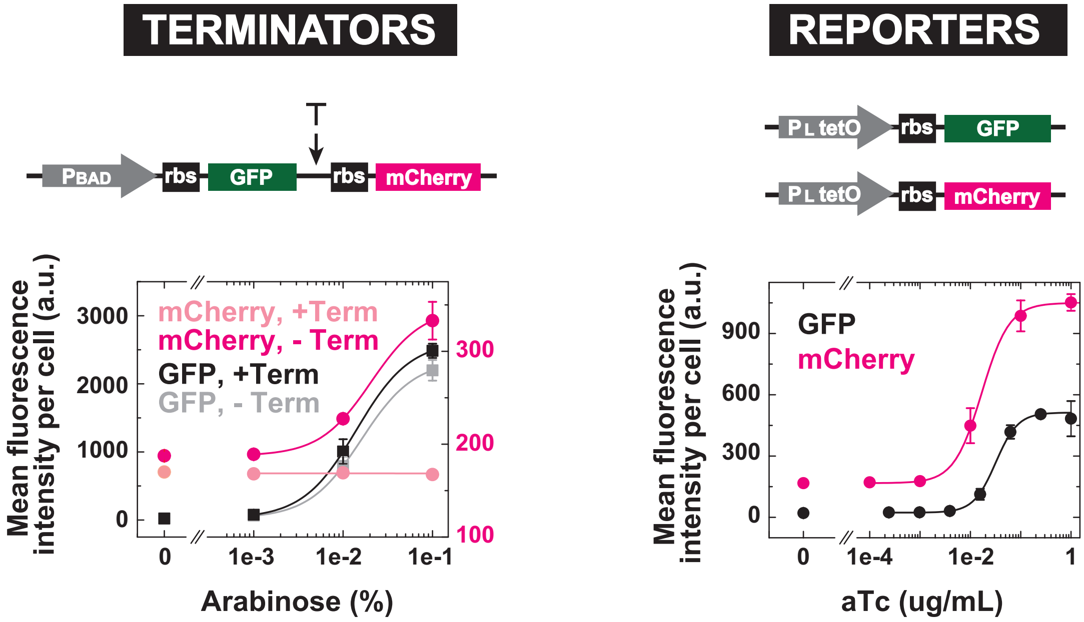
Cells were grown overnight in LB medium, then diluted 1:1000 into LB and induced for 5 hours prior to measurement. Fluorescence was measured via flow cytometry by collecting 10,000 events per replicate. P_{mgrB} was induced with 0–50 mM $MgCl_2$. P_{BAD} was induced with 0–0.01 % w/v arabinose. P_{LtetO} 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.



