Model-driven Engineering of Gene Expression from RNA Replicons

Supplementary Material

Jacob Beal, Tyler E. Wagner, Tasuku Kitada, Odizze Azazgolshani,
Jordan Moberg Parker, Douglas Densmore, Ron Weiss

Contents

1 Exceptions to Replicate Numbers

The following replicates failed, and were omitted from analysis:

- Dose-response time series: Replicate #2 for at hour 3 of lowest dosage.
- Dual transfection experiment: nothing omitted.
- Three-color experiment: Replicate #2 for Mixture #5.

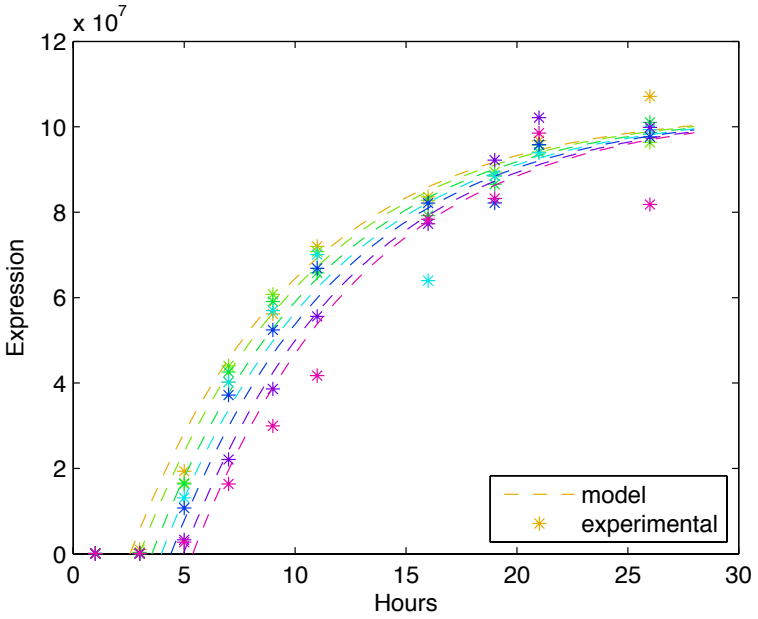
2 Modeling Replication

The RNA replication process is expected to increase the number of copies of RNA exponentially, then saturate at some converged level in response to additional constraints, such as resource scarcity, self-regulation of the NSP complex, cell internal immune response, etc.

This process can be modeled at varying levels of detail. The choice of modeling detail depends criticially, however, on the degree to which a model can be constrained by its observable effects, in this case on fluorescent expression.

In the main text, we have chosen the simplest possible approximation, of a fixed delay before saturation. This model is clearly inadequate in general, as indicated by the fact that the lowest two dosages climb toward saturated fluorescent expression noticeably more slowly. Do we have good enough data, however, to support a more complex model? Supplementary Figure 1 shows a least-squares best fit to a model that adds one more degree of freedom, making the lag to saturation logarithmically dependent on the initial dose. As can be seen, however, adding this degree of freedom does not improve the model, as the errors are similar in size to the entire range of systematic variation predicted by the model.

If the fine structure of the initial transient is deemed important, future investigation could improve this model by using measurements with increased precision, gathering data at finer increments of conditions, or using protocols that can measure replication dynamics directly, such as qRT-PCR.



Supplementary Figure 1: When dose-dependence is included in the model of replication, the observed experimental data has errors on the same scale as the prediction variations enabled by dose-dependence, indicating that such a model enhancement cannot be well-supported by the data in this manuscript.

3 Full Details of Predicted vs. Observed Results

Supplementary Figure 2 presents the fold-error for all predictions vs. time, showing that there is no time that dominates the prediction error.

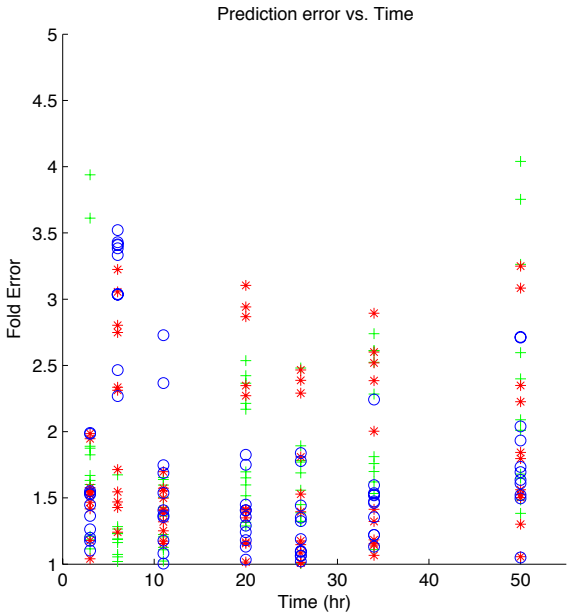
Supplementary Figure 3 compares predicted and experimentally observed geometric mean for all time points and all three-color mixtures. Dosages used for three-color mixtures are listed in the main text and repeated for convenience in Supplementary Table 1.

Supplementary Figures 4 through 10 compare predicted and experimentally observed distributions of fluorescence for every time point and every three-color mixutre. Note that the raggedness of the experimental data at the lowest levels comes from quantization of very low flow cytometry values.

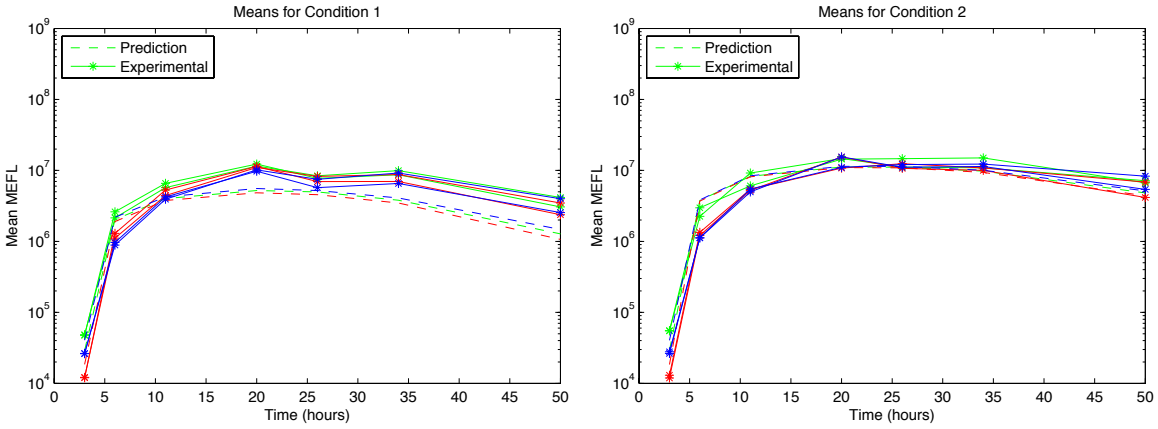
Predictions for Conditions 1 through 4 relate to the observed values in a fairly straightforward manner: differences are generally caused by small differences in the location of the peaks and the relative weight of the transfected and untransfected populations. For Conditions 5 and 6, in which some replicons have very low dosage, the observed behavior is significantly less bimodal than the predictions, with a distribution that "smears" more evenly between low expression and untransfected cells. This suggests that additional sources of variability not currently included in our model may be having a significant impact, and may be worth studying for future inclusion in order to improve accuracy.

Condition	mVenus (ng)	mKate (ng)	EBFP2 (ng)
1	180	180	180
2	540	540	540
3	180	900	720
4	360	360	1080
5	18	180	900
6	720	36	36

Supplementary Table 1: Dosages used for three-replicon mixtures.

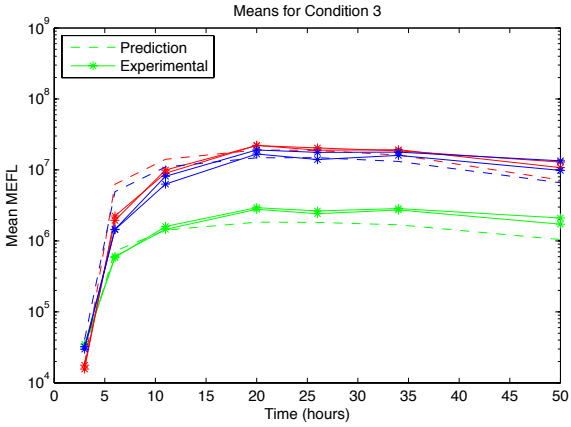


Supplementary Figure 2: The error of predictions is not dominated by samples at any particular time.

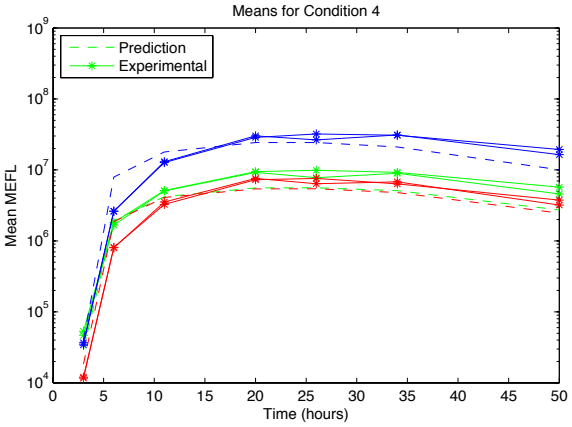


(a) Condition 1

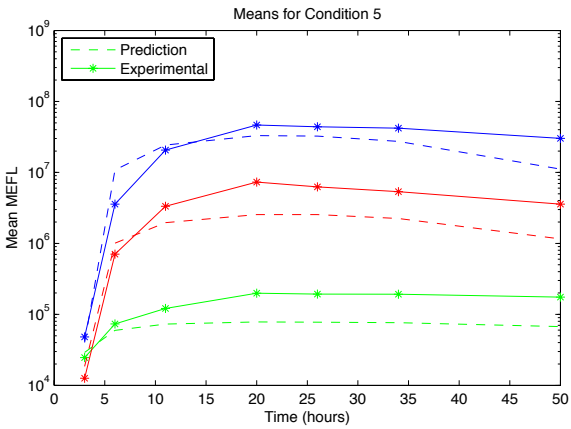
(b) Condition 2



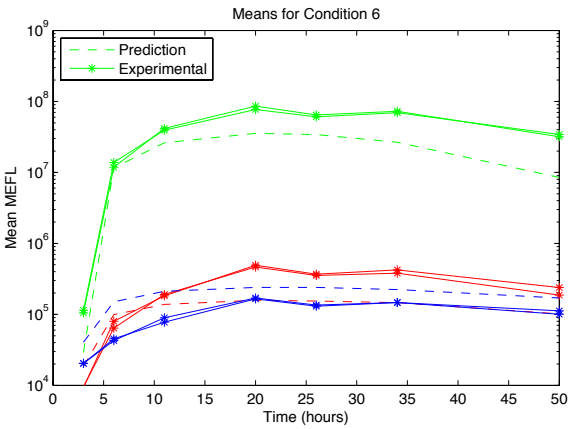
(c) Condition 3



(d) Condition 4

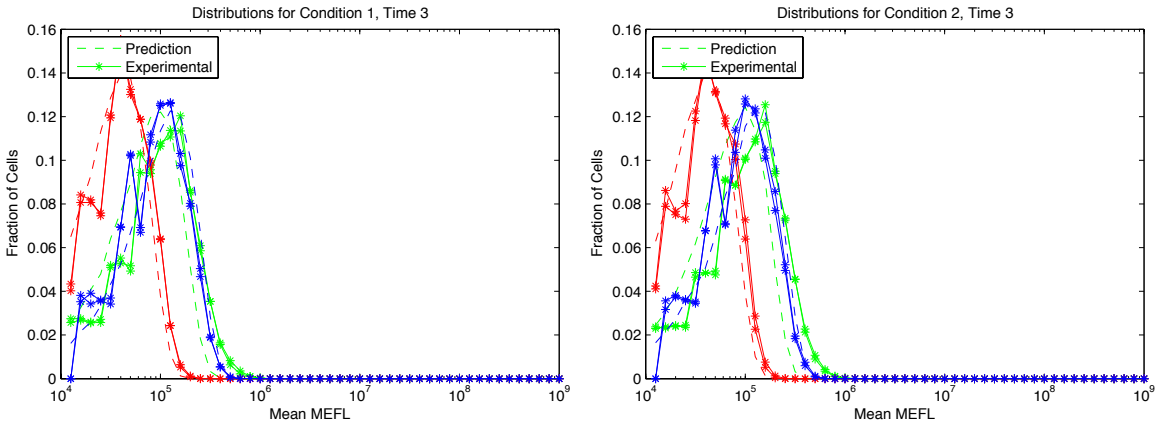


(e) Condition 5



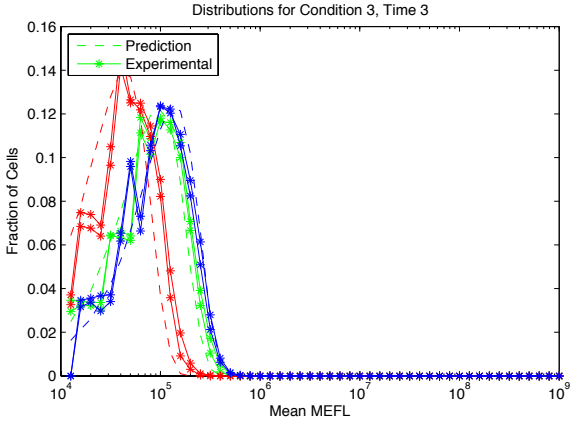
(f) Condition 6

Supplementary Figure 3: Predicted (dashed) vs. experimentally observed (solid) geometric mean fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions and time points.