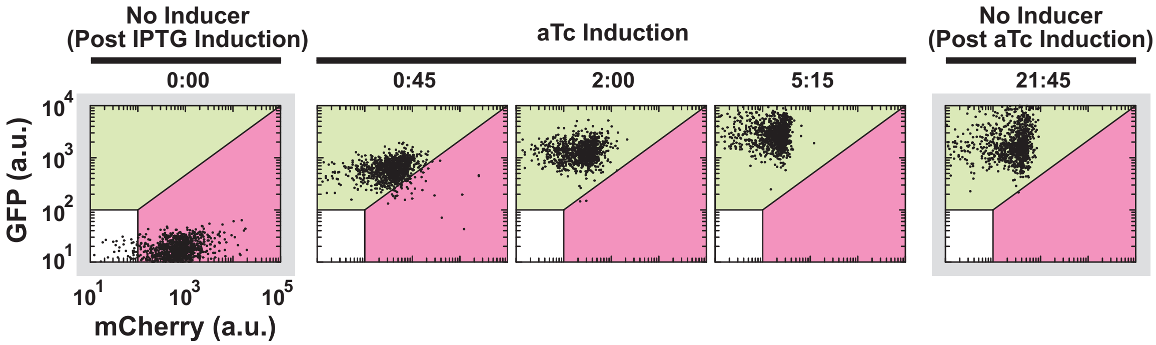
Cells were grown overnight in LB medium, then diluted 1:1000 into LB and induced for 8 hours prior to measurement. Fluorescence was measured via flow cytometry by collecting 10,000 events per replicate. All experiments were conducted without TF (black, 50 mM $MgCl_2$) or with TF (red, 0 mM $MgCl_2$), and dose responses were performed with the TF-specific inducer chemicals, except for cI (0–50 mM $MgCl_2$). 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 µg/ml aTc). Points represent mean values for three experiments ± 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
AclI	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
ScaI-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

```
LOCUS       pKE1-MCS     2376 bp    DNA   CIRCULAR SYN
DEFINITION  Carbenicillin-Resistant, ColE1 ori, Optimized Vector
ACCESSION   pKE1-MCS
FEATURES             Location/Qualifiers
     misc_feature    1..330
                     /note="Multiple Cloning Site"
     terminator      331..435
                     /note="T1"
                     /product="transcriptional terminator from rrnB operon"
     misc_feature    437..437
                     /note="C->T AvrII"
     rep_origin      442..1249
                     /note="ColE1"
                     /product="High Copy Origin of Replication"
     misc_feature    839..839
                     /note="G->C ApaLI"
     misc_feature    1250..1250
                     /note="A->G SpeI"
     terminator      1256..1361
                     /note="T0"
                     /product="transcriptional terminator from phage lambda"
     terminator      complement (1256..1361)
                     /note="T0"
     misc_feature    complement (1367..2376)
                     /note="AmpR"
                     /phenotype="Ampicillin Resistance"
     CDS             complement (1386..2246)
                     /note="Beta-lactamase"
     misc_feature    2273
                     /note="Transcription Start"
     -10_signal      complement (2290..2295)
                     /note="-10 Box"
     -35_signal      complement (2311..2316)
                     /note="-35 Box"
BASE COUNT      587 a    614 c    586 g    589 t
ORIGIN
        1 gacgtctgtg caagtactac tgttctgcag tcacttgaat tcgataccca gctgggtgga
       61 gtgcaccaag gagcatgcga aggaaacgtt tcgcagaagc ttccgcaagg taccactttg
      121 ccgcggagta tttgtacatt tgaaggatcc tcaagtcggc cgcccgttcc atggatactc
      181 gtcgaccatt acgctagccg tctggagctc ggactgctta agtcgctcca tatgctcgtt
      241 cccgggacta cacaattgtc ccccggcgcc agggttgata tctatcgccc tagggaccgt
      301 ctcgagagaa tcaatattaa tccaacgcgt ggcatcaaat aaaacgaaag gctcagtcga
      361 aagactgggc ctttcgtttt atctgttgtt tgtcggtgaa cgctctcctg agtaggacaa
      421 atccgccgcc ctagacttag gcgttcggct gcggcgagcg gtatcagctc actcaaaggc
      481 ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg
      541 ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg
      601 cccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg
      661 actataaaga taccaggcgt ttccccctgg aagctccctc gtgcgctctc ctgttccgac
      721 cctgccgctt accggatacc tgtccgcctt tctcccttcg ggaagcgtgg cgctttctca
      781 tagctcacgc tgtaggtatc tcagttcggt gtaggtcgtt cgctccaagc tgggctgtct
```

```
      841 gcacgaaccc cccgttcagc ccgaccgctg cgccttatcc ggtaactatc gtcttgagtc
      901 caacccggta agacacgact tatcgccact ggcagcagcc actggtaaca ggattagcag
      961 agcgaggtat gtaggcggtg ctacagagtt cttgaagtgg tggcctaact acggctacac
     1021 tagaaggaca gtatttggta tctgcgctct gctgaagcca gttaccttcg gaaaaagagt
     1081 tggtagctct tgatccggca aacaaaccac cgctggtagc ggtggttttt ttgtttgcaa
     1141 gcagcagatt acgcgcagaa aaaaaggatc tcaagaagat cctttgatct tttctacggg
     1201 gtctgacgct cagtggaacg aaaactcacg ttaagggatt ttggtcatgg ctagtgcttg
     1261 gattctcacc aataaaaaac gcccggcggc aaccgagcgt tctgaacaaa tccagatgga
     1321 gttctgaggt cattactgga tctatcaaca ggagtccaag ccaattcgta aacttggtct
     1381 gacagttacc aatgcttaat cagtgaggca cctatctcag cgatctgtct atttcgttca
     1441 tccatagttg cctgactccc cgtcgtgtag ataactacga tacgggaggg cttaccatct
     1501 ggccccagtg ctgcaatgat accgcgagac ccacgctcac cggctccaga tttatcagca
     1561 ataaaccagc cagccggaag ggccgagcgc agaagtggtc ctgcaacttt atccgcctcc
     1621 atccagtcta ttaattgttg ccgggaagct agagtaagta gttcgccagt taatagtttg
     1681 cgcagcgttg ttgccattgc tacaggcatc gtggtgtcac gctcgtcgtt tggtatggct
     1741 tcattcagct ccggttccca acgatcaagg cgagttacat gatcccccat gttgtgcaaa
     1801 aaagcggtta gctccttcgg tcctccgatc gttgtcagaa gtaagttggc cgcagtgtta
     1861 tcactcatgg ttatggcagc actgcataat tctcttactg tcatgccatc cgtaagatgc
     1921 ttttctgtga ctggtgagta ttcaaccaag tcattctgag aatagtgtat gcggcgaccg
     1981 agttgctctt gcccggcgtc aatacgggat aataccgcgc cacatagcag aactttaaaa
     2041 gtgctcatca ttggaaagcg ttcttcgggg cgaaaactct caaggatctt accgctgttg
     2101 agatccagtt cgatgtaacc cactcgcgca cccaactgat cttcagcatc ttttactttc
     2161 accagcgttt ctgggtgagc aaaaacagga aggcaaaatg ccgcaaaaaa gggaataagg
     2221 gcgacacgga aatgttgaat actcatactc ttcctttttc agtattattg aagcatttat
     2281 cagggttatt gtctcatgag cggatacata tttgaatgta tttagaaaaa taaacaaata
     2341 ggggttccgc gcacatttcc ccgaaaagtg ccacct
//

LOCUS       pKE2_MCS     2317 bp    DNA   CIRCULAR SYN
DEFINITION  Kanamycin-Resistant, ColE1 ori, Optimized Vector
ACCESSION   pKE2_MCS
FEATURES             Location/Qualifiers
     misc_feature    1..330
                     /note="Multiple Cloning Site"
     terminator      331..435
                     /note="T1"
                     /product="transcriptional terminator from rrnB operon"
     misc_feature    complement (437..437)
                     /note="C->T AvrII"
     rep_origin      442..1249
                     /note="ColE1"
                     /product="High Copy Origin of Replication"
     misc_feature    complement (839..839)
                     /note="G->C ApaLI"
     misc_feature    complement (1250..1250)
                     /note="A->G SpeI"
     terminator      1256..1361
                     /note="T0"
                     /product="transcriptional terminator from phage lambda"
     CDS             complement (1387..2181)
                     /note="KanR"
     misc_feature    complement (1367..2317)
                     /note="KanR Region"

BASE COUNT      543 a    668 c    611 g    495 t
```

```
ORIGIN
        1 gacgtctgtg caagtactac tgttctgcag tcacttgaat tcgataccca gctgggtgga
       61 gtgcaccaag gagcatgcga aggaaacgtt tcgcagaagc ttccgcaagg taccactttg
      121 ccgcggagta tttgtacatt tgaaggatcc tcaagtcggc cgcccgttcc atggatactc
      181 gtcgaccatt acgctagccg tctggagctc ggactgctta agtcgctcca tatgctcgtt
      241 cccgggacta cacaattgtc ccccggcgcc agggttgata tctatcgccc tagggaccgt
      301 ctcgagagaa tcaatattaa tccaacgcgt ggcatcaaat aaaacgaaag gctcagtcga
      361 aagactgggc ctttcgtttt atctgttgtt tgtcggtgaa cgctctcctg agtaggacaa
      421 atccgccgcc ctagacttag gcgttcggct gcggcgagcg gtatcagctc actcaaaggc
      481 ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg
      541 ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg
      601 cccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg
      661 actataaaga taccaggcgt ttccccctgg aagctccctc gtgcgctctc ctgttccgac
      721 cctgccgctt accggatacc tgtccgcctt tctcccttcg ggaagcgtgg cgctttctca
      781 tagctcacgc tgtaggtatc tcagttcggt gtaggtcgtt cgctccaagc tgggctgtct
      841 gcacgaaccc cccgttcagc ccgaccgctg cgccttatcc ggtaactatc gtcttgagtc
      901 caacccggta agacacgact tatcgccact ggcagcagcc actggtaaca ggattagcag
      961 agcgaggtat gtaggcggtg ctacagagtt cttgaagtgg tggcctaact acggctacac
     1021 tagaaggaca gtatttggta tctgcgctct gctgaagcca gttaccttcg gaaaaagagt
     1081 tggtagctct tgatccggca aacaaaccac cgctggtagc ggtggttttt ttgtttgcaa
     1141 gcagcagatt acgcgcagaa aaaaaggatc tcaagaagat cctttgatct tttctacggg
     1201 gtctgacgct cagtggaacg aaaactcacg ttaagggatt ttggtcatgg ctagtgcttg
     1261 gattctcacc aataaaaaac gcccggcggc aaccgagcgt tctgaacaaa tccagatgga
     1321 gttctgaggt cattactgga tctatcaaca ggagtccaag ccaattctcg aaccccagag
     1381 tcccgctcag aagaactcgt caagaaggcg atagaaggcg atgcgctgcg aatcgggagc
     1441 ggcgataccg taaagcacga ggaagcggtc agcccattcg ccgccaagct cttcagcaat
     1501 atcacgggta gccaacgcta tgtcctgata gcggtccgcc acacccagcc ggccacagtc
     1561 gatgaatcca gaaaagcggc cattttccac catgatattc ggcaagcagg catcgccgtg
     1621 ggtcacgacg agatcctcgc cgtcgggcat acgcgccttg agcctggcga acagttcggc
     1681 tggcgcgagc ccctgatgct cttcgtccag atcatcctga tcgacaagac cggcttccat
     1741 ccgagtacgt gctcgctcga tgcgatgttt cgcttggtgg tcgaatgggc aggtagccgg
     1801 atcaagcgta tgcagccgcc gcattgcatc agccatgatg gatactttct cggcaggagc
     1861 aaggtgagat gacaggagat cctgccccgg cacttcgccc aatagcagcc agtcccttcc
     1921 cgcttcagtg acaacgtcga gcacagccgc gcaaggaacg cccgtcgtgg ccagccacga
     1981 tagccgcgct gcctcgtctt gcagttcatt cagggcaccg gacaggtcgg tcttgacaaa
     2041 aagaaccggg cgaccctgcg ctgacagccg gaacacggcg gcatcagagc agccgattgt
     2101 ctgttgtgcc cagtcatagc cgaatagcct ctccacccaa gccgccggag aacctgcgtg
     2161 caatccatct tgttcaatca tgcgaaacga tcctcatcct gtctcttgat cagatcttga
     2221 tcccctgcgc catcagatcc ttggcggcaa gaaagccatc cagtttactt tgcagggctt
     2281 cccaacctta ccagagggcg gcccaactgg caattcc
//

LOCUS       pKE3_MCS     2337 bp    DNA   CIRCULAR SYN
DEFINITION  Chloramphenicol-Resistant, ColE1 ori, Optimized Vector
ACCESSION   pKE3_MCS
FEATURES             Location/Qualifiers
     misc_feature    1..330
                     /note="Multiple Cloning Site"
     terminator      331..435
                     /note="T1"
                     /product="transcriptional terminator from rrnB operon"
     misc_feature    437..437
                     /note="C->T AvrII"
     rep_origin      442..1249
                     /note="ColE1"
```

```
                     /product="High Copy Origin of Replication"
     misc_feature    839..839
                     /note="G->C ApaLI"
     misc_feature    1250..1250
                     /note="A->G SpeI"
     terminator      1256..1361
                     /note="T0"
                     /product="transcriptional terminator from phage lambda"
     CDS             complement (1256..1361)
                     /note="T0"
     misc_feature    1420..1420
                     /note="C->T ScaI"
     misc_feature    1525..1525
                     /note="T->C SspI"
     misc_feature    1537..1537
                     /note="C->T NcoI"
     misc_feature    1750..1750
                     /note="G->C AclI"
     misc_feature    1834..1834
                     /note="C->T EcoRI"
     misc_feature    1939..1939
                     /note="G->A PvuII"
     CDS             complement (1393..2052)
                     /note="cat"
                     /product="Chloramphenicol Acetyltransferase"
     rbs             complement (2065..2071)
                     /note="Shine-Dalgarno region"
     -10_signal     complement (2093..2098)
                     /note="-10 box"
     -35_signal      complement (2117..2122)
                     /note="-35 box"
     promoter        complement (2076..2125)
                     /note="Promoter for cat"
BASE COUNT      599 a    582 c    566 g    590 t
ORIGIN
        1 gacgtctgtg caagtactac tgttctgcag tcacttgaat tcgataccca gctgggtgga
       61 gtgcaccaag gagcatgcga aggaaacgtt tcgcagaagc ttccgcaagg taccactttg
      121 ccgcggagta tttgtacatt tgaaggatcc tcaagtcggc cgcccgttcc atggatactc
      181 gtcgaccatt acgctagccg tctggagctc ggactgctta agtcgctcca tatgctcgtt
      241 cccgggacta cacaattgtc ccccggcgcc agggttgata tctatcgccc tagggaccgt
      301 ctcgagagaa tcaatattaa tccaacgcgt ggcatcaaat aaaacgaaag gctcagtcga
      361 aagactgggc ctttcgtttt atctgttgtt tgtcggtgaa cgctctcctg agtaggacaa
      421 atccgccgcc ctagacttag gcgttcggct gcggcgagcg gtatcagctc actcaaaggc
      481 ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg
      541 ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg
      601 cccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg
      661 actataaaga taccaggcgt ttccccctgg aagctccctc gtgcgctctc ctgttccgac
      721 cctgccgctt accggatacc tgtccgcctt tctcccttcg ggaagcgtgg cgctttctca
      781 tagctcacgc tgtaggtatc tcagttcggt gtaggtcgtt cgctccaagc tgggctgtct
      841 gcacgaaccc cccgttcagc ccgaccgctg cgccttatcc ggtaactatc gtcttgagtc
      901 caacccggta agacacgact tatcgccact ggcagcagcc actggtaaca ggattagcag
      961 agcgaggtat gtaggcggtg ctacagagtt cttgaagtgg tggcctaact acggctacac
     1021 tagaaggaca gtatttggta tctgcgctct gctgaagcca gttaccttcg gaaaaagagt
     1081 tggtagctct tgatccggca aacaaaccac cgctggtagc ggtggttttt ttgtttgcaa
     1141 gcagcagatt acgcgcagaa aaaaaggatc tcaagaagat cctttgatct tttctacggg
```

```
     1201 gtctgacgct cagtggaacg aaaactcacg ttaagggatt ttggtcatgg ctagtgcttg
     1261 gattctcacc aataaaaaac gcccggcggc aaccgagcgt tctgaacaaa tccagatgga
     1321 gttctgaggt cattactgga tctatcaaca ggagtccaag ccaattctgc accaataact
     1381 gccttaaaaa aattacgccc cgccctgcca ctcatcgcaa tactgttgta attcattaag
     1441 cattctgccg acatggaagc catcacagac ggcatgatga acctgaatcg ccagcggcat
     1501 cagcaccttg tcgccttgcg tatagtattt gcccatagtg aaaacggggg cgaagaagtt
     1561 gtccatattg gccacgttta aatcaaaact ggtgaaactc acccagggat tggctgagac
     1621 gaaaaacata ttctcaataa accctttagg gaaataggcc aggttttcac cgtaacacgc
     1681 cacatcttgc gaatatatgt gtagaaactg ccggaaatcg tcgtggtatt cactccagag
     1741 cgatgaaaag gtttcagttt gctcatggaa aacggtgtaa caagggtgaa cactatccca
     1801 tatcaccagc tcaccgtctt tcattgccat acgaaattcc ggatgagcat tcatcaggcg
     1861 ggcaagaatg tgaataaagg ccggataaaa cttgtgctta tttttcttta cggtctttaa
     1921 aaaggccgta atatccagtt gaacggtctg gttataggta cattgagcaa ctgactgaaa
     1981 tgcctcaaaa tgttctttac gatgccattg ggatatatca acggtggtat atccagtgat
     2041 ttttttctcc attttagctt ccttagctcc tgaaaatctc gataactcaa aaaatacgcc
     2101 cggtagtgat cttatttcat tatggtgaaa gttggaacct cttacgtgcc gatcaacgtc
     2161 tcattttcgc caaaagttgg cccagggctt cccggtatca acagggacac caggatttat
     2221 ttattctgcg aagtgatctt ccgtcacagg tatttattcg gcgcaaagtg cgtcgggtga
     2281 tgctgccaac ttactgattt agtgtatgat ggtgtttttg aggtgctcca gtggctt
//
```

```
LOCUS       GFPmut3b-LAA 756 bp    DNA   LINEAR   SYN

DEFINITION  Gene (Fluorescent Reporter)
ACCESSION   pKLi011
FEATURES             Location/Qualifiers
     CDS             1..756
                     /note="GFPmut3b-LAA "
     primer_bind     1..20
                     /note="Fw. Primer Homology"
     misc_feature    complement (99..118)
                     /note="spKDL022"
     misc_feature    168..168
                     /note="A->G NcoI"
     misc_feature    231..231
                     /note="T->C NdeI"
     misc_feature    276..276
                     /note="T->C BsrGI"
     misc_feature    561..561
                     /note="A->G MfeI"
     misc_feature    675..675
                     /note="A->C PvuII"
     misc_signal     715..756
                     /note="LAA tag"
     primer_bind     complement (737..756)
                     /note="Rev. Primer Homology"
BASE COUNT      254 a    152 c    156 g    194 t
ORIGIN
        1 atgcgtaaag gagaagaact tttcactgga gttgtcccaa ttcttgttga attagatggt
       61 gatgttaatg ggcacaaatt ttctgtcagt ggagagggtg aaggtgatgc aacatacgga
      121 aaacttaccc ttaaatttat ttgcactact ggaaaactac ctgttccgtg gccaacactt
      181 gtcactactt tcggttatgg tgttcaatgc tttgcgagat acccagatca catgaaacag
      241 catgactttt tcaagagtgc catgcccgaa ggttacgtac aggaaagaac tatatttttc
      301 aaagatgacg ggaactacaa gacacgtgct gaagtcaagt ttgaaggtga tacccttgtt
      361 aatagaatcg agttaaaagg tattgatttt aaagaagatg gaaacattct tggacacaaa
      421 ttggaataca actataactc acacaatgta tacatcatgg cagacaaaca aaagaatgga
      481 atcaaagtta acttcaaaat tagacacaac attgaagatg gaagcgttca actagcagac
      541 cattatcaac aaaatactcc gattggcgat ggccctgtcc ttttaccaga caaccattac
      601 ctgtccacac aatctgccct ttcgaaagat cccaacgaaa agagagacca catggtcctt
      661 cttgagtttg taaccgctgc tgggattaca catggcatgg atgaactata caaaactagc
      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
```

```
                     /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 gagtatgccg gtgtctctta tcagaccgtt
       61 tcccgcgtgg tgaaccaggc cagccacgtt tctgcgaaaa cgcgggaaaa agtggaagcg
      121 gcgatggcgg agctgaatta cattcccaac cgcgtggcac aacaactggc gggcaaacag
      181 tcgttgctga ttggcgttgc cacctccagt ctggccctgc acgcgccgtc gcaaattgtc
      241 gcggcgatta aatctcgcgc cgatcaactg ggtgccagcg tggtggtgtc gatggtagaa
      301 cgaagcggcg tcgaagcctg taaagcggcg gttcacaatc ttctcgcgca gcgcgtcagt
      361 gggctgatca ttaactatcc gctggatgac caggatgcca ttgctgtgga agctgcctgc
      421 actaatgttc cggcgttatt tcttgatgtc tctgaccaga cacccatcaa cagtattatt
      481 ttctcccatg aagacggtac gcgactgggc gtggagcatc tggtcgcatt gggtcaccag
      541 caaatcgcgc tgttagcggg cccattaagt tctgtctcgg cgcgtctgcg tctggctggc
      601 tggcataaat atctcactcg caatcaaatt cagccgatag cggaacggga aggcgactgg
      661 agtgccatgt ccggttttca acaaaccatg caaatgctga atgagggcat cgttcccact
      721 gcgatgctgg ttgccaacga tcagatggcg ctgggcgcaa tgcgcgccat taccgagtcc
      781 gggctgcgcg ttggtgcgga catctcggta gtgggatacg acgataccga agacagctca
      841 tgttatatcc cgccgttaac caccatcaaa caggattttc gcctgctggg gcaaaccagc
      901 gtggaccgct tgctgcaact ctctcagggc caggcggtga agggcaatca actgttgccc
      961 gtctcactgg tgaaaagaaa aaccaccctg gctcccaata cgcaaaccgc ctctccccgc
     1021 gcgttggccg attcattaat gcaactggca cgacaggttt cccgactgga aagcgggcag
     1081 actagcgcag cgaacgacga aaattacgcc cttgcagcgt 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 atgtctcgtt tagataaaag taaagtgatt aacagcgcat tagagctgct taatgaggtc
```

```
       61 ggaatcgaag gtttaacaac ccgtaaactc gcccagaagc taggtgtaga gcagcctaca
      121 ttgtattggc atgtaaaaaa taagcgggct ttgctcgacg ccttagccat tgagatgtta
      181 gataggcacc atactcactt ttgcccttta gaaggggaaa gctggcaaga ttttttacgt
      241 aataacgcta aaagttttag atgtgcttta ctaagtcatc gcgatggagc aaaagtacat
      301 ttaggtacac ggcctacaga aaaacagtat gaaactctcg aaaatcaatt agccttttta
      361 tgccaacaag gtttttcact agagaatgca ttatatgcac tcagcgctgt ggggcatttt
      421 actttaggtt gcgtattgga agatcaagag catcaagtcg ctaaagaaga aagggaaaca
      481 cctactactg atagtatgcc gccattatta cgacaagcta tcgaattatt tgatcaccaa
      541 ggtgcagagc cagccttctt attcggcctt gaattgatca tctgcggatt agaaaaacaa
      601 cttaaatgtg aaagtgggtc tactagcgca gcgaacgacg aaaattacgc ccttgcagcg
      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 aaaaaaagaa aaatgaactt ggcttatccc aggaatctgt cgcagacaag
      121 atggggatgg ggcagtcagg cgttggtgct ttatttaatg gcatcaatgc attaaatgct
      181 tataacgccg cattgcttgc aaaaattctc aaagttagcg ttgaagaatt tagcccttca
      241 atcgccagag aaatctacga gatgtatgaa gcggttagta tgcagccgtc acttagaagt
      301 gagtatgagt accctgtttt ttctcatgtt caggcaggga tgttctcacc tgagcttaga
      361 acctttacca aaggtgatgc ggagagatgg gtaagcacaa ccaaaaaagc cagtgattct
      421 gcattctggc ttgaggttga aggtaattcc atgaccgcac caacaggctc caagccgagc
      481 tttcctgacg gaatgttaat tctcgttgac cctgagcagg ctgttgagcc aggtgatttc
      541 tgcatagcca gacttggggg tgatgagttt accttcaaga aactgatcag ggatagcggt
      601 caggtgtttt tacaaccact aaacccacag tacccaatga tcccatgcaa tgagagttgt
      661 tccgttgtgg ggaaagttat cgctagtcag tggcctgaag agacgtttgg cactagcgca
      721 gcgaacgacg aaaattacgc ccttgcagcg tga
//

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