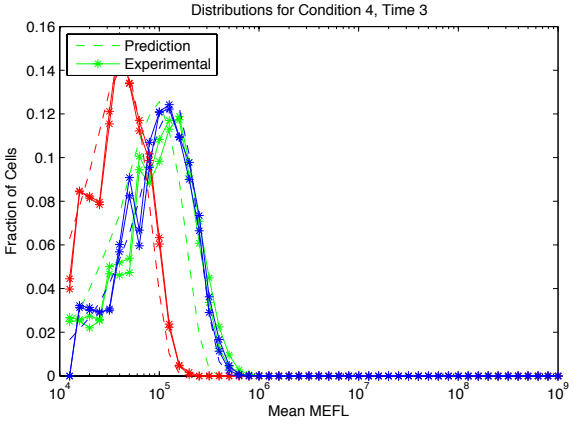


(a) Condition 1

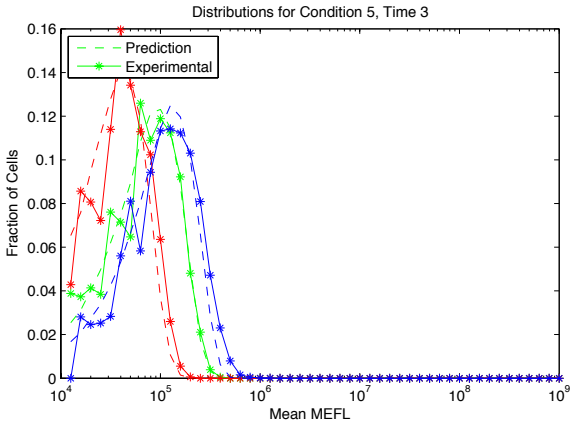
(b) Condition 2



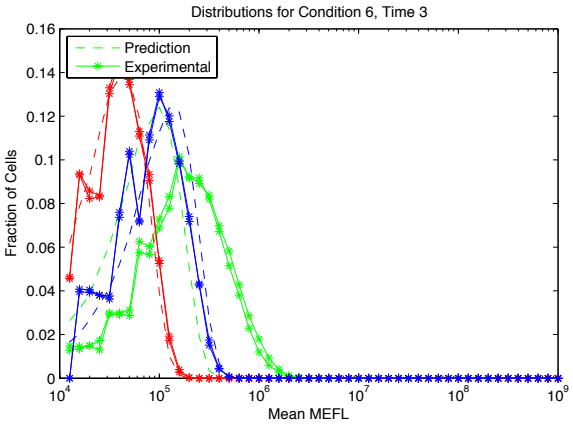
(c) Condition 3



(d) Condition 4

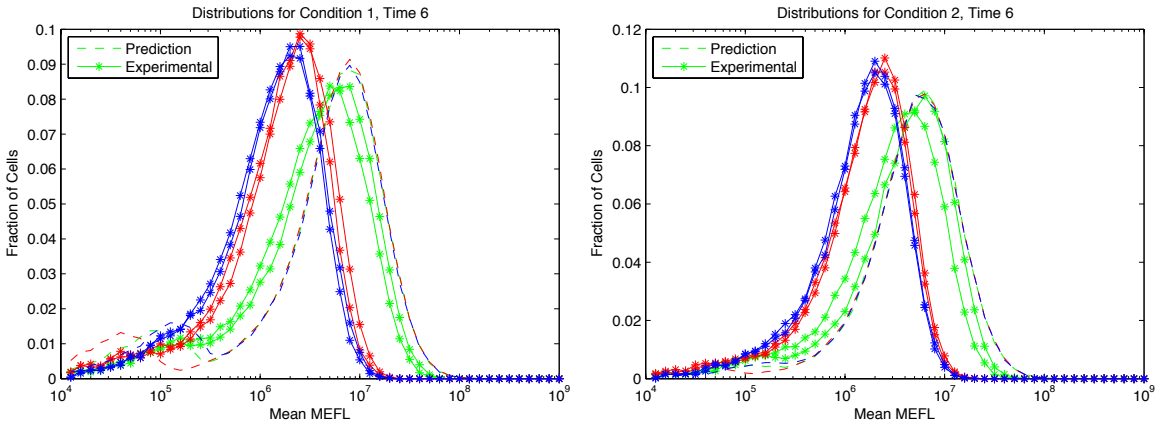


(e) Condition 5



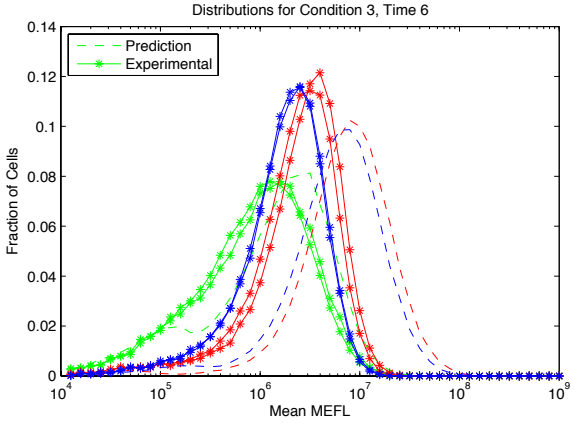
(f) Condition 6

Supplementary Figure 4: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 3 hours post-transfection.

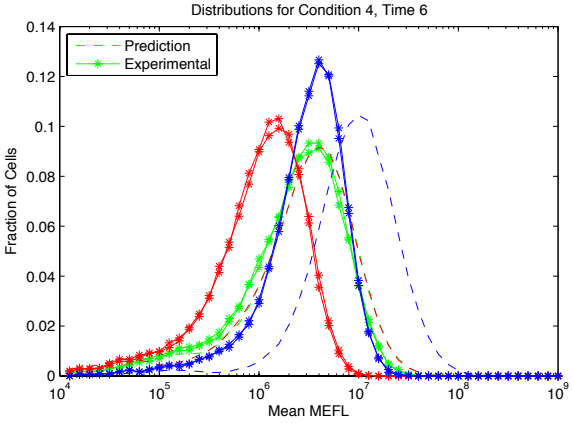


(a) Condition 1

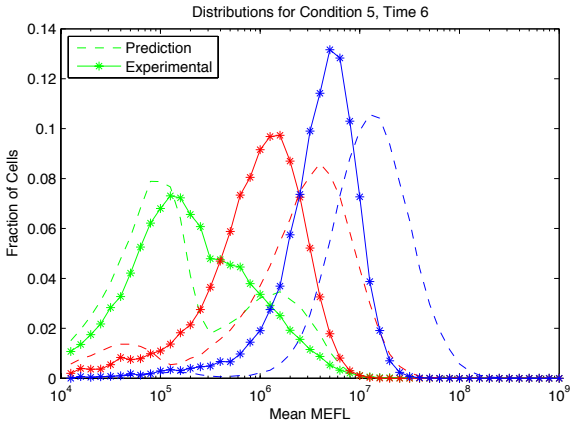
(b) Condition 2



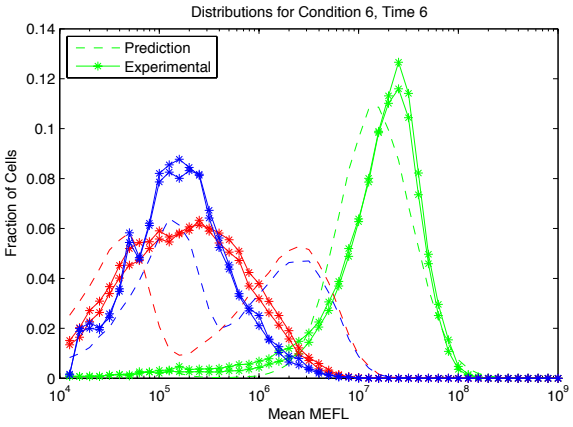
(c) Condition 3



(d) Condition 4

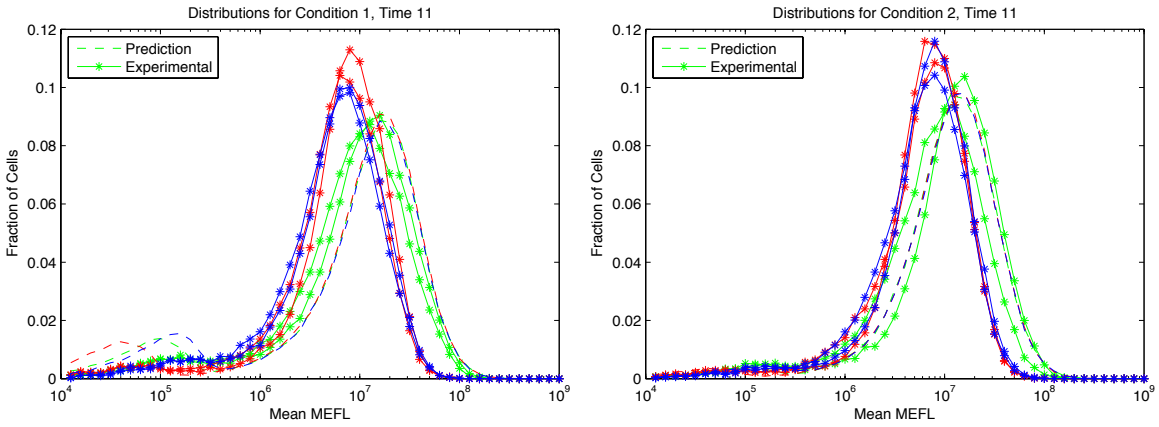


(e) Condition 5



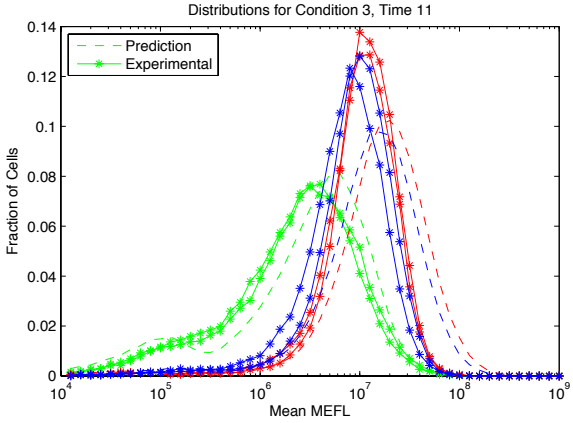
(f) Condition 6

Supplementary Figure 5: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 6 hours post-transfection.

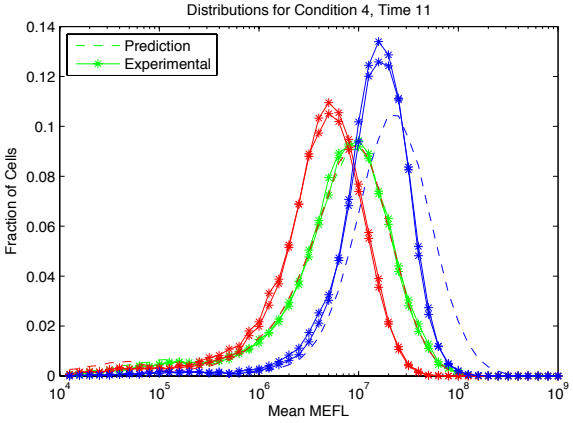


(a) Condition 1

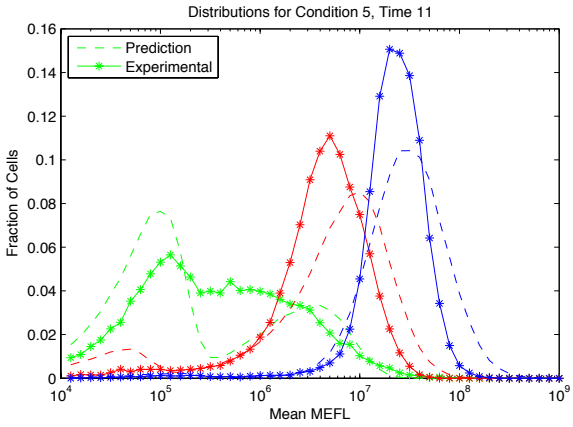
(b) Condition 2



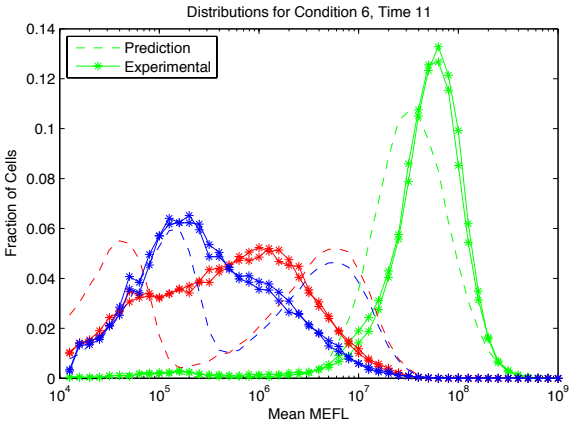
(c) Condition 3



(d) Condition 4

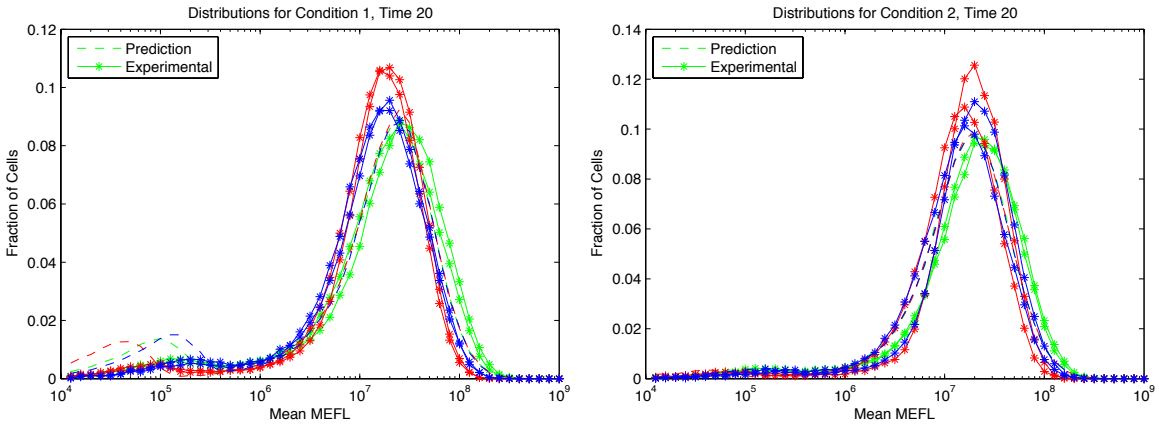


(e) Condition 5



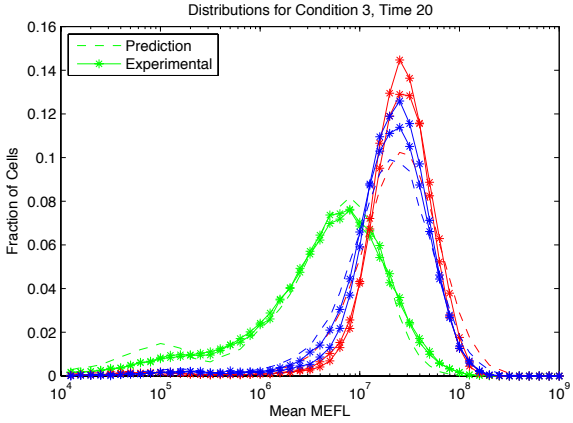
(f) Condition 6

Supplementary Figure 6: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 11 hours post-transfection.

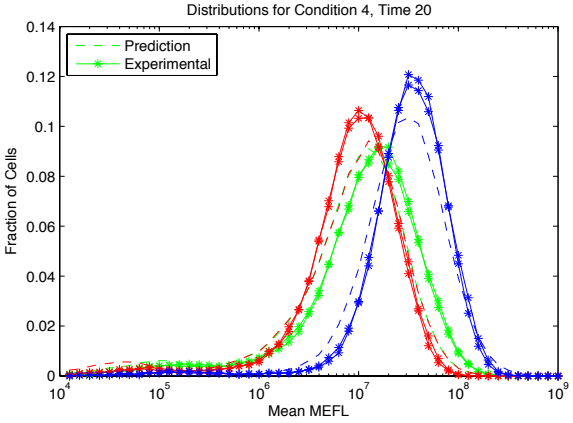


(a) Condition 1

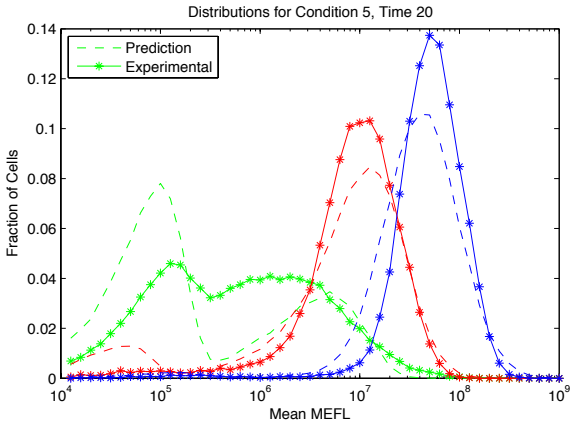
(b) Condition 2



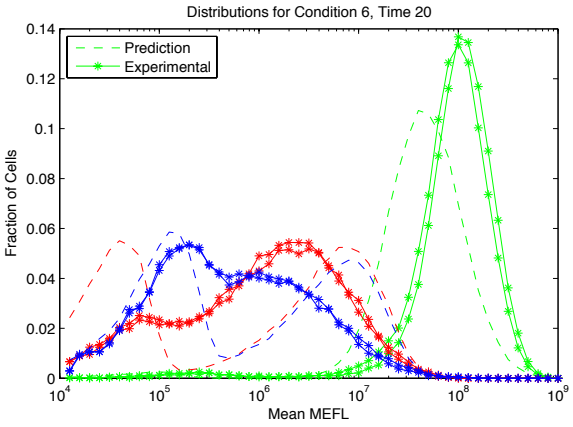
(c) Condition 3



(d) Condition 4

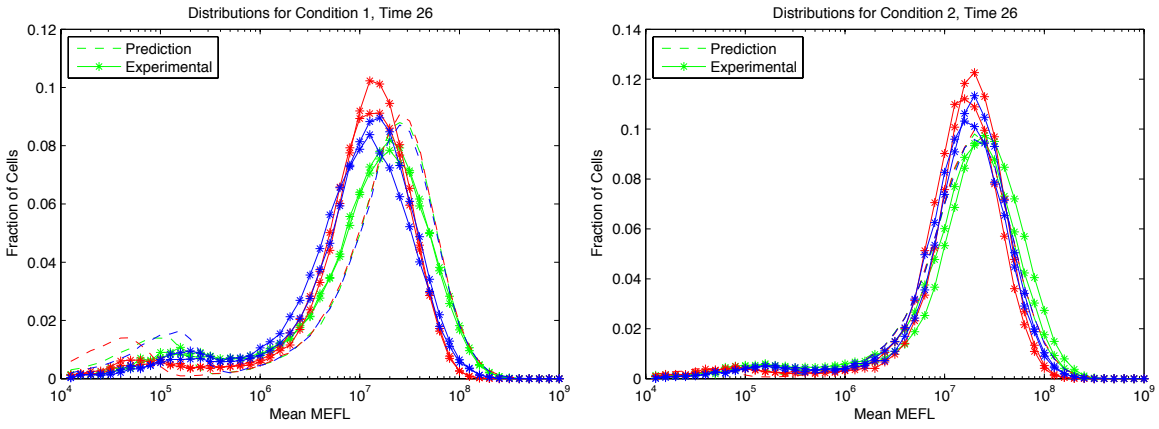


(e) Condition 5



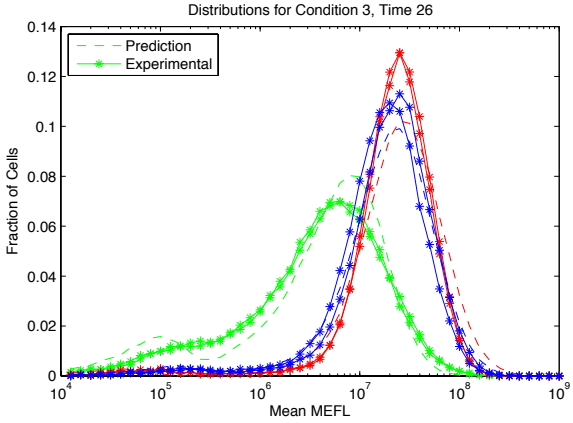
(f) Condition 6

Supplementary Figure 7: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 20 hours post-transfection.