```
                     /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 tcagtttgtg
       61 gaaaggtttg aaagaccttc aggggaaaaa atagcatcat gtgctgctga actaacctat
      121 ttatgttgga tgattactca taacggaaca gcaatcaaga gagccacatt catgagctat
      181 aatactatca taagcaattc gctgagtttc gatattgtca acaaatcact ccagtttaaa
      241 tacaagacgc aaaaagcaac aattctggaa gcctcattaa agaaattaat tcctgcttgg
      301 gaatttacaa ttattcctta caatggacaa aaacatcaat ctgacatcac tgatattgta
      361 agtagtttgc aattacagtt cgaatcatcg gaagaagcag ataagggaaa tagccacagt
      421 aaaaaaatgc ttaaagcact tctaagtgag ggtgaaagca tctgggagat cactgagaaa
      481 atactaaatt cgtttgagta tacctctcgc tttacaaaaa caaaaacttt ataccaattc
      541 ctcttcctag ctactttcat caactgtgga agattcagcg atattaagaa cgtggacccg
      601 aaatcattta aattagtcca aaataagtat ctgggagtaa taatccagtg tttagtgaca
      661 gagacaaaga caagcgttag taggcacata tacttcttta gcgcaagggg taggatcgat
      721 ccacttgtat atttggatga atttttgagg aactctgaac cagtcctaaa acgagtaaat
      781 aggaccggca attcttcaag caacaaacag gaataccaat tattaaaaga taacttagtc
      841 agatcgtaca acaaggcttt gaagaaaaat gcgccttatc caatctttgc tataaagaat
      901 ggcccaaaat ctcacattgg aagacatttg atgacctcat ttctgtcaat gaagggccta
      961 acggagttga ctaatgttgt gggaaattgg agcgataagc gtgcttctgc cgtggccagg
     1021 acaacgtata ctcatcagat aacagcaata cctgatcact acttcgcgct agtttctcgg
     1081 tactatgcgt atgatccaat atcaaaggaa atgatagcat tgaaggatga gactaatccg
     1141 attgaggagt ggcagcatat agaacagcta aagggtagtg ctgaaggaag catacgatac
     1201 cccgcatgga atgggataat atcacaggag gtactagact acctttcatc ctacataaat
     1261 agacgcatag cagcaaacga cgaaaactac gctttagcag 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 cttggactcc tgttgataga tccagtaatg acctcagaac tccatctgga
       61 tttgttcaga acgctcggtt gccgccgggc gttttttatt ggtgagaatc caagcagtag
      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 gctagaggca tcaaataaaa cgaaaggctc agtcgaaaga ctgggccttt cgttttatct
       61 gttgtttgtc ggtgaacgct ctcctgagta ggacaaatcc gccgggagcg gatttgaacg
      121 ctgcgaagca acggcccgga gggtggcggg caggacgccc gccataaact gccaggcatc
      181 aaattaagca gaaggccatc ctgacggatg gcctttttgc 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 tccctatcag 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 acagataacc atctgcggtg ataaattatc tctggcggtg ttgacataaa taccactggc
       61 ggtgatactg agcacatcag caggacgcac tgaccaccat gaaggtgacg ctcttaaaaa
      121 ttaagccctg aagaagggca gcattcaaag cagaaggctt tggggtgtgt gatacgaaac
      181 gaagcattgg ccgtaagtgc gattccggat tagctgccaa tgtgccaatc gcggggggtt
      241 ttcgttcagg actacaactg ccacacacca ccaaagctaa ctgacaggag aatccagatg
      301 gatgcacaaa cacgccgccg cgaacgtcgc gcagagaaac aggctcaatg gaaagcagca
      361 aatcccctgt tggttggggt aagcgcaaaa ccagttccga aagatttttt taactataaa
      421 cgctgatgga agcgtttatg cggaagaggt aaagcccttc ccgagtaaca aaaaaacaac
      481 agcataaata 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
```

```
                     /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 tgcatcagac attgccgtca ctgcgtcttt tactggctct
       61 tctcgctaac caaaccggta accccgctta ttaaaagcat tctgtaacaa agcgggacca
      121 aagccatgac aaaagcgcgt aacaaaagtg tctataatca cggcagaaaa gtccacattg
      181 attatttgca cggcgtcaca ctttgctatg ccatagcatt tttatccata agattagcgg
      241 atcatacctg acgcttttta 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 gcgcttcaag
       61 gttcacatgg agggctccgt gaacggccac gagttcgaga tcgagggcga gggcgagggc
      121 cgcccctacg agggcaccca gaccgccaag ctgaaggtga ccaagggtgg cccccctgccc
      181 ttcgcctggg acatcctgtc ccctcagttc atgtacggct ccaaggccta cgtgaagcac
      241 cccgccgaca tccccgacta cttgaagctg tccttccccg agggcttcaa gtgggagcgc
      301 gtgatgaact tcgaggacgg cggcgtggtg accgtgaccc aggactcctc cctgcaagac
      361 ggcgagttca tctacaaggt gaagctgcgc ggcaccaact tcccctccga cggccccgta
      421 atgcagaaga agactatggg ctgggaggcc tcctccgagc ggatgtaccc cgaggacggc
      481 gcgctgaagg gcgagatcaa gcagaggctg aagctgaagg acggcggcca ctacgacgct
      541 gaggtcaaga ccacctacaa ggccaagaag cccgtgcaac tgcccggcgc gtacaacgtc
      601 aacatcaagt tggacatcac ctcccacaac gaggactaca ccatcgtgga acagtacgaa
      661 cgcgccgagg gccgccactc caccggcggc atggacgagc tgtataagac tagcgcagcg
      721 aacgacgaaa attacgccct 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 tgataaaaaa atcggatttt ttggcaattc catcggagga gtataaaggt
       61 attctaagtc ttcgttatca agtgtttaag caaagacttg agtgggactt agttgtagaa
      121 aataaccttg aatcagatga gtatgataac tcaaatgcag aatatattta tgcttgtgat
      181 gatactgaaa atgtaagtgg atgctggcgt ttattaccta caacaggtga ttatatgctg
      241 aaaagtgttt ttcctgaatt gcttggtcaa cagagtgctc ccaaagatcc taatatagtc
      301 gaattaagtc gttttgctgt aggtaaaaat agctcaaaga taaataactc tgctagtgaa
      361 attacaatga aactatttga agctatatat aaacacgctg ttagtcaagg tattacagaa
      421 tatgtaacag taacatcaac agcaatagag cgatttttaa agcgtattaa agttccttgt
      481 catcgtattg gagacaaaga aattcatgta ttaggtgata ctaaatcggt tgtattgtct
      541 atgcctatta atgaacagtt taaaaaagca gtcttaaatg ctgcaaacga cgaaaactac
      601 gctttagtag cttaataa
//

LOCUS       Plux-cI     97 bp    DNA   LINEAR   SYN
DEFINITION  Hybrid Promoter
ACCESSION   pKLi048
FEATURES             Location/Qualifiers
     primer_bind     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             Location/Qualifiers
     CDS             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"
```

```
     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 atgcttatct gatatgacta aaatggtaca ttgtgaatac
      121 tatttactcg cgatcattta tcctcattct atggttaaat ctgatatttc aatcctagat
      181 aattacccta aaaaatggag gcagtattat gatgacgcta atttaataaa atatgatcct
      241 atagtagatt attctaactc caatcattca ccaattaatt ggaatatatt tgaaaacaat
      301 gctgtaaata aaaaatctcc aaatgtaatt aaagaagcga aaacatcagg tcttatcact
      361 gggtttagtt tccctattca tacggctaac aatggcttcg gaatgcttag ttttgcacat
      421 tcagaaaaag acaactatat agatagttta tttttacatg cgtgtatgaa cataccatta
      481 attgttcctt ctctagttga taattatcga aaaataaata tagcaaataa taaatcaaac
      541 aacgatttaa ccaaaagaga aaaagaatgt ttagcgtggg cgtgcgaagg aaaaagctct
      601 tgggatattt caaaaatctt aggttgcagt gagcgtactg tcactttcca tttaaccaat
      661 gcgcaaatga aactcaatac aacaaaccgc tgccaaagta tttctaaagc aattttaaca
      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"
```

```
BASE COUNT      186 a    231 c    246 g    216 t
ORIGIN
        1 atggctgaag cgcaaatcga tcccctgctg ccgggatact cgtttaacgc ccatctggtg
       61 gcgggtttaa cgccgattga ggccaacggt tatctcgatt tttttatcga ccgaccgctg
      121 ggaatgaaag gttatattct caatctcacc attcgcggtc agggggtggt gaaaaatcag
      181 ggacgagaat ttggttgccg accgggtgat attttgctgt tcccgccagg agagattcat
      241 cactacggtc gtcatccgga ggctcgcgaa tggtatcacc agtgggttta ctttcgtccg
      301 cgcgcctact ggcatgaatg gcttaactgg ccgtcaatct ttgccaatac gggtttcttt
      361 cgcccggatg aagcgcacca gccgcatttc agcgacctgt ttgggcaaat cattaacgcc
      421 gggcaagggg aagggcgcta ttcggagctg ctggcgataa atctgcttga gcaactgtta
      481 ctgcggcgca tgggtgcgat taacgtgtcg ctccatccac cgatggataa tcgggtacgc
      541 gaggcttgtc agtacatcag cgatcacctg gcagacagca attttgacat cgccagcgtc
      601 gcacagcatg tttgcttgtc gccgtcgcgt ctgtcacatc ttttccgcca gcagttaggg
      661 attagcgtcc tgagctggcg cgaggaccaa cgcattagtc aggcgaagct gcttttgagc
      721 actacccgga tgcctatcgc caccgtcggt cgcaatgttg gttttgacga tcaactctat
      781 ttctcgcgag tatttaaaaa atgcaccggg gccagcccga gcgagtttcg tgccggtact
      841 agcgcagcga acgacgaaaa ttacgccctt gcagcgtga
//

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="araI1"
     misc_feature    414..414
                     /note="C->A BamHI"
     misc_feature    418..418
                     /note="G->T HindIII"
     protein_bind    425..444
                     /note="Os"
     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
        1 aattgtgagc ggataacaat ttcacacagg gccctcggac accgaggaga atgtcaagag
       61 gcgaacacac aacgtcttgg agcgccagag gaggaacgag ctaaaacgga gctttttttgc
      121 cctgcgtgac cagatcccgg agttggaaaa caatgaaaag gcccccaagg tagttatcct
      181 taaaaaagcc acagcataca tcctgtccgt ccaagcagag gagcaaaagc tcatttctga
      241 agaggacttg ttgcggaaac gacgagaaca gttgaaacac aaacttgaac agctacggaa
      301 ctcttgtgcg taaggaaaag taaggaaaac gattccttct aacagaaatg tcctgagtca
```

```
      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 tctttattta gcatgagaaa
       61 aataaagttg aaggtggcgt tatattaaac gcgcttgcta taagagtatt ttactcagga
      121 gtgagaatct ggttatttat tgcccttaac cattatcgac cacgatattg cttttgcgta
      181 acagcgggca atctgttatc cccaaaaaac cacttttagt gtgcaagtat tgtaccgtgc
      241 tggtgcctct ggcagtcaga taggtacatt gcaaacctaa tcctgcggca ttctctttgc
      301 ttccaatcaa aacgccatat ccgctgagta ataatcctat ccataccagt gctatcagca
      361 taactgtgcg aatgatgaat cgcattacaa cctcttctct ttttatgttc gcttaatcgt
      421 agcggcaata tgcgctgaag caagcgactc attccgaaaa agcacgaata tcgacatagt
      481 taggcgctgt ttaactaacc catgctagtt taatgacata aggtaggtga 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 tgcgtgttat atatcattaa
       61 aacggtaatg gattgacatt tgattctaat aaattggatt tttgtcacac tattgtatcg
      121 ctgggaatac aattacttaa cataagcacc tgtaggatcg tacaggttta cgcaagaaaa
      181 tggtttgtta tagtcgaata 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 gcgcttcaag
       61 gttcacatgg agggctccgt gaacggccac gagttcgaga tcgagggcga gggcgagggc
      121 cgcccctacg agggcaccca gaccgccaag ctgaaggtga ccaagggtgg cccccctgccc
      181 ttcgcctggg acatcctgtc ccctcagttc atgtacggct ccaaggccta cgtgaagcac
      241 cccgccgaca tccccgacta cttgaagctg tccttccccg agggcttcaa gtgggagcgc
      301 gtgatgaact tcgaggacgg cggcgtggtg accgtgaccc aggactcctc cctgcaagac
      361 ggcgagttca tctacaaggt gaagctgcgc ggcaccaact tcccctccga cggccccgta
      421 atgcagaaga agactatggg ctgggaggcc tcctccgagc ggatgtaccc cgaggacggc
      481 gcgctgaagg gcgagatcaa gcagaggctg aagctgaagg acggcggcca ctacgacgct
      541 gaggtcaaga ccacctacaa ggccaagaag cccgtgcaac tgcccggcgc gtacaacgtc
      601 aacatcaagt tggacatcac ctcccacaac gaggactaca ccatcgtgga acagtacgaa
      661 cgcgccgagg gccgccactc caccggcggc atggacgagc tgtataagac tagcgcagcg
      721 aacgacgaaa attacgccct tgcagcgtga
//

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"
```

```
     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 cattggtcaa
       61 cgaatgcatt gcgcaatcac accaaaggtt 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 cattggtcaa
       61 cgaatgcatt gcgcaatcac accaaaggtt caatgcaaag gtttctatgg tcaagagagc
      121 catcgatagc ttaatacaaa agggatacct acagagggga gacgatggtg aatcgtatgc
      181 ttaccttgct taatcatctt tgaaggcttg tgctgatcga acgaagcaaa tcctacgagt
      241 aaatacataa
//