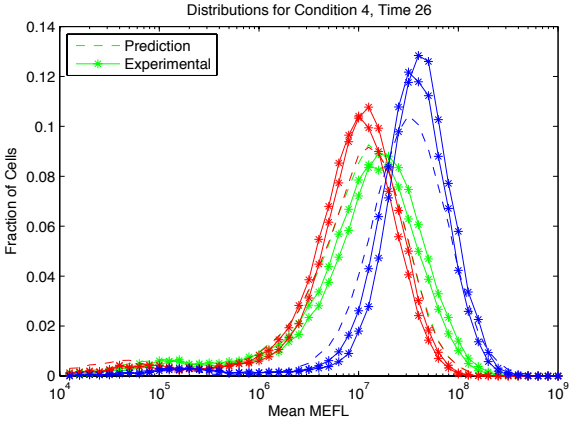


(a) Condition 1

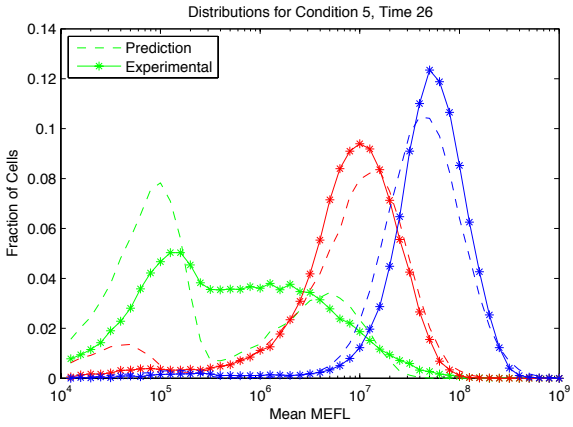
(b) Condition 2



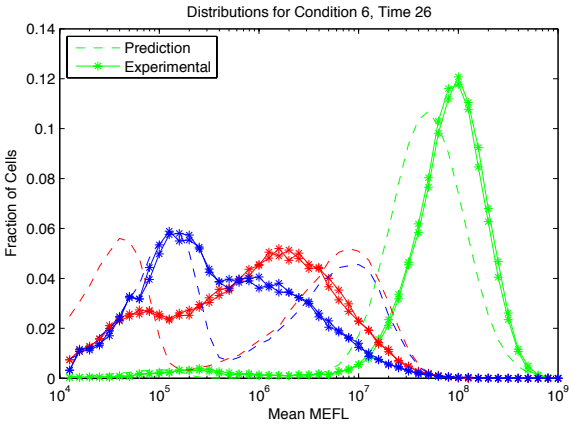
(c) Condition 3



(d) Condition 4

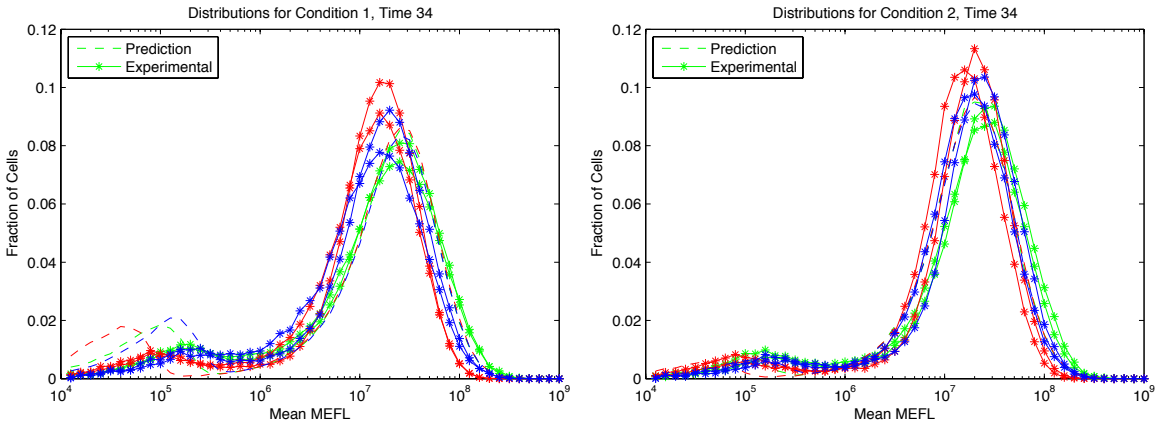


(e) Condition 5



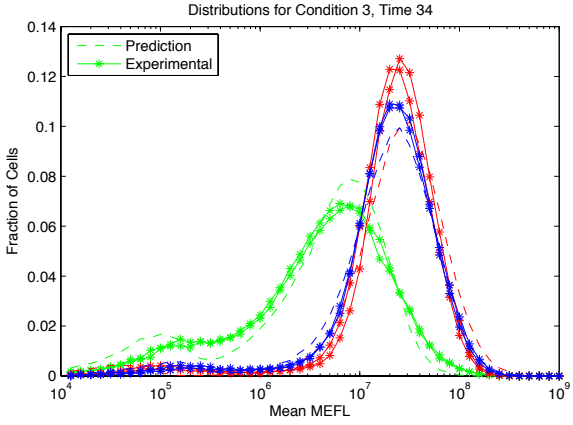
(f) Condition 6

Supplementary Figure 8: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 26 hours post-transfection.

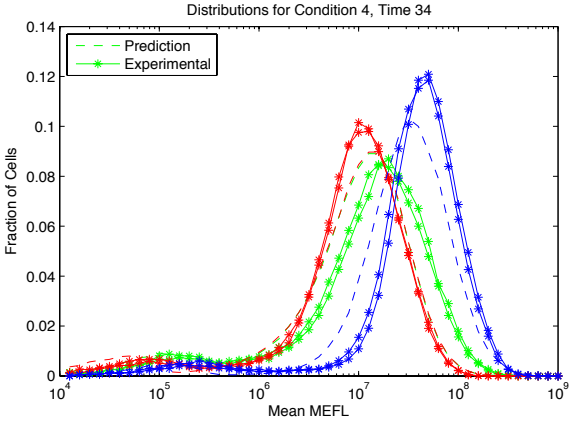


(a) Condition 1

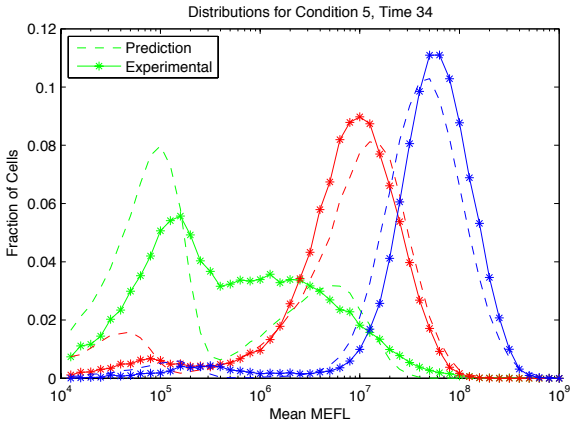
(b) Condition 2



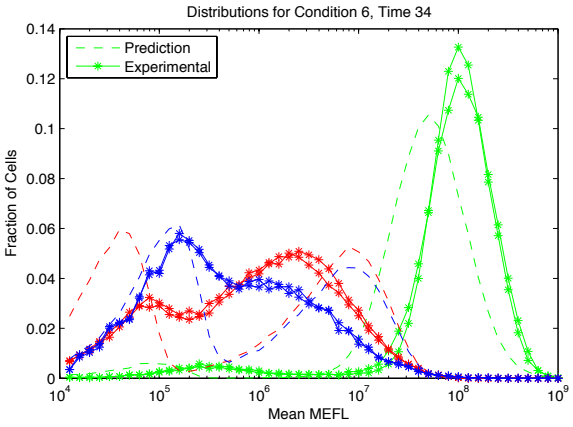
(c) Condition 3



(d) Condition 4

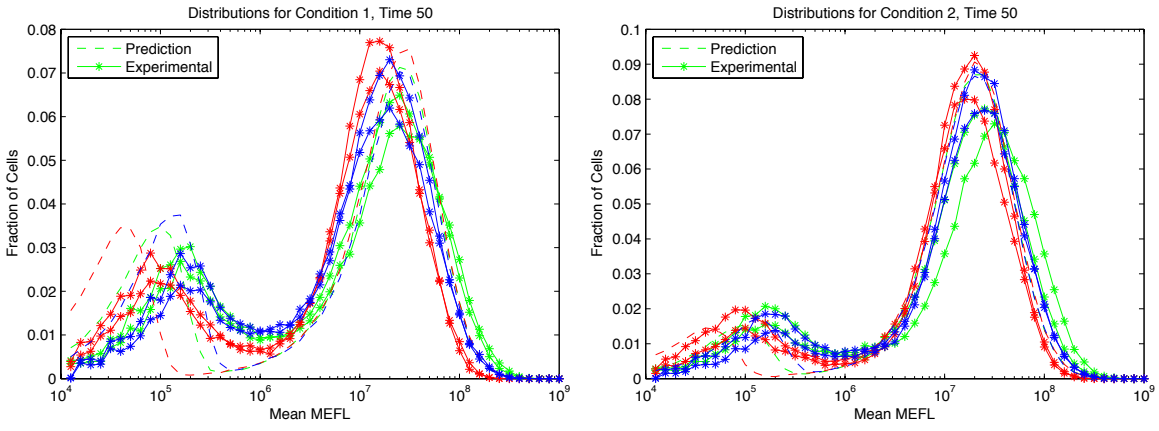


(e) Condition 5



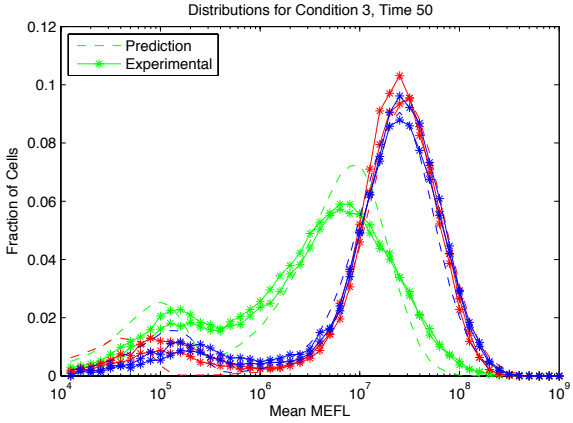
(f) Condition 6

Supplementary Figure 9: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 34 hours post-transfection.

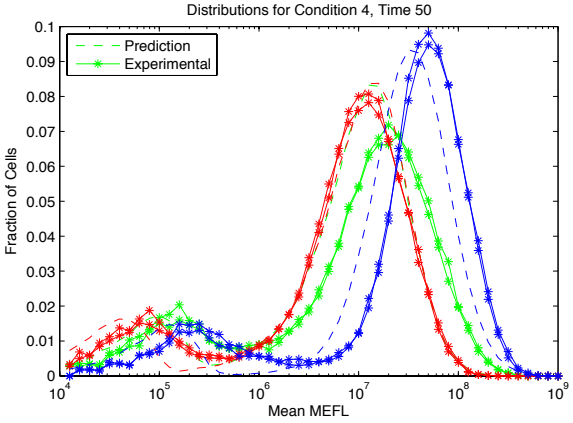


(a) Condition 1

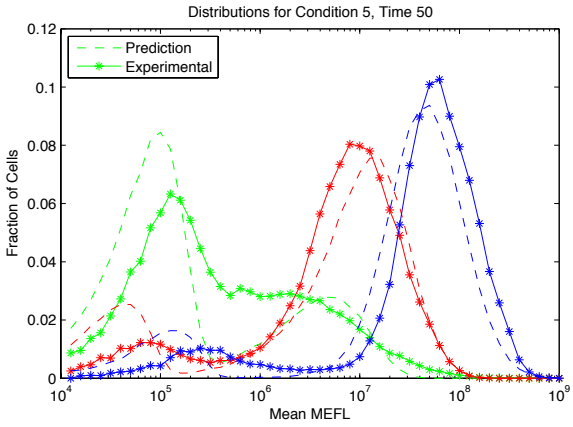
(b) Condition 2



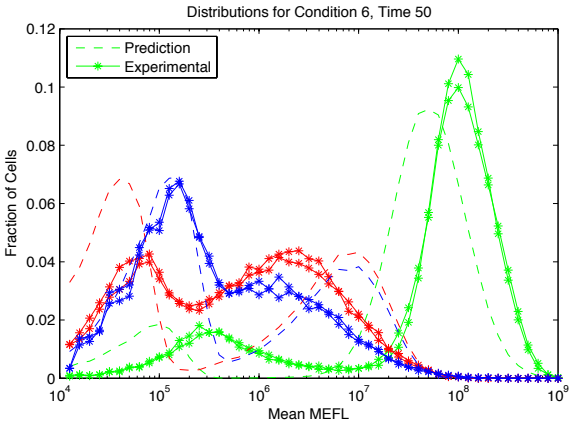
(c) Condition 3



(d) Condition 4



(e) Condition 5



(f) Condition 6

Supplementary Figure 10: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 50 hours post-transfection.