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 cattggtcaa
```

```
       61 cgaatgcatt gcgcaatcac accaaaggtt caatgcaaag gtttctatgg tcaagagagc
      121 catcgatagc ttaatacaaa agggatacct acagagggga gacgatggtg aatcgtatgc
      181 ttaccttgct taatcatctt tgaaggcttg tgctgatcga acgaagcaaa tcctacgagt
      241 aaatacataa gcgtatacat atatatatat atatatatat atatatatat atgtatatat
      301 atatatgtgt gtgtgtgtaa ttgtgtgtat tcaactgaac tatgaagagt ctttgacctc
      361 ttgagaatct catagtatga agatatggca cttctctttc cgttgtaaca tcctttaccg
      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="Ptrc-2"
     misc_feature    2231..2262
                     /note="rbsKDL028-SDM"
     CDS             2263..2886
                     /note="tetR"
     misc_feature    2929..3003
                     /note="Ptrc-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 tgttctgcag tcacttgaat tcgataccca gctgttattt
       61 gtatagttca tccatgccat gtgtaatccc agcagcggtt acaaactcaa gaaggaccat
      121 gtggtctctc ttttcgttgg gatctttcga aagggcagat tgtgtggaca ggtaatggtt
      181 gtctggtaaa aggacagggc catcgccaat cggagtattt tgttgataat ggtctgctag
      241 ttgaacgctt ccatcttcaa tgttgtgtct aattttgaag ttaactttga ttccattctt
      301 ttgtttgtct gccatgatgt atacattgtg tgagttatag ttgtattcca atttgtgtcc
      361 aagaatgttt ccatcttctt taaaatcaat accttttaac tcgattctat taacaagggt
      421 atcaccttca aacttgactt cagcacgtgt cttgtagttc ccgtcatctt tgaaaaatat
```

```
 481 agttctttcc tgtacgtaac cttcgggcat ggcactcttg aaaaagtcat gctgtttcat
 541 gtgatctggg tatctcgcaa agcattgaac accataaccg aaagtagtga caagtgttgg
 601 ccacggaaca ggtagttttc cagtagtgca aataaattta agggtaagtt ttccgtatgt
 661 tgcatcacct tcaccctctc cactgacaga aaatttgtgc ccattaacat caccatctaa
 721 ttcaacaaga attgggacaa ctccagtgaa aagttcttct cctttacgca tgtgcactac
 781 ctccttggtt gtctatgcta tgctgatcta caactggcat gcggtcagtg cgtcctgctg
 841 atgtgctcag tatctctatc actgataggg atgtcaatct ctatcactga tagggaaacg
 901 tttcgcagaa gcttccgcaa ggtaccactt tgccgcggtc actgcccgct ttccagtcgg
 961 gaaacctgtc gtgccagttg cattaatgaa tcggccaacg cgcggggaga ggcggtttgc
1021 gtattgggag ccagggtggt ttttcttttc accagtgaga cgggcaacag ttgattgccc
1081 ttcaccgcct ggccctgaga gagttgcagc aagcggtcca cgctggtttg ccccagcagg
1141 cgaaaatcct gtttgatggt ggttaacggc gggatataac atgagctgtc ttcggtatcg
1201 tcgtatccca ctaccgagat gtccgcacca acgcgcagcc cggactcggt aatggcgcgc
1261 attgcgccca gcgccatctg atcgttggca accagcatcg cagtgggaac gatgccctca
1321 ttcagcattt gcatggtttg ttgaaaaccg gacatggcac tccagtcgcc ttcccgttcc
1381 gctatcggct gaatttgatt gcgagtgaga tatttatgcc agccagccag acgcagacgc
1441 gccgagacag aacttaatgg gcccgctaac agcgcgattt gctggtgacc caatgcgacc
1501 agatgctcca cgcccagtcg cgtaccgtct tcatgggaga aaataatact gttgatgggt
1561 gtctggtcag agacatcaag aaataacgcc ggaacattag tgcaggcagc ttccacagca
1621 atggcatcct ggtcatccag cggatagtta atgatcagcc cactgacgcg ctgcgcgaga
1681 agattgtgaa ccgccgcttt acaggcttcg acgccgcttc gttctaccat cgacaccacc
1741 acgctggcac ccagttgatc ggcgcgagat ttaatcgccg cgacaatttg cgacggcgcg
1801 tgcagggcca gactggaggt ggcaacgcca atcagcaacg actgtttgcc cgccagttgt
1861 tgtgccacgc ggttgggaat gtaattcagc tccgccatcg ccgcttccac tttttcccgc
1921 gttttcgcag aaacgtggct ggcctggttc accacgcggg aaacggtctg ataagagaca
1981 ccggcatact ctgcgacatc gtacagcgtt actggtttca ttgtacaccc tctccttata
2041 ttgggaccta tggatccggt cagtgcgtcc tgctgatgtg ctcagtatct ctatcactga
2101 tagggatgtc aatctctatc actgataggg acggccgccc gttccatggc tgaaatgagc
2161 tgttgacaat taatcatccg gctcgtataa tgtgtggaat tgtgagcgga taacaatttc
2221 acacgtcgac cgccttcggc gaagctaggg acgagagcta gcatgtctcg tttagataaa
2281 agtaaagtga ttaacagcgc attagagctg cttaatgagg tcggaatcga aggtttaaca
2341 acccgtaaac tcgcccagaa gctaggtgta gagcagccta cattgtattg gcatgtaaaa
2401 aataagcggg ctttgctcga cgccttagcc attgagatgt tagataggca ccatactcac
2461 ttttgccctt tagaagggga aagctggcaa gatttttac gtaataacgc taaaagtttt
2521 agatgtgctt tactaagtca tcgcgatgga gcaaaagtac atttaggtac acggcctaca
2581 gaaaaacagt atgaaactct cgaaaatcaa ttagcctttt tatgccaaca aggtttttca
2641 ctagagaatg cattatatgc actcagcgct gtggggcatt ttactttagg ttgcgtattg
2701 gaagatcaag agcatcaagt cgctaaagaa gaaagggaaa cacctactac tgatagtatg
2761 ccgccattat tacgacaagc tatcgaatta tttgatcacc aaggtgcaga gccagccttc
2821 ttattcggcc ttgaattgat catctgcgga ttagaaaaac aacttaaatg tgaaagtggg
2881 tcttgagagc tcggactgct taagtcgctc catatgctcg ttcccgggct gaaatgagct
2941 gttgacaatt aatcatccgg ctcgtataat gtgtggaatt gtgagcggat aacaatttca
3001 caccaattga gtattaacta tcgttcaact gatagggagg cgccgatggt gagcaagggc
3061 gaggaggata acatggccat catcaaggag ttcatgcgct tcaaggttca catggagggc
3121 tccgtgaacg gccacgagtt cgagatcgag ggcgagggcg agggccgccc ctacgagggc
3181 acccagaccg ccaagctgaa ggtgaccaag ggtggccccc tgcccttcgc ctgggacatc
3241 ctgtcccctc agttcatgta cggctccaag gcctacgtga agcaccccgc cgacatcccc
3301 gactacttga agctgtcctt ccccgagggc ttcaagtggg agcgcgtgat gaacttcgag
3361 gacggcggcg tggtgaccgt gacccaggac tcctccctgc aagacggcga gttcatctac
3421 aaggtgaagc tgcgcggcac caacttcccc tccgacggcc ccgtaatgca gaagaagact
3481 atgggctggg aggcctcctc cgagcggatg taccccgagg acggcgcgct gaagggcgag
3541 atcaagcaga ggctgaagct gaaggacggc ggccactacg acgctgaggt caagaccacc
3601 tacaaggcca agaagcccgt gcaactgccc ggcgcgtaca acgtcaacat caagttggac
3661 atcacctccc acaacgagga ctacaccatc gtggaacagt acgaacgcgc cgagggccgc
3721 cactccaccg gcggcatgga cgagctgtat aagtaagata tctatcgccc tagggaccgt
```

```
     3781 ctcgagagaa tcaatattaa tccaacgcgt ggcatcaaat aaaacgaaag gctcagtcga
     3841 aagactgggc ctttcgtttt atctgttgtt tgtcggtgaa cgctctcctg agtaggacaa
     3901 atccgccgcc ctagacttag gcgttcggct gcggcgagcg gtatcagctc actcaaaggc
     3961 ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg
     4021 ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg
     4081 cccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa acccgacagg
     4141 actataaaga taccaggcgt ttccccctgg aagctccctc gtgcgctctc ctgttccgac
     4201 cctgccgctt accggatacc tgtccgcctt tctcccttcg ggaagcgtgg cgctttctca
     4261 tagctcacgc tgtaggtatc tcagttcggt gtaggtcgtt cgctccaagc tgggctgtct
     4321 gcacgaaccc cccgttcagc ccgaccgctg cgccttatcc ggtaactatc gtcttgagtc
     4381 caacccggta agacacgact tatcgccact ggcagcagcc actggtaaca ggattagcag
     4441 agcgaggtat gtaggcggtg ctacagagtt cttgaagtgg tggcctaact acggctacac
     4501 tagaaggaca gtatttggta tctgcgctct gctgaagcca gttaccttcg gaaaaagagt
     4561 tggtagctct tgatccggca aacaaaccac cgctggtagc ggtggttttt ttgtttgcaa
     4621 gcagcagatt acgcgcagaa aaaaaggatc tcaagaagat cctttgatct tttctacggg
     4681 gtctgacgct cagtggaacg aaaactcacg ttaagggatt ttggtcatgg ctagtgcttg
     4741 gattctcacc aataaaaaac gcccggcggc aaccgagcgt tctgaacaaa tccagatgga
     4801 gttctgaggt cattactgga tctatcaaca ggagtccaag ccaattctcg aaccccagag
     4861 tcccgctcag aagaactcgt caagaaggcg atagaaggcg atgcgctgcg aatcgggagc
     4921 ggcgataccg taaagcacga ggaagcggtc agcccattcg ccgccaagct cttcagcaat
     4981 atcacgggta gccaacgcta tgtcctgata gcggtccgcc acacccagcc ggccacagtc
     5041 gatgaatcca gaaaagcggc cattttccac catgatattc ggcaagcagg catcgccgtg
     5101 ggtcacgacg agatcctcgc cgtcgggcat acgcgccttg agcctggcga acagttcggc
     5161 tggcgcgagc ccctgatgct cttcgtccag atcatcctga tcgacaagac cggcttccat
     5221 ccgagtacgt gctcgctcga tgcgatgttt cgcttggtgg tcgaatgggc aggtagccgg
     5281 atcaagcgta tgcagccgcc gcattgcatc agccatgatg gatactttct cggcaggagc
     5341 aaggtgagat gacaggagat cctgccccgg cacttcgccc aatagcagcc agtcccttcc
     5401 cgcttcagtg acaacgtcga gcacagccgc gcaaggaacg cccgtcgtgg ccagccacga
     5461 tagccgcgct gcctcgtctt gcagttcatt cagggcaccg gacaggtcgg tcttgacaaa
     5521 aagaaccggg cgaccctgcg ctgacagccg gaacacggcg gcatcagagc agccgattgt
     5581 ctgttgtgcc cagtcatagc cgaatagcct ctccacccaa gccgccggag aacctgcgtg
     5641 caatccatct tgttcaatca tgcgaaacga tcctcatcct gtctcttgat cagatcttga
     5701 tcccctgcgc catcagatcc ttggcggcaa gaaagccatc cagtttactt tgcagggctt
     5761 cccaacctta ccagagggcg gcccaactgg caattcc
//