4 Replicon Sequences

```
LOCUS       pTK035                 10544 bp    DNA     circular UNA 20-JUN-2013
DEFINITION  SP6 SIN P1234 (P726S ncp) SGP mKate.
ACCESSION   urn.local...1371481879831.1250
VERSION     urn.local...1371481879831.1250
KEYWORDS    .
SOURCE      Sindbis
  ORGANISM  Sindbis
            .
FEATURES             Location/Qualifiers
     5'UTR           1..59
                     /label="5'UTR"
     CDS             60..1679
                     /label="nsP1"
     primer_bind     227..247
                     /label="TK_RTPCR_FP"
     primer_bind     227..247
                     /label="1st forward primer"
     primer_bind     227..247
                     /label="2nd forward primer"
     primer_bind     227..247
                     /label="3rd forward primer"
     primer_bind     227..247
                     /label="4th forward primer"
     primer_bind     227..247
                     /label="5th forward primer"
     misc_feature    311..330
                     /note="Geneious type: DNA probe bind"
                     /label="1st probe"
     misc_feature    311..330
                     /note="Geneious type: DNA probe bind"
                     /label="2nd probe"
     misc_feature    311..330
                     /note="Geneious type: DNA probe bind"
                     /label="3rd probe"
     misc_feature    complement(358..378)
                     /note="Geneious type: primer_bind_reverse"
                     /label="3rd reverse primer"
     misc_feature    complement(359..379)
                     /note="Geneious type: primer_bind_reverse"
                     /label="4th reverse primer"
     misc_feature    complement(360..379)
                     /note="Geneious type: primer_bind_reverse"
                     /label="1st reverse primer"
     misc_feature    complement(360..379)
                     /note="Geneious type: primer_bind_reverse"
                     /label="2nd reverse primer"
     misc_feature    complement(360..379)
                     /note="Geneious type: primer_bind_reverse"
                     /label="3rd reverse primer"
     misc_feature    complement(366..385)
                     /note="Geneious type: primer_bind_reverse"
                     /label="2nd reverse primer"
     misc_feature    complement(366..384)
                     /note="Geneious type: primer_bind_reverse"
                     /label="5th reverse primer"
     CDS             1680..4100
                     /label="nsP2"
     primer_bind     2517..2538
                     /label="TK-SIN-ClaI-ncp-seq-F1"
     misc_feature    2992
                     /note="Geneious type: Editing History Replacement"
                     /label="C"
     misc_feature    3579
                     /note="Geneious type: Editing History Replacement"
                     /label="A"
     misc_feature    3855
                     /note="Geneious type: Mutation"
                     /label="P726S ncp mutation"
     misc_feature    3855
                     /note="Geneious type: Editing History Replacement"
                     /label="C"
     CDS             4101..5747
                     /label="nsP3"
     primer_bind     complement(4443..4462)
                     /label="TK-SIN-AvrII-ncp-seq-R1"
```

```
     misc_feature    5748..5750
                     /note="Geneious type: Stop codon"
                     /label="Opal stop codon"
     CDS             5751..7601
                     /label="nsP4"
     primer_bind     6086..6105
                     /label="TK-SIN-ts-seq-F1"
     promoter        7500..7611
                     /label="Subgenomic promoter (98/14 enhanced)"
     promoter        7579..7602
                     /label="Subgenomic promoter (19/5 minimal)"
     misc_feature    7598..7646
                     /label="Subgenomic RNA 5' UTR"
     primer_bind     7637..7676
                     /label="SGP-BsiWI-mKate-F"
     CDS             7653..8366
                     /label="mKate"
     primer_bind     complement(7953..7972)
                     /label="mKate-seq-R"
     primer_bind     complement(8343..8382)
                     /label="UTR-XhoI-mKate-R"
     3'UTR           <8367..8588
                     /label="3'UTR"
     misc_feature    8397
                     /note="Geneious type: Editing History Insertion"
                     /label
     misc_feature    8588
                     /note="Geneious type: Editing History Replacement"
                     /label="A"
     CDS             8820..9677
                     /label="AmpR"
     promoter        10527..10544
                     /label="SP6"
ORIGIN
        1 attgacggcg tagtacacac tattgaatca aacagccgac caattgcact accatcacaa
       61 tggagaagcc agtagtaaac gtagacgtag acccccagag tccgtttgtc gtgcaactgc
      121 aaaaaagctt cccgcaattt gaggtagtag cacagcaggt cactccaaat gaccatgcta
      181 atgccagagc attttcgcat ctggccagta aactaatcga gctggaggtt cctaccacag
      241 cgacgatctt ggacataggc agcgcaccgg ctcgtagaat gttttccgag caccagtatc
      301 attgtgtctg ccccatgcgt agtccagaag acccggaccg catgatgaaa tacgccagta
      361 aactggcgga aaaagcgtgc aagattacaa acaagaactt gcatgagaag attaaggatc
      421 tccggaccgt acttgatacg ccggatgctg aaacaccatc gctctgcttt cacaacgatg
      481 ttacctgcaa catgcgtgcc gaatattccg tcatgcagga cgtgtatatc aacgctcccg
      541 gaactatcta tcatcaggct atgaaaggcg tgcggaccct gtactggatt ggcttcgaca
      601 ccacccagtt catgttctcg gctatggcag gttcgtaccc tgcgtacaac accaactggg
      661 ccgacgagaa agtccttgaa gcgcgtaaca tcggactttg cagcacaaag ctgagtgaag
      721 gtaggacagg aaaattgtcg ataatgagga agaaggagtt gaagcccggg tcgcgggttt
      781 atttctccgt aggatcgaca ctttatccag aacacagagc cagcttgcag agctggcatc
      841 ttccatcggt gttccacttg aatggaaagc agtcgtacac ttgccgctgt gatacagtgg
      901 tgagttgcga aggctacgta gtgaagaaaa tcaccatcag tcccgggatc acgggagaaa
      961 ccgtgggata cgcggttaca cacaatagcg agggcttctt gctatgcaaa gttactgaca
     1021 cagtaaaagg agaacgggta tcgttccctg tgtgcacgta catcccggcc accatatgcg
     1081 atcagatgac tggtataatg gccacggata tatcacctga cgatgcacaa aaacttctgg
     1141 ttgggctcaa ccagcgaatt gtcattaacg gtaggactaa caggaacacc aacaccatgc
     1201 aaaattacct tctgccgatc atagcacaag ggttcagcaa atgggctaag gagcgcaagg
     1261 atgatcttga taacgagaaa atgctgggta ctagagaacg caagcttacg tatggctgct
     1321 tgtgggcgtt tcgcactaag aaagtacatt cgttttatcg cccacctgga acgcagacct
     1381 gcgtaaaagt cccagcctct tttagcgctt ttcccatgtc gtccgtatgg acgacctctt
     1441 tgcccatgtc gctgaggcag aaattgaaac tggcattgca accaaagaag gaggaaaaac
     1501 tgctgcaggt ctcggaggaa ttagtcatgg aggccaaggc tgcttttgag gatgctcagg
     1561 aggaagccag agcggagaag ctccgagaag cacttccacc attagtggca gacaaaggca
     1621 tcgaggcagc cgcagaagtt gtctgcgaag tggaggggct ccaggcggac atcggagcag
     1681 cattagttga aaccccgcgc ggtcacgtaa ggataatacc tcaagcaaat gaccgtatga
     1741 tcggacagta tatcgttgtc tcgccaaact ctgtgctgaa gaatgccaaa ctcgcaccag
     1801 cgcacccgct agcagatcag gttaagatca taacacactc cggaagatca ggaaggtacg
     1861 cggtcgaacc atacgacgct aaagtactga tgccagcagg aggtgccgta ccatggccag
     1921 aattcctagc actgagtgag agcgccacgt tagtgtacaa cgaaagagag tttgtgaacc
     1981 gcaaactata ccacattgcc atgcatggcc ccgccaagaa tacagaagag gagcagtaca
     2041 aggttacaaa ggcagagctt gcagaaacag agtacgtgtt tgacgtggac aagaagcgtt
     2101 gcgttaagaa ggaagaagcc tcaggtctgg tcctctcggg agaactgacc aaccctccct
     2161 atcatgagct agctctggag ggactgaaga cccgacctgc ggtcccgtac aaggtcgaaa
     2221 caataggagt gataggcaca ccggggtcgg gcaagtcagc tattatcaag tcaactgtca
     2281 cggcacgaga tcttgttacc agcggaaaga aagaaaattg tcgcgaaatt gaggccgacg
     2341 tgctaagact gaggggtatg cagattacgt cgaagacagt agattcggtt atgctcaacg
     2401 gatgccacaa agccgtagaa gtgctgtacg ttgacgaagc gttcgcgtgc cacgcaggag
     2461 cactacttgc cttgattgct atcgtcaggc cccgcaagaa ggtagtacta tgcggagacc
     2521 ccatgcaatg cggattcttc aacatgatgc aactaaaggt acatttcaat caccctgaaa
```