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)
                     /note="lacI"
     misc_feature    complement (2022..2051)
                     /note="rbsKDL026"
     misc_feature    complement (2058..2131)
                     /note="PLtetO"
```

```
     misc_feature    2150..2692
                     /note="PmgrB"
     misc_feature    2699..2726
                     /note="rbsKDL032d"
     CDS             2727..3350
                     /note="tetR"
     CDS             3394..4272
                     /note="AraC_LAA_Keasling"
     misc_feature    4279..4401
                     /note="T0"
     misc_feature    4293..4387
                     /note="T0"
     misc_feature    4408..4893
                     /note="Plac/ara
     misc_feature    4900..4935
                     /note="rbsKDL027"
     CDS             4936..5646
                     /note="mCherry"
     misc_feature    5701..5805
                     /note="T1"
                     /product="transcriptional terminator from rrnB operon"
     misc_feature    5812..6619
                     /note="ColE1"
                     /product="High Copy Origin of Replication"
     misc_feature    6626..6731
                     /note="T0"
                     /product="transcriptional terminator from phage lambda"
     CDS             complement (6757..7551)
                     /note="KanR"
BASE COUNT     1928 a   1971 c   1955 g   1833 t
ORIGIN
        1 gacgtctgtg caagtactac tgttctgcag tcacttgaat tcgataccca gctgttattt
       61 gtatagttca tccatgccat gtgtaatccc agcagcggtt acaaactcaa gaaggaccat
      121 gtggtctctc ttttcgttgg gatctttcga aagggcagat tgtgtggaca ggtaatggtt
      181 gtctggtaaa aggacagggc catcgccaat cggagtattt tgttgataat ggtctgctag
      241 ttgaacgctt ccatcttcaa tgttgtgtct aattttgaag ttaactttga ttccattctt
      301 ttgtttgtct gccatgatgt atacattgtg tgagttatag ttgtattcca atttgtgtcc
      361 aagaatgttt ccatcttctt taaaatcaat accttttaac tcgattctat taacaagggt
      421 atcaccttca aacttgactt cagcacgtgt cttgtagttc ccgtcatctt tgaaaaatat
      481 agttctttcc tgtacgtaac cttcgggcat ggcactcttg aaaaagtcat gctgtttcat
      541 gtgatctggg tatctcgcaa agcattgaac accataaccg aaagtagtga caagtgttgg
      601 ccacggaaca ggtagttttc cagtagtgca aataaattta agggtaagtt ttccgtatgt
      661 tgcatcacct tcaccctctc cactgacaga aaatttgtgc ccattaacat caccatctaa
      721 ttcaacaaga attgggacaa ctccagtgaa aagttcttct cctttacgca tgtgcactac
      781 ctccttggtt gtctatgcta tgctgatcta caactggcat gcggtcagtg cgtcctgctg
      841 atgtgctcag tatctctatc actgataggg atgtcaatct ctatcactga tagggaaacg
      901 tttcgcagaa gcttccgcaa ggtaccactt tgccgcggtc actgcccgct ttccagtcgg
      961 gaaacctgtc gtgccagttg cattaatgaa tcggccaacg cgcggggaga ggcggtttgc
     1021 gtattgggag ccagggtggt ttttcttttc accagtgaga cgggcaacag ttgattgccc
     1081 ttcaccgcct ggccctgaga gagttgcagc aagcggtcca cgctggtttg ccccagcagg
     1141 cgaaaatcct gtttgatggt ggttaacggc gggatataac atgagctgtc ttcggtatcg
     1201 tcgtatccca ctaccgagat gtccgcacca acgcgcagcc cggactcggt aatggcgcgc
     1261 attgcgccca gcgccatctg atcgttggca accagcatcg cagtgggaac gatgccctca
     1321 ttcagcattt gcatggtttg ttgaaaaccg gacatggcac tccagtcgcc ttcccgttcc
     1381 gctatcggct gaatttgatt gcgagtgaga tatttatgcc agccagccag acgcagacgc
```

```
1441 gccgagacag aacttaatgg gcccgctaac agcgcgattt gctggtgacc caatgcgacc
1501 agatgctcca cgcccagtcg cgtaccgtct tcatgggaga aaataatact gttgatgggt
1561 gtctggtcag agacatcaag aaataacgcc ggaacattag tgcaggcagc ttccacagca
1621 atggcatcct ggtcatccag cggatagtta atgatcagcc cactgacgcg ctgcgcgaga
1681 agattgtgaa ccgccgcttt acaggcttcg acgccgcttc gttctaccat cgacaccacc
1741 acgctggcac ccagttgatc ggcgcgagat ttaatcgccg cgacaatttg cgacggcgcg
1801 tgcagggcca gactggaggt ggcaacgcca atcagcaacg actgtttgcc cgccagttgt
1861 tgtgccacgc ggttgggaat gtaattcagc tccgccatcg ccgcttccac tttttcccgc
1921 gttttcgcag aaacgtggct ggcctggttc accacgcggg aaacggtctg ataagagaca
1981 ccggcatact ctgcgacatc gtacagcgtt actggtttca ttgtacaccc tctccttata
2041 ttgggaccta tggatccggt cagtgcgtcc tgctgatgtg ctcagtatct ctatcactga
2101 tagggatgtc aatctctatc actgataggg acggccgccc gttccatggg atgagagtaa
2161 gaacctgtcg gaatatcaaa cagacaggtt ctttatttag catgagaaaa ataaagttga
2221 aggtggcgtt atattaaacg cgcttgctat aagagtattt tactcaggag tgagaatctg
2281 gttatttatt gcccttaacc attatcgacc acgatattgc ttttgcgtaa cagcgggcaa
2341 tctgttatcc ccaaaaaacc acttttagtg tgcaagtatt gtaccgtgct ggtgcctctg
2401 gcagtcagat aggtacattg caaacctaat cctgcggcat tctctttgct tccaatcaaa
2461 acgccatatc cgctgagtaa taatcctatc cataccagtg ctatcagcat aactgtgcga
2521 atgatgaatc gcattacaac ctcttctctt tttatgttcg cttaatcgta gcggcaatat
2581 gcgctgaagc aagcgactca ttccgaaaaa gcacgaatat cgacatagtt aggcgctgtt
2641 taactaaccc atgctagttt aatgacataa ggtaggtgaa acggagattg gagtcgaccg
2701 cataggagac aataagaggg gctagcatgt ctcgtttaga taaaagtaaa gtgattaaca
2761 gcgcattaga gctgcttaat gaggtcggaa tcgaaggttt aacaacccgt aaactcgccc
2821 agaagctagg tgtagagcag cctacattgt attggcatgt aaaaaataag cgggctttgc
2881 tcgacgcctt agccattgag atgttagata ggcaccatac tcacttttgc cctttagaag
2941 gggaaagctg gcaagatttt ttacgtaata acgctaaaag ttttagatgt gctttactaa
3001 gtcatcgcga tggagcaaaa gtacatttag gtacacggcc tacagaaaaa cagtatgaaa
3061 ctctcgaaaa tcaattagcc tttttatgcc aacaaggttt ttcactagag aatgcattat
3121 atgcactcag cgctgtgggg cattttactt taggttgcgt attggaagat caagagcatc
3181 aagtcgctaa agaagaaagg gaaacaccta ctactgatag tatgccgcca ttattacgac
3241 aagctatcga attatttgat caccaaggtg cagagccagc cttcttattc ggccttgaat
3301 tgatcatctg cggattagaa aaacaactta aatgtgaaag tgggtcttga gagctcggac
3361 tgcttaagat aaatataaat aaggacaata atcatggctg aagcgcaaat cgatcccctg
3421 ctgccgggat actcgtttaa cgcccatctg gtggcgggtt taacgccgat tgaggccaac
3481 ggttatctcg atttttttat cgaccgaccg ctgggaatga aaggttatat tctcaatctc
3541 accattcgcg gtcagggggt ggtgaaaaat cagggacgag aatttggttg ccgaccgggt
3601 gatattttgc tgttcccgcc aggagagatt catcactacg gtcgtcatcc ggaggctcgc
3661 gaatggtatc accagtgggt ttactttcgt ccgcgcgcct actggcatga atggcttaac
3721 tggccgtcaa tctttgccaa tacgggtttc tttcgcccgg atgaagcgca ccagccgcat
3781 ttcagcgacc tgtttgggca aatcattaac gccgggcaag ggaagggcg ctattcggag
3841 ctgctggcga taaatctgct tgagcaactg ttactgcggc gcatgggtgc gattaacgtg
3901 tcgctccatc caccgatgga taatcgggta cgcgaggctt gtcagtacat cagcgatcac
3961 ctggcagaca gcaattttga catcgccagc gtcgcacagc atgtttgctt gtcgccgtcg
4021 cgtctgtcac atcttttccg ccagcagtta gggattagcg tcctgagctg gcgcgaggac
4081 caacgcatta gtcaggcgaa gctgcttttg agcactaccc ggatgcctat cgccaccgtc
4141 ggtcgcaatg ttggttttga cgatcaactc tatttctcgc gagtatttaa aaaatgcacc
4201 ggggccagcc cgagcgagtt tcgtgccggt actagcgcag cgaacgacga aaattacgcc
4261 gcatcagttt gacatatgcg agaattggct tggactcctg ttgatagatc cagtaatgac
4321 ctcagaactc catctggatt tgttcagaac gctcggttgc cgccgggcgt ttttttattgg
4381 tgagaatcca agcagtagtc acccgggaat tgtgagcgga taacaatttc acacagggcc
4441 ctcggacacc gaggagaatg tcaagaggcg aacacacaac gtcttggagc gccagaggag
4501 gaacgagcta aaacggagct ttttgccct gcgtgaccag atcccggagt tggaaaacaa
4561 tgaaaaggcc cccaaggtag ttatccttaa aaaagccaca gcatacatcc tgtccgtcca
4621 agcagaggag caaaagctca tttctgaaga ggacttgttg cggaaacgac gagaacagtt
4681 gaaacacaaa cttgaacagc tacggaactc ttgtgcgtaa ggaaaagtaa ggaaaacgat
```