2581 aagacatatg caccaagaca ttctacaagt atatctcccg gcgttgcaca cagccagtta
2641 cagctattgt atcgacactg cattacgatg gaaagatgaa aaccacgaac ccgtgcaaga
2701 agaacattga aatcgatatt acaggggcca caaagccgaa gccaggggat atcatcctga
2761 catgtttccg cgggtgggtt aagcaattgc aaatcgacta tcccggacat gaagtaatga
2821 cagccgcggc ctcacaaggg ctaaccagaa aaggagtgta tgccgtccgg caaaaagtca
2881 atgaaaaccc actgtacgcg atcacatcag agcatgtgaa cgtgttgctc acccgcactg
2941 aggacaggct agtgtggaaa accttgcagg gcgacccatg gattaagcag ctcactaaca
3001 tacctaaagg aaactttcag gctactatag aggactggga agctgaacac aagggaataa
3061 ttgctgcaat aaacagcccc actccccgtg ccaatccgtt cagctgcaag accaacgttt
3121 gctgggcgaa agcattggaa ccgatactag ccacggccgg tatcgtactt accggttgcc
3181 agtggagcga actgttccca cagtttgcgg atgacaaacc acattcggcc atttacgcct
3241 tagacgtaat ttgcattaag tttttcggca tggacttgac aagcggactg ttttctaaac
3301 agagcatccc actaacgtac catcccgccg attcagcgag gccggtagct cattgggaca
3361 acagcccagg aacccgcaag tatgggtacg atcacgccat tgccgccgaa ctctcccgta
3421 gatttccggt gttccagcta gctgggaagg gcacacaact tgatttgcag acgggagaa
3481 ccagagttat ctctgcacag cataacctgg tcccggtgaa ccgcaatctt cctcacgcct
3541 tagtccccga gtacaaggag aagcaacccg gcccggtcga aaaattcttg aaccagttca
3601 aacaccactc agtacttgtg gtatcagagg aaaaaattga agctccccgt aagagaatcg
3661 aatggatcgc cccgattggc atagccggtg cagataagaa ctacaacctg gctttcgggt
3721 ttccgccgca ggcacggtac gacctggtgt tcatcaacat tggaactaaa tacagaaacc
3781 accactttca gcagtgcgaa gaccatgcgg cgaccttaaa aacccttttcg cgttcggccc
3841 tgaattgcct taactcagga ggcaccctcg tggtgaagtc ctatggctac gccgaccgca
3901 acagtgagga cgtagtcacc gctcttgcca gaaagtttgt cagggtgtct gcagcgagac
3961 cagattgtgt ctcaagcaat acagaaatgt acctgatttt ccgacaacta gacaacagcc
4021 gtacacggca attcaccccg caccatctga attgcgtgat ttcgtccgtg tatgagggta
4081 caagagatgg agttggagcc gcgccgtcat accgcaccaa aagggagaat attgctgact
4141 gtcaagagga agcagttgtc aacgcagcca atccgctggg tagaccaggc gaaggagtct
4201 gccgtgccat ctataaacgt tggccgacca gttttaccga ttcagccacg gagacaggca
4261 ccgcaagaat gactgtgtgc ctaggaaaga aagtgatcca cgcggtcggc cctgatttcc
4321 ggaagcaccc agaagcagaa gccttgaaat tgctacaaaa cgcctaccat gcagtggcag
4381 acttagtaaa tgaacataac atcaagtctg tcgccattcc actgctatct acaggcattt
4441 acgcagccgg aaaagaccgc cttgaagtat cacttaactg cttgacaacc gcgctagaca
4501 gaactgacgc ggacgtaacc atctattgcc tggataagaa gtggaaggaa agaatcgacg
4561 cggcactcca acttaaggag tctgtaacag agctgaagga tgaagatatg gagatcgacg
4621 atgagttagt atggatccat ccagacagtt gcttgaaggg aagaaaggga ttcagtacta
4681 caaaaggaaa attgtattcg tacttcgaag gcaccaaatt ccatcaagca gcaaaagaca
4741 tggcggagat aaaggtcctg ttccctaatg accaggaaag taatgaacaa ctgtgtgcct
4801 acatattggg tgagaccatg gaagcaatcc gcgaaaagtg cccggtcgac cataacccgt
4861 cgtctagccc gcccaaaacg ttgccgtgcc tttgcatgta tgccatgacg ccagaaaggg
4921 tccacagact tagaagcaat aacgtcaaag aagttacagt atgctcctcc accccccttc
4981 ctaagcacaa aattaagaat gttcagaagg ttcagtgcac gaaagtagtc ctgtttaatc
5041 cgcacactcc cgcattcgtt cccgcccgta agtacataga agtgccagaa cagcctaccg
5101 ctcctcctgc acaggccgag gaggcccccg aagttgtagc gacaccgtca ccatctacag
5161 ctgataacac ctcgcttgat gtcacagaca tctcactgga tatggatgac agtagcgaag
5221 gctcactttt ttcgagcttt agcggatcgg acaactctat tactagtatg gacagttggt
5281 cgtcaggacc tagttcacta gagatagtag accgaaggca ggtggtggtg gctgacgttc
5341 atgccgtcca agagcctgcc cctattccac cgccaaggct aaagaagatg gcccgcctgg
5401 cagcggcaag aaaagagccc actccaccgg caagcaatag ctctgagtcc ctccacctct
5461 cttttggtgg ggtatccatg tccctcggat caattttcga cggagagacg gcccgccagg
5521 cagcggtaca acccctggca acaggcccca cggatgtgcc tatgtctttc ggatcgtttt
5581 ccgacggaga gattgatgag ctgagccgca gagtaactga gtccgaaccc gtcctgtttg
5641 gatcatttga accgggcgaa gtgaactcaa ttatatcgtc ccgatcagcc gtatcttttc
5701 cactacgcaa gcagagacgt agacgcagga gcaggaggac tgaatactga ctaaccgggg
5761 taggtgggta catattttcg acggacacag gccctgggca cttgcaaaag aagtccgttc
5821 tgcagaacca gcttacagaa ccgaccttgg agcgcaatgt cctggaaaga attcatgccc
5881 cggtgctcga cacgtcgaaa gaggaacaac tcaaactcag gtaccagatg atgcccaccg
5941 aagccaacaa aagtaggtac cagtctcgta aagtagaaaa tcagaaagcc ataaccactg
6001 agcgactact gtcaggacta cgactgtata actctgccac agatcagcca gaatgctata
6061 agatcaccta tccgaaacca ttgtactcca gtagcgtacc ggcgaactac tccgatccac
6121 agttcgctgt agctgtctgt aacaactatc tgcatgagaa ctatccgaca gtagcatctt
6181 atcagattac tgacgagtac gatgcttact tggatatggt agacgggaca gtcgcctgcc
6241 tggatactgc aaccttctgc cccgctaagc ttagaagtta cccgaaaaaa catgagtata
6301 gagccccgaa tatccgcagt gcggttccat cagcgatgca gaacacgcta caaaatgtgc
6361 tcattgccgc aactaaaaga aattgcaacg tcacgcagat gcgtgaactg ccaacactgg
6421 actcagcgac attcaatgtc gaatgctttc gaaaatatgc atgtaatgac gagtattggg
6481 aggagttcgc tcggaagcca attaggatta ccactgagtt tgtcaccgca tatgtagcta
6541 gactgaaagg ccctaaggcc gccgcactat ttgcaaagac gtataatttg gtcccattgc
6601 aagaagtgcc tatggataga ttcgtcatgg acatgaaaag agacgtgaaa gttacaccag
6661 gcacgaaaca cacagaagaa agaccgaaag tacaagtgat acaagccgca gaacccctgg
6721 cgactgctta cttatgcggg attcaccggg aattagtgcg taggcttacg gccgtcttgc
6781 ttccaaacat tcacacgctt tttgacatgt cggcggagga ttttgatgca atcatagcag
6841 aacacttcaa gcaaggcgac ccggtactgg agacggatat cgcatcattc gacaaaagcc
6901 aagacgacgc tatggcgtta accggtctga tgatcttgga ggacctgggt gtggatcaac
6961 cactactcga cttgatcgag tgcgcctttg gagaaatatc atccacccat ctacctacgg
7021 gtactcgttt taaattcggg gcgatgatga aatccggaat gttcctcaca ctttttgtca
7081 acacagtttt gaatgtcgtt atcgccagca gagtactaga agagcggctt aaaacgtcca
7141 gatgtgcagc gttcattggc gacgacaaca tcatacatgg agtagtatct gacaaagaaa

```
     7201 tggctgagag gtgcgccacc tggctcaaca tggaggttaa gatcatcgac gcagtcatcg
     7261 gtgagagacc accttacttc tgcggcggat ttatcttgca agattcggtt acttccacag
     7321 cgtgccgcgt ggcggatccc ctgaaaaggc tgtttaagtt gggtaaaccg ctcccagccg
     7381 acgacgagca agacgaagac agaagacgcg ctctgctaga tgaaacaaag gcgtggttta
     7441 gagtaggtat aacaggcact ttagcagtgg ccgtgacgac ccggtatgag gtagacaata
     7501 ttacacctgt cctactggca ttgagaactt ttgcccagag caaaagagca ttccaagcca
     7561 tcagagggga aataaagcat ctctacggtg gtcctaaata gtcagcatag tacatttcat
     7621 ctgactaata ctacaacacc accacccgta cgatggtgtc taagggcgaa gagctgatta
     7681 aggagaacat gcacatgaag ctgtacatgg agggcaccgt gaacaaccac cacttcaagt
     7741 gcacatccga gggcgaaggc aagccctacg agggcaccca gaccatgaga atcaaggtgg
     7801 tcgagggcgg ccctctcccc ttcgccttcg acatcctggc taccagcttc atgtacggca
     7861 gcaaaacctt catcaaccac acccagggca tccccgactt ctttaagcag tccttccctg
     7921 agggcttcac atgggagaga gtcaccacat acgaagacgg gggcgtgctg accgctaccc
     7981 aggacaccag cctccaggac ggctgcctca tctacaacgt caagatcaga ggggtgaact
     8041 tcccatccaa cggccctgtg atgcagaaga aaacactcgg ctgggaggcc tccaccgaga
     8101 tgctgtaccc cgctgacggc ggcctggaag gcagaagcga catggccctg aagctcgtgg
     8161 gcgggggcca cctgatctgc aacttgaaga ccacatacag atccaagaaa cccgctaaga
     8221 acctcaagat gcccggcgtc tactatgtgg acagaagact ggaaagaatc aaggaggccg
     8281 acaaagagac ctacgtcgag cagcacgagg tggctgtggc cagatactgc gacctcccta
     8341 gcaaactggg gcacaaactt aattgactcg aggaagcgca gtacataatg ctgcgcagtg
     8401 ttgccacata accactatat taaccattta tctagcggac gccaaaaact caatgtattt
     8461 ctgaggaagc gtggtgcata atgccacgca gcgtctgcat aacttttatt atttctttta
     8521 ttaatcaaca aaattttgtt tttaacattt caaaaaaaaa aaaaaaaaaa aaaaaaaaaa
     8581 aaaaaaaggg gaattcgagc tcgggaatta attcttgaag acgaaagggc ctcgtgatac
     8641 gcctattttt ataggttaat gtcatgataa taatggtttc ttagacgtca ggtggcactt