```
     4741 tccttctaac agaaatgtcc tgagcaatca cctatgaact gtcgattcga gcatagcatt
     4801 tttatccata agattagcgg atcataatct ttacaatttt gagcgctcac aattatgata
     4861 gattcagttg tgagcggata acaatttcac acacaattga gtattaacta tcgttcaact
     4921 gatagggagg cgccgatggt gagcaagggc gaggaggata acatggccat catcaaggag
     4981 ttcatgcgct tcaaggttca catggagggc tccgtgaacg gccacgagtt cgagatcgag
     5041 ggcgagggcg agggccgccc ctacgagggc acccagaccg ccaagctgaa ggtgaccaag
     5101 ggtggccccc tgcccttcgc ctgggacatc ctgtcccctc agttcatgta cggctccaag
     5161 gcctacgtga agcaccccgc cgacatcccc gactacttga agctgtcctt ccccgagggc
     5221 ttcaagtggg agcgcgtgat gaacttcgag gacggcggcg tggtgaccgt gacccaggac
     5281 tcctccctgc aagacggcga gttcatctac aaggtgaagc tgcgcggcac caacttcccc
     5341 tccgacggcc ccgtaatgca gaagaagact atgggctggg aggcctcctc cgagcggatg
     5401 taccccgagg acggcgcgct gaagggcgag atcaagcaga ggctgaagct gaaggacggc
     5461 ggccactacg acgctgaggt caagaccacc tacaaggcca agaagcccgt gcaactgccc
     5521 ggcgcgtaca acgtcaacat caagttggac atcacctccc acaacgagga ctacaccatc
     5581 gtggaacagt acgaacgcgc cgagggccgc cactccaccg gcggcatgga cgagctgtat
     5641 aagtaagata tctatcgccc tagggaccgt ctcgagagaa tcaatattaa tccaacgcgt
     5701 ggcatcaaat aaaacgaaag gctcagtcga aagactgggc ctttcgtttt atctgttgtt
     5761 tgtcggtgaa cgctctcctg agtaggacaa atccgccgcc ctagacttag gcgttcggct
     5821 gcggcgagcg gtatcagctc actcaaaggc ggtaatacgg ttatccacag aatcagggga
     5881 taacgcagga aagaacatgt gagcaaaagg ccagcaaaag gccaggaacc gtaaaaaggc
     5941 cgcgttgctg gcgtttttcc ataggctccg cccccctgac gagcatcaca aaaatcgacg
     6001 ctcaagtcag aggtggcgaa acccgacagg actataaaga taccaggcgt ttccccctgg
     6061 aagctccctc gtgcgctctc ctgttccgac cctgccgctt accggatacc tgtccgcctt
     6121 tctcccttcg ggaagcgtgg cgctttctca tagctcacgc tgtaggtatc tcagttcggt
     6181 gtaggtcgtt cgctccaagc tgggctgtct gcacgaaccc cccgttcagc ccgaccgctg
     6241 cgccttatcc ggtaactatc gtcttgagtc caacccggta agacacgact tatcgccact
     6301 ggcagcagcc actggtaaca ggattagcag agcgaggtat gtaggcggtg ctacagagtt
     6361 cttgaagtgg tggcctaact acggctacac tagaaggaca gtatttggta tctgcgctct
     6421 gctgaagcca gttaccttcg gaaaaagagt tggtagctct tgatccggca aacaaaccac
     6481 cgctggtagc ggtggttttt ttgtttgcaa gcagcagatt acgcgcagaa aaaaaggatc
     6541 tcaagaagat cctttgatct tttctacggg gtctgacgct cagtggaacg aaaactcacg
     6601 ttaagggatt ttggtcatgg ctagtgcttg gattctcacc aataaaaaac gcccggcggc
     6661 aaccgagcgt tctgaacaaa tccagatgga gttctgaggt cattactgga tctatcaaca
     6721 ggagtccaag ccaattctcg aaccccagag tcccgctcag aagaactcgt caagaaggcg
     6781 atagaaggcg atgcgctgcg aatcgggagc ggcgataccg taaagcacga ggaagcggtc
     6841 agcccattcg ccgccaagct cttcagcaat atcacgggta gccaacgcta tgtcctgata
     6901 gcggtccgcc acacccagcc ggccacagtc gatgaatcca gaaaagcggc cattttccac
     6961 catgatattc ggcaagcagg catcgccgtg ggtcacgacg agatcctcgc cgtcgggcat
     7021 acgcgccttg agcctggcga acagttcggc tggcgcgagc ccctgatgct cttcgtccag
     7081 atcatcctga tcgacaagac cggcttccat ccgagtacgt gctcgctcga tgcgatgttt
     7141 cgcttggtgg tcgaatgggc aggtagccgg atcaagcgta tgcagccgcc gcattgcatc
     7201 agccatgatg gatactttct cggcaggagc aaggtgagat gacaggagat cctgccccgg
     7261 cacttcgccc aatagcagcc agtcccttcc cgcttcagtg acaacgtcga gcacagccgc
     7321 gcaaggaacg cccgtcgtgg ccagccacga tagccgcgct gcctcgtctt gcagttcatt
     7381 cagggcaccg gacaggtcgg tcttgacaaa aagaaccggg cgaccctgcg ctgacagccg
     7441 gaacacggcg gcatcagagc agccgattgt ctgttgtgcc cagtcatagc cgaatagcct
     7501 ctccacccaa gccgccggag aacctgcgtg caatccatct tgttcaatca tgcgaaacga
     7561 tcctcatcct gtctcttgat cagatcttga tcccctgcgc catcagatcc ttggcggcaa
     7621 gaaagccatc cagtttactt tgcagggctt cccaacctta ccagagggcg gcccaactgg
     7681 caattcc
//

LOCUS       pKDL108     8096 bp    DNA   CIRCULAR SYN
DEFINITION  4FFL
ACCESSION   pKDL108
```

```
FEATURES             Location/Qualifiers
     misc_feature    complement (7208..1)
                     /note="KanR"
     CDS             complement (55..810)
                     /note="LuxR"
     rbs             complement (811..855)
                     /note="rbsKDL025"
     promoter        complement (862..935)
                     /note="PLtetO"
     terminator      complement (942..1172)
                     /note="T1T2"
     CDS             complement (1203..2285)
                     /note="lacI"
     rbs             complement (2286..2315)
                     /note="rbsKDL026"
     misc_feature    complement (2322..2540)
                     /note="Plux"
     misc_feature    2559..3101
                     /note="PmgrB"
     misc_feature    3108..3135
                     /note="rbsKDL032d"
     CDS             3136..3759
                     /note="tetR"
     misc_feature    3778..3802
                     /note="rbs"
     CDS             3803..4681
                     /note="AraC_ASV_Keasling"
     misc_feature    4688..4810
                     /note="T0"
     misc_feature    4702..4796
                     /note="T0"
     misc_feature    4817..5302
                     /note="Plac/ara"
     misc_feature    5309..5344
                     /note="rbsKDL027"
     CDS             5345..6055
                     /note="mCherry"
     misc_feature    6110..6214
                     /note="T1"
                     /product="transcriptional terminator from rrnB operon"
     misc_feature    6221..7028
                     /note="ColE1"
                     /product="High Copy Origin of Replication"
     misc_feature    7035..7140
                     /note="T0"
                     /product="transcriptional terminator from phage lambda"
     CDS             complement (7166..7960)
                     /note="KanR"
BASE COUNT     2076 a   2026 c   2010 g   1984 t
ORIGIN
        1 gacgtctgtg caagtactac tgttctgcag tcacttgaat tcgataccca gctgttatta
       61 atttttaaag tatgggcaat cgattgctcc tgttaaaatt gctttagaaa tactttggca
      121 gcggtttgtt gtattgagtt tcatttgcgc attggttaaa tggaaagtga cagtacgctc
      181 actgcaacct aagattttg aaatatccca agagcttttt ccttcgcacg cccacgctaa
      241 acattctttt tctcttttgg ttaaatcgtt gtttgattta ttatttgcta tatttatttt
```

```
 301 tcgataatta tcaactagag aaggaacaat taatggtatg ttcatacacg catgtaaaaa
 361 taaactatct atatagttgt ctttttctga atgtgcaaaa ctaagcattc cgaagccatt
 421 gttagccgta tgaataggga aactaaaccc agtgataaga cctgatgttt tcgcttcttt
 481 aattacattt ggagattttt tatttacagc attgttttca aatatattcc aattaattgg
 541 tgaatgattg gagttagaat aatctactat aggatcatat tttattaaat tagcgtcatc
 601 ataatactgc ctccattttt tagggtaatt atctaggatt gaaatatcag atttaaccat
 661 agaatgagga taaatgatcg cgagtaaata gtattcacaa tgtaccattt tagtcatatc
 721 agataagcat tgattaatat cattattgct tctacacgct ttaattttat taattattct
 781 gtatgtgtcg tcggcattta tgtttttcat gtgcactacc tccttggttg tctatgctat
 841 gctgatctac aactggcatg cggtcagtgc gtcctgctga tgtgctcagt atctctatca
 901 ctgataggga tgtcaatctc tatcactgat agggaaacgt tttgtagaa acgcaaaaag
 961 gccatccgtc aggatggcct tctgcttaat ttgatgcctg gcagtttatg gcgggcgtcc
1021 tgcccgccac cctccgggcc gttgcttcgc agcgttcaaa tccgctcccg gcggatttgt
1081 cctactcagg agagcgttca ccgacaaaca acagataaaa cgaaaggccc agtctttcga
1141 ctgagccttt cgttttattt gatgcctcta gcaagcttcc gcaaggtacc actttgccgc
1201 ggtcactgcc cgctttccag tcgggaaacc tgtcgtgcca gttgcattaa tgaatcggcc
1261 aacgcgcggg gagaggcggt ttgcgtattg ggagccaggg tggtttttct tttcaccagt
1321 gagacgggca acagttgatt gcccttcacc gcctggccct gagagagttg cagcaagcgg
1381 tccacgctgg tttgccccag caggcgaaaa tcctgtttga tggtggttaa cggcgggata
1441 taacatgagc tgtcttcggt atcgtcgtat cccactaccg agatgtccgc accaacgcgc
1501 agcccggact cggtaatggc gcgcattgcg cccagcgcca tctgatcgtt ggcaaccagc
1561 atcgcagtgg gaacgatgcc ctcattcagc atttgcatgg tttgttgaaa accggacatg
1621 gcactccagt cgccttcccg ttccgctatc ggctgaattt gattgcgagt gagatattta
1681 tgccagccag ccagacgcag acgcgccgag acagaactta atgggcccgc taacagcgcg
1741 atttgctggt gacccaatgc gaccagatgc tccacgccca gtcgcgtacc gtcttcatgg
1801 gagaaaataa tactgttgat gggtgtctgg tcagagacat caagaaataa cgccggaaca
1861 ttagtgcagg cagcttccac agcaatggca tcctggtcat ccagcggata gttaatgatc
1921 agcccactga cgcgctgcgc gagaagattg tgaaccgccg ctttacaggc ttcgacgccg
1981 cttcgttcta ccatcgacac caccacgctg gcacccagtt gatcggcgcg agatttaatc
2041 gccgcgacaa tttgcgacgg cgcgtgcagg gccagactgg aggtggcaac gccaatcagc
2101 aacgactgtt tgcccgccag ttgttgtgcc acgcggttgg gaatgtaatt cagctccgcc
2161 atcgccgctt ccactttttc ccgcgttttc gcagaaacgt ggctggcctg gttcaccacg
2221 cgggaaacgg tctgataaga gacaccggca tactctgcga catcgtacag cgttactggt
2281 ttcattgtac accctctcct tatattggga cctatggatc cggtcagtgc gtcctgctga
2341 tattcgacta taacaaacca ttttcttgcg taaacctgta cgatcctaca ggtgcttatg
2401 ttaagtaatt gtattcccag cgatacaata gtgtgacaaa aatccaattt attagaatca
2461 aatgtcaatc cattaccgtt ttaatgatat ataacacgca aaacttgcga caaacaatag
2521 gtaaggataa agagatgggt cggccgcccg ttccatggga tgagagtaag aacctgtcgg
2581 aatatcaaac agacaggttc tttatttagc atgagaaaaa taaagttgaa ggtggcgtta
2641 tattaaacgc gcttgctata agagtatttt actcaggagt gagaatctgg ttatttattg
2701 cccttaacca ttatcgacca cgatattgct tttgcgtaac agcgggcaat ctgttatccc
2761 caaaaaacca cttttagtgt gcaagtattg taccgtgctg gtgcctctgg cagtcagata
2821 ggtacattgc aaacctaatc ctgcggcatt ctctttgctt ccaatcaaaa cgccatatcc
2881 gctgagtaat aatcctatcc ataccagtgc tatcagcata actgtgcgaa tgatgaatcg
2941 cattacaacc tcttctcttt ttatgttcgc ttaatcgtag cggcaatatg cgctgaagca
3001 agcgactcat tccgaaaaag cacgaatatc gacatagtta ggcgctgttt aactaaccca
3061 tgctagttta atgacataag gtaggtgaaa cggagattgg agtcgaccgc ataggagaca
3121 ataagagggg ctagcatgtc tcgtttagat aaaagtaaag tgattaacag cgcattagag
3181 ctgcttaatg aggtcggaat cgaaggttta acaacccgta aactcgccca gaagctaggt
3241 gtagagcagc ctacattgta ttggcatgta aaaaataagc gggctttgct cgacgcctta
3301 gccattgaga tgttagatag gcaccatact cacttttgcc ctttagaagg ggaaagctgg
3361 caagattttt tacgtaataa cgctaaaagt tttagatgtg ctttactaag tcatcgcgat
3421 ggagcaaaag tacatttagg tacacggcct acagaaaaac agtatgaaac tctcgaaaat
3481 caattagcct ttttatgcca acaaggtttt tcactagaga atgcattata tgcactcagc
3541 gctgtggggc attttacttt aggttgcgta ttggaagatc aagagcatca agtcgctaaa
```