     8701 ttcggggaaa tgtgcgcgga acccctattt gtttatttttt ctaaatacat tcaaatatgt
     8761 atccgctcat gagacaataa ccctgataaa tgcttcaata atattgaaaa aggaagagta
     8821 tgagtattca acatttccgt gtcgccctta ttccctttttt tgcggcattt tgccttcctg
     8881 tttttgctca cccagaaacg ctggtgaaag taaaagatgc tgaagatcag ttgggtgcac
     8941 gagtgggtta catcgaactg gatctcaaca gcggtaagat ccttgagagt tttcgccccg
     9001 aagaacgttt tccaatgatg agcactttta aagttctgct atgtggcgcg gtattatccc
     9061 gtgttgacgc cgggcaagag caactcggtc gccgcataca ctattctcag aatgacttgg
     9121 ttgagtactc accagtcaca gaaaagcatc ttacggatgg catgacagta agagaattat
     9181 gcagtgctgc cataaccatg agtgataaca ctgcggccaa cttacttctg acaacgatcg
     9241 gaggaccgaa ggagctaacc gcttttttgc acaacatggg ggatcatgta actcgccttg
     9301 atcgttggga accggagctg aatgaagcca taccaaacga cgagcgtgac accacgatgc
     9361 ctgtagcaat ggcaacaacg ttgcgcaaac tattaactgg cgaactactt actctagctt
     9421 cccggcaaca attaatagac tggatggagg cggataaagt tgcaggacca cttctgcgct
     9481 cggcccttcc ggctggctgg tttattgctg ataaatctgg agccggtgag cgtgggtctc
     9541 gcggtatcat tgcagcactg gggccagatg gtaagccctc ccgtatcgta gttatctaca
     9601 cgacggggag tcaggcaact atggatgaac gaaatagaca gatcgctgag ataggtgcct
     9661 cactgattaa gcattggtaa ctgtcagacc aagtttactc atatatactt tagattgatt
     9721 taaaacttca tttttaattt aaaaggatct aggtgaagat ccttttgat aatctcatga
     9781 ccaaaatccc ttaacgtgag ttttcgttcc actgagcgtc agaccccgta gaaaagatca
     9841 aaggatcttc ttgagatcct ttttttctgc gcgtaatctg ctgcttgcaa acaaaaaaac
     9901 caccgctacc agcggtggtt tgtttgccgg atcaagagct accaactctt tttccgaagg
     9961 taactggctt cagcagagcg cagataccaa atactgtcct tctagtgtag ccgtagttag
    10021 gccaccactt caagaactct gtagcaccgc ctacatacct cgctctgcta atcctgttac
    10081 cagtggctgc tgccagtggc gataagtcgt gtcttaccgg gttggactca agacgatagt
    10141 taccggataa ggcgcagcgg tcgggctgaa cgggggttc gtgcacacag cccagcttgg
    10201 agcgaacgac ctacaccgaa ctgagatacc tacagcgtga gcattgagaa agcgccacgc
    10261 ttcccgaagg gagaaaggcg gacaggtatc cggtaagcgg cagggtcgga acaggagagc
    10321 gcacgaggga gcttccaggg ggaaacgcct ggtatcttta tagtcctgtc gggtttcgcc
    10381 acctctgact tgagcgtcga tttttgtgat gctcgtcagg ggggcggagc ctatggaaaa
    10441 acgccagcaa cgcgagctcg tatggacata ttgtcgttag aacgcggcta caattaatac
    10501 ataaccttat gtatcataca catacgattt aggtgacact atag
//
LOCUS       pTK036                 10550 bp    DNA     circular UNA 26-NOV-2012
DEFINITION  SP6 SIN P1234 (P726S ncp) SGP EBFP2.
ACCESSION   urn.local...1371481879834.1251
VERSION     urn.local...1371481879834.1251
KEYWORDS    .
SOURCE      Sindbis
  ORGANISM  Sindbis
            .
FEATURES             Location/Qualifiers
     5'UTR           1..59
                     /label="5'UTR"
     CDS             60..1679
                     /label="nsP1"
     CDS             1680..4100
                     /label="nsP2"
     primer_bind     2517..2538
                     /label="TK-SIN-ClaI-ncp-seq-F1"
     misc_feature    2992
                     /note="Geneious type: Editing History Replacement"
                     /label="C"
```

```
     misc_feature    3579
                     /note="Geneious type: Editing History Replacement"
                     /label="A"
     misc_feature    3855
                     /note="Geneious type: Mutation"
                     /label="P726S ncp mutation"
     misc_feature    3855
                     /note="Geneious type: Editing History Replacement"
                     /label="C"
     CDS             4101..5747
                     /label="nsP3"
     primer_bind     complement(4443..4462)
                     /label="TK-SIN-AvrII-ncp-seq-R1"
     misc_feature    5748..5750
                     /note="Geneious type: Stop codon"
                     /label="Opal stop codon"
     CDS             5751..7601
                     /label="nsP4"
     primer_bind     6086..6105
                     /label="TK-SIN-ts-seq-F1"
     promoter        7500..7611
                     /label="Subgenomic promoter (98/14 enhanced)"
     promoter        7579..7602
                     /label="Subgenomic promoter (19/5 minimal)"
     misc_feature    7598..7646
                     /label="Subgenomic RNA 5' UTR"
     primer_bind     7637..7676
                     /label="SGP-BsiWI-YFP-F"
     CDS             7653..8372
                     /label="EBFP2"
     primer_bind     complement(8349..8388)
                     /label="UTR-XhoI-YFP-R"
     3'UTR           <8373..8594
                     /label="3'UTR"
     misc_feature    8403
                     /note="Geneious type: Editing History Insertion"
                     /label
     misc_feature    8594
                     /note="Geneious type: Editing History Replacement"
                     /label="A"
     CDS             8826..9683
                     /label="AmpR"
     promoter        10533..10550
                     /label="SP6"
ORIGIN
        1 attgacggcg tagtacacac tattgaatca aacagccgac caattgcact accatcacaa
       61 tggagaagcc agtagtaaac gtagacgtag acccccagag tccgtttgtc gtgcaactgc
      121 aaaaaagctt cccgcaattt gaggtagtag cacagcaggt cactccaaat gaccatgcta
      181 atgccagagc attttcgcat ctggccagta aactaatcga gctggaggtt cctaccacag
      241 cgacgatctt ggacataggc agcgcaccgg ctcgtagaat gttttccgag caccagtatc
      301 attgtgtctg ccccatgcgt agtccagaag acccggaccg catgatgaaa tacgccagta
      361 aactggcgga aaaagcgtgc aagattacaa acaagaactt gcatgagaag attaaggatc
      421 tccggaccgt acttgatacg ccggatgctg aaacaccatc gctctgcttt cacaacgatg
      481 ttacctgcaa catgcgtgcc gaatattccg tcatgcagga cgtgtatatc aacgctcccg
      541 gaactatcta tcatcaggct atgaaaggcg tgcggaccct gtactggatt ggcttcgaca
      601 ccacccagtt catgttctcg gctatggcag gttcgtaccc tgcgtacaac accaactggg
      661 ccgacgagaa agtccttgaa gcgcgtaaca tcggactttg cagcacaaag ctgagtgaag
      721 gtaggacagg aaaattgtcg ataatgagga agaaggagtt gaagcccggg tcgcgggttt
      781 atttctccgt aggatcgaca ctttatccag aacacagagc cagcttgcag agctggcatc
      841 ttccatcggt gttccacttg aatggaaagc agtcgtacac ttgccgctgt gatacagtgg
      901 tgagttgcga aggctacgta gtgaagaaaa tcaccatcag tcccgggatc acgggagaaa
      961 ccgtgggata cgcggttaca cacaatagcg agggcttctt gctatgcaaa gttactgaca
     1021 cagtaaaagg agaacgggta tcgttccctg tgtgcacgta catcccggcc accatatgcg
     1081 atcagatgac tggtataatg gccacggata tatcacctga cgatgcacaa aaacttctgg
     1141 ttgggctcaa ccagcgaatt gtcattaacg gtaggactaa caggaacacc aacaccatgc
     1201 aaaattacct tctgccgatc atagcacaag ggttcagcaa atgggctaag gagcgcaagg
     1261 atgatcttga taacgagaaa atgctgggta ctagagaacg caagcttacg tatggctgct
     1321 tgtgggcgtt tcgcactaag aaagtacatt cgttttatcg cccacctgga acgcagacct
     1381 gcgtaaaagt cccagcctct tttagcgctt ttcccatgtc gtccgtatgg acgacctctt
     1441 tgcccatgtc gctgaggcag aaattgaaac tggcattgca accaaagaag gaggaaaaac
     1501 tgctgcaggt ctcggaggaa ttagtcatgg aggccaaggc tgcttttgag gatgctcagg
     1561 aggaagccag agcggagaag ctccgagaag cacttccacc attagtggca gacaaaggca
     1621 tcgaggcagc cgcagaagtt gtctgcgaag tggaggggct ccaggcggac atcggagcag
     1681 cattagttga aaccccgcgc ggtcacgtaa ggataatacc tcaagcaaat gaccgtatga
     1741 tcggacagta tatcgttgtc tcgccaaact ctgtgctgaa gaatgccaaa ctcgcaccag
     1801 cgcacccgct agcagatcag gttaagatca taacacactc cggaagatca ggaaggtacg
     1861 cggtcgaacc atacgacgct aaagtactga tgccagcagg aggtgccgta ccatggccag
```