```
3601 gaagaaaggg aaacacctac tactgatagt atgccgccat tattacgaca agctatcgaa
3661 ttatttgatc accaaggtgc agagccagcc ttcttattcg gccttgaatt gatcatctgc
3721 ggattagaaa aacaacttaa atgtgaaagt gggtcttgag agctcggact gcttaagata
3781 aatataaata aggacaataa tcatggctga agcgcaaatc gatcccctgc tgccgggata
3841 ctcgtttaac gcccatctgg tggcgggttt aacgccgatt gaggccaacg gttatctcga
3901 tttttttatc gaccgaccgc tgggaatgaa aggttatatt ctcaatctca ccattcgcgg
3961 tcaggggggtg gtgaaaaatc agggacgaga atttggttgc cgaccgggtg atattttgct
4021 gttcccgcca ggagagattc atcactacgg tcgtcatccg gaggctcgcg aatggtatca
4081 ccagtgggtt tactttcgtc cgcgcgccta ctggcatgaa tggcttaact ggccgtcaat
4141 ctttgccaat acgggtttct ttcgcccgga tgaagcgcac cagccgcatt tcagcgacct
4201 gtttgggcaa atcattaacg ccgggcaagg ggaagggcgc tattcggagc tgctggcgat
4261 aaatctgctt gagcaactgt tactgcggcg catgggtgcg attaacgtgt cgctccatcc
4321 accgatggat aatcgggtac gcgaggcttg tcagtacatc agcgatcacc tggcagacag
4381 caattttgac atcgccagcg tcgcacagca tgtttgcttg tcgccgtcgc gtctgtcaca
4441 tcttttccgc cagcagttag ggattagcgt cctgagctgg cgcgaggacc aacgcattag
4501 tcaggcgaag ctgcttttga gcactacccg gatgcctatc gccaccgtcg gtcgcaatgt
4561 tggttttgac gatcaactct atttctcgcg agtatttaaa aaatgcaccg gggccagccc
4621 gagcgagttt cgtgccggta ctagcgcagc gaacgacgaa aattacgccg catcagtttg
4681 acatatgcga gaattggctt ggactcctgt tgatagatcc agtaatgacc tcagaactcc
4741 atctggattt gttcagaacg ctcggttgcc gccgggcgtt ttttattggt gagaatccaa
4801 gcagtagtca cccgggaatt gtgagcggat aacaatttca cacagggccc tcggacaccg
4861 aggagaatgt caagaggcga acacacaacg tcttggagcg ccagaggagg aacgagctaa
4921 aacggagctt ttttgccctg cgtgaccaga tcccggagtt ggaaaacaat gaaaaggccc
4981 ccaaggtagt tatccttaaa aaagccacag catacatcct gtccgtccaa gcagaggagc
5041 aaaagctcat ttctgaagag gacttgttgc ggaaacgacg agaacagttg aaacacaaac
5101 ttgaacagct acggaactct tgtgcgtaag gaaaagtaag gaaaacgatt ccttctaaca
5161 gaaatgtcct gagcaatcac ctatgaactg tcgattcgag catagcattt ttatccataa
5221 gattagcgga tcataatctt tacaattttg agcgctcaca attatgatag attcagttgt
5281 gagcggataa caatttcaca cacaattgag tattaactat cgttcaactg atagggaggc
5341 gccgatggtg agcaagggcg aggaggataa catggccatc atcaaggagt tcatgcgctt
5401 caaggttcac atggagggct ccgtgaacgg ccacgagttc gagatcgagg gcgagggcga
5461 gggccgcccc tacgagggca cccagaccgc caagctgaag gtgaccaagg gtggcccccct
5521 gcccttcgcc tgggacatcc tgtcccctca gttcatgtac ggctccaagg cctacgtgaa
5581 gcaccccgcc gacatccccg actacttgaa gctgtccttc cccgagggct tcaagtggga
5641 gcgcgtgatg aacttcgagg acggcggcgt ggtgaccgtg acccaggact cctccctgca
5701 agacggcgag ttcatctaca aggtgaagct gcgcggcacc aacttcccct ccgacggccc
5761 cgtaatgcag aagaagacta tgggctggga ggcctcctcc gagcggatgt accccgagga
5821 cggcgcgctg aagggcgaga tcaagcagag gctgaagctg aaggacggcg gccactacga
5881 cgctgaggtc aagaccacct acaaggccaa gaagcccgtg caactgcccg gcgcgtacaa
5941 cgtcaacatc aagttggaca tcacctccca caacgaggac tacaccatcg tggaacagta
6001 cgaacgcgcc gagggccgcc actccaccgg cggcatggac gagctgtata agtaagatat
6061 ctatcgccct agggaccgtc tcgagagaat caatattaat ccaacgcgtg gcatcaaata
6121 aaacgaaagg ctcagtcgaa agactgggcc tttcgtttta tctgttgttt gtcggtgaac
6181 gctctcctga gtaggacaaa tccgccgccc tagacttagg cgttcggctg cggcgagcgg
6241 tatcagctca ctcaaaggcg gtaatacggt tatccacaga atcaggggat aacgcaggaa
6301 agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg
6361 cgtttttcca taggctccgc ccccctgacg agcatcacaa aaatcgacgc tcaagtcaga
6421 ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga agctccctcg
6481 tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt ctcccttcgg
6541 gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg taggtcgttc
6601 gctccaagct gggctgtctg cacgaacccc ccgttcagcc cgaccgctgc gccttatccg
6661 gtaactatcg tcttgagtcc aacccggtaa gacacgactt atcgccactg gcagcagcca
6721 ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc ttgaagtggt
6781 ggcctaacta cggctacact agaaggacag tatttggtat ctgcgctctg ctgaagccag
6841 ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc gctggtagcg
```

```
     6901 gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct caagaagatc
     6961 ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt taagggattt
     7021 tggtcatggc tagtgcttgg attctcacca ataaaaaacg cccggcggca accgagcgtt
     7081 ctgaacaaat ccagatggag ttctgaggtc attactggat ctatcaacag gagtccaagc
     7141 caattctcga accccagagt cccgctcaga agaactcgtc aagaaggcga tagaaggcga
     7201 tgcgctgcga atcgggagcg gcgataccgt aaagcacgag gaagcggtca gcccattcgc
     7261 cgccaagctc ttcagcaata tcacgggtag ccaacgctat gtcctgatag cggtccgcca
     7321 cacccagccg gccacagtcg atgaatccag aaaagcggcc attttccacc atgatattcg
     7381 gcaagcaggc atcgccgtgg gtcacgacga gatcctcgcc gtcgggcata cgcgccttga
     7441 gcctggcgaa cagttcggct ggcgcgagcc cctgatgctc ttcgtccaga tcatcctgat
     7501 cgacaagacc ggcttccatc cgagtacgtg ctcgctcgat gcgatgtttc gcttggtggt
     7561 cgaatgggca ggtagccgga tcaagcgtat gcagccgccg cattgcatca gccatgatgg
     7621 atactttctc ggcaggagca aggtgagatg acaggagatc ctgccccggc acttcgccca
     7681 atagcagcca gtcccttccc gcttcagtga caacgtcgag cacagccgcg caaggaacgc
     7741 ccgtcgtggc cagccacgat agccgcgctg cctcgtcttg cagttcattc agggcaccgg
     7801 acaggtcggt cttgacaaaa agaaccgggc gaccctgcgc tgacagccgg aacacggcgg
     7861 catcagagca gccgattgtc tgttgtgccc agtcatagcc gaatagcctc tccacccaag
     7921 ccgccggaga acctgcgtgc aatccatctt gttcaatcat gcgaaacgat cctcatcctg
     7981 tctcttgatc agatcttgat cccctgcgcc atcagatcct tggcggcaag aaagccatcc
     8041 agtttacttt gcagggcttc ccaaccttac cagagggcgg cccaactggc aattcc
//
```