1921 aattcctagc actgagtgag agcgccacgt tagtgtacaa cgaaagagag tttgtgaacc
1981 gcaaactata ccacattgcc atgcatggcc ccgccaagaa tacagaagag gagcagtaca
2041 aggttacaaa ggcagagctt gcagaaacag agtacgtgtt tgacgtggac aagaagcgtt
2101 gcgttaagaa ggaagaagcc tcaggtctgg tcctctcggg agaactgacc aaccctccct
2161 atcatgagct agctctggag ggactgaaga cccgacctgc ggtcccgtac aaggtcgaaa
2221 caataggagt gataggcaca ccggggtcgg gcaagtcagc tattatcaag tcaactgtca
2281 cggcacgaga tcttgttacc agcggaaaga aagaaaattg tcgcgaaatt gaggccgacg
2341 tgctaagact gaggggtatg cagattacgt cgaagacagt agattcggtt atgctcaacg
2401 gatgccacaa agccgtagaa gtgctgtacg ttgacgaagc gttcgcgtgc cacgcaggag
2461 cactacttgc cttgattgct atcgtcaggc cccgcaagaa ggtagtacta tgcggagacc
2521 ccatgcaatg cggattcttc aacatgatgc aactaaaggt acatttcaat caccctgaaa
2581 aagacatatg caccaagaca ttctacaagt atatctcccg gcgttgcaca cagccagtta
2641 cagctattgt atcgacactg cattacgatg gaaagatgaa aaccacgaac ccgtgcaaga
2701 agaacattga aatcgatatt acaggggcca caaagccgaa gccaggggat atcatcctga
2761 catgtttccg cgggtgggtt aagcaattgc aaatcgacta tcccggacat gaagtaatga
2821 cagccgcggc ctcacaaggg ctaaccagaa aaggagtgta tgccgtccgg caaaaagtca
2881 atgaaaaccc actgtacgcg atcacatcag agcatgtgaa cgtgttgctc acccgcactg
2941 aggacaggct agtgtggaaa accttgcagg gcgacccatg gattaagcag ctcactaaca
3001 tacctaaagg aaactttcag gctactatag aggactggga agctgaacac aagggaataa
3061 ttgctgcaat aaacagcccc actccccgtg ccaatccgtt cagctgcaag accaacgttt
3121 gctgggcgaa agcattggaa ccgatactag ccacggccgg tatcgtactt accggttgcc
3181 agtggagcga actgttccca cagtttgcgg atgacaaacc acattcggcc atttacgcct
3241 tagacgtaat ttgcattaag tttttcggca tggacttgac aagcggactg ttttctaaac
3301 agagcatccc actaacgtac catcccgccg attcagcgag gccggtagct cattgggaca
3361 acagcccagg aacccgcaag tatgggtacg atcacgccat tgccgccgaa ctctcccgta
3421 gatttccggt gttccagcta gctgggaagg gcacacaact tgatttgcag acggggagaa
3481 ccagagttat ctctgcacag cataacctgg tcccggtgaa ccgcaatctt cctcacgcct
3541 tagtccccga gtacaaggag aagcaacccg gcccggtcga aaaattcttg aaccagttca
3601 aacaccactc agtacttgtg gtatcagagg aaaaaattga agctccccgt aagagaatcg
3661 aatggatcgc cccgattggc atagccggtg cagataagaa ctacaacctg gctttcgggt
3721 ttccgccgca ggcacggtac gacctggtgt tcatcaacat tggaactaaa tacagaaacc
3781 accactttca gcagtgcgaa gaccatgcgg cgaccttaaa aacccttcg cgttcggccc
3841 tgaattgcct taactcagga ggcaccctcg tggtgaagtc ctatggctac gccgaccgca
3901 acagtgagga cgtagtcacc gctcttgcca gaaagtttgt cagggtgtct gcagcgagac
3961 cagattgtgt ctcaagcaat acagaaatgt acctgatttt ccgacaacta gacaacagcc
4021 gtacacggca attcaccccg caccatctga attgcgtgat ttcgtccgtg tatgagggta
4081 caagagatgg agttggagcc gcgccgtcat accgcaccaa aagggagaat attgctgact
4141 gtcaagagga agcagttgtc aacgcagcca atccgctggg tagaccaggc gaaggagtct
4201 gccgtgccat ctataaacgt tggccgacca gttttaccga ttcagccacg gagacaggca
4261 ccgcaagaat gactgtgtgc ctaggaaaga aagtgatcca cgcggtcggc cctgatttcc
4321 ggaagcaccc agaagcagaa gccttgaaat tgctacaaaa cgcctaccat gcagtggcag
4381 acttagtaaa tgaacataac atcaagtctg tcgccattcc actgctatct acaggcattt
4441 acgcagccgg aaaagaccgc cttgaagtat cacttaactg cttgacaacc gcgctagaca
4501 gaactgacgc ggacgtaacc atctattgcc tggataagaa gtggaaggaa agaatcgacg
4561 cggcactcca acttaaggag tctgtaacag agctgaagga tgaagatatg gagatcgacg
4621 atgagttagt atggatccat ccagacagtt gcttgaaggg aagaaaggga ttcagtacta
4681 caaaaggaaa attgtattcg tacttcgaag gcaccaaatt ccatcaagca gcaaaagaca
4741 tggcggagat aaaggtcctg ttccctaatg accaggaaag taatgaacaa ctgtgtgcct
4801 acatattggg tgagaccatg gaagcaatcc gcgaaaagtg cccggtcgac cataacccgt
4861 cgtctagccc gcccaaaacg ttgccgtgcc tttgcatgta tgccatgacg ccagaaaggg
4921 tccacagact tagaagcaat aacgtcaaag aagttacagt atgctcctcc accccccttc
4981 ctaagcacaa aattaagaat gttcagaagg ttcagtgcac gaaagtagtc ctgtttaatc
5041 cgcacactcc cgcattcgtt cccgcccgta agtacataga agtgccagaa cagcctaccg
5101 ctcctcctgc acaggccgag gaggcccccg aagttgtagc gacaccgtca ccatctacag
5161 ctgataacac ctcgcttgat gtcacagaca tctcactgga tatggatgac agtagcgaag
5221 gctcactttt ttcgagcttt agcggatcgg acaactctat tactagtatg gacagttggt
5281 cgtcaggacc tagttcacta gagatagtag accgaaggca ggtggtggtg gctgacgttc
5341 atgccgtcca agagcctgcc cctattccac cgccaaggct aaagaagatg gcccgcctgg
5401 cagcggcaag aaaagagccc actccaccgg caagcaatag ctctgagtcc ctccacctct
5461 cttttggtgg ggtatccatg tccctcggat caattttcga cggagagacg gcccgccagg
5521 cagcggtaca acccctggca acaggcccca cggatgtgcc tatgtctttc ggatcgtttt
5581 ccgacggaga gattgatgag ctgagccgca gagtaactga gtccgaaccc gtcctgtttg
5641 gatcatttga accgggcgaa gtgaactcaa ttatatcgtc ccgatcagcc gtatcttttc
5701 cactacgcaa gcagagacgt agacgcagga gcaggaggac tgaatactga ctaaccgggg
5761 taggtgggta catattttcg acggacacag gccctgggca cttgcaaaag aagtccgttc
5821 tgcagaacca gcttacagaa ccgaccttgg agcgcaatgt cctggaaaga attcatgccc
5881 cggtgctcga cacgtcgaaa gaggaacaac tcaaactcag gtaccagatg atgcccaccg
5941 aagccaacaa aagtaggtac cagtctcgta aagtagaaaa tcagaaagcc ataaccactg
6001 agcgactact gtcaggacta cgactgtata actctgccac agatcagcca gaatgctata
6061 agatcaccta tccgaaacca ttgtactcca gtagcgtacc ggcgaactac tccgatccac
6121 agttcgctgt agctgtctgt aacaactatc tgcatgagaa ctatccgaca gtagcatctt
6181 atcagattac tgacgagtac gatgcttact tggatatggt agacgggaca gtcgcctgcc
6241 tggatactgc aaccttctgc cccgctaagc ttagaagtta cccgaaaaaa catgagtata
6301 gagccccgaa tatccgcagt gcggttccat cagcgatgca gaacacgcta caaaatgtgc
6361 tcattgccgc aactaaaaga aattgcaacg tcacgcagat gcgtgaactg ccaacactgg
6421 actcagcgac attcaatgtc gaatgctttc gaaaatatgc atgtaatgac gagtattggg
6481 aggagttcgc tcggaagcca attaggatta ccactgagtt tgtcaccgca tatgtagcta

```
     6541 gactgaaagg ccctaaggcc gccgcactat ttgcaaagac gtataatttg gtcccattgc
     6601 aagaagtgcc tatggataga ttcgtcatgg acatgaaaag agacgtgaaa gttacaccag
     6661 gcacgaaaca cacagaagaa agaccgaaag tacaagtgat acaagccgca gaacccctgg
     6721 cgactgctta cttatgcggg attcaccggg aattagtgcg taggcttacg gccgtcttgc
     6781 ttccaaacat tcacacgctt tttgacatgt cggcggagga ttttgatgca atcatagcag
     6841 aacacttcaa gcaaggcgac ccggtactgg agacggatat cgcatcattc gacaaaagcc
     6901 aagacgacgc tatggcgtta accggtctga tgatcttgga ggacctgggt gtggatcaac
     6961 cactactcga cttgatcgag tgcgcctttg gagaaatatc atccacccat ctacctacgg
     7021 gtactcgttt taaattcggg gcgatgatga aatccggaat gttcctcaca ctttttgtca
     7081 acacagtttt gaatgtcgtt atcgccagca gagtactaga agagcggctt aaaacgtcca
     7141 gatgtgcagc gttcattggc gacgacaaca tcatacatgg agtagtatct gacaaagaaa
     7201 tggctgagag gtgcgccacc tggctcaaca tggaggttaa gatcatcgac gcagtcatcg
     7261 gtgagagacc accttacttc tgcggcggat ttatcttgca agattcggtt acttccacag
     7321 cgtgccgcgt ggcggatccc ctgaaaaggc tgtttaagtt gggtaaaccg ctcccagccg
     7381 acgacgagca agacgaagac agaagacgcg ctctgctaga tgaaacaaag gcgtggttta
     7441 gagtaggtat aacaggcact ttagcagtgg ccgtgacgac ccggtatgag gtagacaata
     7501 ttacacctgt cctactggca ttgagaactt ttgcccagag caaaagagca ttccaagcca
     7561 tcagagggga aataaagcat ctctacggtg gtcctaaata gtcagcatag tacatttcat
     7621 ctgactaata ctacaacacc accacccgta cgatggtgag caagggcgag gagctgttca
     7681 ccggggtggt gcccatcctg gtcgagctgg acggcgacgt aaacggccac aagttcagcg
     7741 tgaggggcga gggcgagggc gatgccacca acggcaagct gaccctgaag ttcatctgca
     7801 ccaccggcaa gctgcccgtg ccctggccca ccctcgtgac caccctgagc cacggcgtgc
     7861 agtgcttcgc ccgctacccc gaccacatga agcagcacga cttcttcaag tccgccatgc
     7921 ccgaaggcta cgtccaggag cgcaccatct tcttcaagga cgacggcacc tacaagaccc
     7981 gcgccgaggt gaagttcgag ggcgacaccc tagtgaaccg catcgagctg aagggcgtcg
     8041 acttcaagga ggacggcaac atcctggggc acaagctgga gtacaacttc aacagccaca
     8101 acatctatat catggccgtc aagcagaaga acggcatcaa ggtgaacttc aagatccgcc
     8161 acaacgtgga ggacggcagc gtgcagctcg ccgaccacta ccagcagaac accccatcg
     8221 gcgacggccc cgtgctgctg cccgacagcc actacctgag cacccagtcc gtgctgagca
     8281 aagaccccaa cgagaagcgc gatcacatgg tcctgctgga gttccgcacc gccgccggga
     8341 tcactctcgg catggacgag ctgtacaagt aactcgagga agcgcagtac ataatgctgc
     8401 gcagtgttgc cacataacca ctatattaac catttatcta gcggacgcca aaaactcaat
     8461 gtatttctga ggaagcgtgg tgcataatgc cacgcagcgt ctgcataact tttattattt
     8521 cttttattaa tcaacaaaat tttgttttta acatttcaaa aaaaaaaaaa aaaaaaaaaa
     8581 aaaaaaaaaa aaaggggaat tcgagctcgg gaattaattc ttgaagacga aagggcctcg
     8641 tgatacgcct atttttatag gttaatgtca tgataataat ggtttcttag acgtcaggtg
     8701 gcacttttcg gggaaatgtg cgcggaaccc ctatttgttt atttttctaa atacattcaa
     8761 atatgtatcc gctcatgaga caataaccct gataaatgct tcaataatat tgaaaaagga
     8821 agagtatgag tattcaacat ttccgtgtcg cccttattcc ctttttgcg gcattttgcc
     8881 ttcctgtttt tgctcaccca gaaacgctgg tgaaagtaaa agatgctgaa gatcagttgg
     8941 gtgcacgagt gggttacatc gaactggatc tcaacagcgg taagatcctt gagagttttc
     9001 gccccgaaga acgttttcca atgatgagca cttttaaagt tctgctatgt ggcgcggtat
     9061 tatcccgtgt tgacgccggg caagagcaac tcggtcgccg catacactat tctcagaatg
     9121 acttggttga gtactcacca gtcacagaaa agcatcttac ggatggcatg acagtaagag
     9181 aattatgcag tgctgccata accatgagtg ataacactgc ggccaactta cttctgacaa
     9241 cgatcggagg accgaaggag ctaaccgctt ttttgcacaa catgggggat catgtaactc
     9301 gccttgatcg ttgggaaccg gagctgaatg aagccatacc aaacgacgag cgtgacacca
     9361 cgatgcctgt agcaatggca acaacgttgc gcaaactatt aactggcgaa ctacttactc
     9421 tagcttcccg gcaacaatta atagactgga tggaggcgga taaagttgca ggaccacttc
     9481 tgcgctcggc ccttccggct ggctggttta ttgctgataa atctggagcc ggtgagcgtg
     9541 ggtctcgcgg tatcattgca gcactggggc cagatggtaa gccctcccgt atcgtagtta
     9601 tctacacgac ggggagtcag gcaactatgg atgaacgaaa tagacagatc gctgagatag
     9661 gtgcctcact gattaagcat tggtaactgt cagaccaagt ttactcatat atactttaga
     9721 ttgatttaaa acttcatttt taatttaaaa ggatctaggt gaagatcctt tttgataatc
     9781 tcatgaccaa aatcccttaa cgtgagtttt cgttccactg agcgtcagac cccgtagaaa
     9841 agatcaaagg atcttcttga gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa
     9901 aaaaaccacc gctaccagcg gtggtttgtt tgccggatca agagctacca actcttttc
     9961 cgaaggtaac tggcttcagc agagcgcaga taccaaatac tgtccttcta gtgtagccgt
    10021 agttaggcca ccacttcaag aactctgtag caccgcctac atacctcgct ctgctaatcc
    10081 tgttaccagt ggctgctgcc agtggcgata agtcgtgtct taccgggttg gactcaagac
    10141 gatagttacc ggataaggcg cagcggtcgg gctgaacggg gggttcgtgc acacagccca
    10201 gcttggagcg aacgacctac accgaactga gatacctaca gcgtgagcat tgagaaagcg
    10261 ccacgcttcc cgaagggaga aaggcggaca ggtatccggt aagcggcagg gtcggaacag
    10321 gagagcgcac gagggagctt ccagggggaa acgcctggta tctttatagt cctgtcgggt
    10381 ttcgccacct ctgacttgag cgtcgatttt tgtgatgctc gtcaggggggg cggagcctat
    10441 ggaaaaacgc cagcaacgcg agctcgtatg gacatattgt cgttagaacg cggctacaat
    10501 taatacataa ccttatgtat catacacata cgatttaggt gacactatag
//
LOCUS       pTK050                 10550 bp    DNA     circular UNA 11-JUL-2013
DEFINITION  SP6 SIN P1234 (P726S ncp) SGP mVenus.
ACCESSION   urn.local...1371481879923.1265
VERSION     urn.local...1371481879923.1265
KEYWORDS    .
SOURCE      Sindbis
  ORGANISM  Sindbis
            .
FEATURES             Location/Qualifiers
```

```
     5'UTR           1..59
                     /label="5'UTR"
     CDS             60..1679
                     /label="nsP1"
     misc_feature    153..209
                     /label="51 nt CSE"
     CDS             1680..4100
                     /label="nsP2"
     primer_bind     2517..2538
                     /label="TK-SIN-ClaI-ncp-seq-F1"
     misc_feature    2992
                     /note="Geneious type: Editing History Replacement"
                     /label="C"
     misc_feature    3579
                     /note="Geneious type: Editing History Replacement"
                     /label="A"
     misc_feature    3855
                     /note="Geneious type: Mutation"
                     /label="P726S ncp mutation"
     CDS             4101..5747
                     /label="nsP3"
     primer_bind     complement(4443..4462)
                     /label="TK-SIN-AvrII-ncp-seq-R1"
     misc_feature    5748..5750
                     /note="Geneious type: Stop codon"
                     /label="Opal stop codon"
     CDS             5751..7601
                     /label="nsP4"
     primer_bind     6086..6105
                     /label="TK-SIN-ts-seq-F1"
     promoter        7500..7611
                     /label="Subgenomic promoter (98/14 enhanced)"
     promoter        7579..7602
                     /label="Subgenomic promoter (19/5 minimal)"
     misc_feature    7598..7646
                     /label="Subgenomic RNA 5' UTR"
     primer_bind     7637..7676
                     /label="SGP-BsiWI-YFP-F"
     CDS             7653..8372
                     /label="mVenus"
     primer_bind     8069..8089
                     /label="TK_RT-PCR_mVenus_Fwd"
     misc_feature    8108..8131
                     /note="Geneious type: DNA probe bind"
                     /label="TK_RT-PCR_mVenus_TaqMan"
     misc_feature    complement(8148..8166)
                     /note="Geneious type: primer_bind_reverse"
                     /label="TK_RT-PCR_mVenus_Rev"
     primer_bind     complement(8349..8388)
                     /label="UTR-XhoI-YFP-R"
     3'UTR           8373..8594
                     /label="3'UTR"
     misc_feature    8403
                     /note="Geneious type: Editing History Insertion"
                     /label
     primer_bind     complement(8440..8462)
                     /label="TK-SIN-3'UTR-seq-R"
     misc_feature    8594
                     /note="Geneious type: Editing History Replacement"
                     /label="A"
     CDS             8826..9683
                     /label="AmpR"
     promoter        10533..10550
                     /label="SP6"
ORIGIN
        1 attgacggcg tagtacacac tattgaatca aacagccgac caattgcact accatcacaa
       61 tggagaagcc agtagtaaac gtagacgtag acccccagag tccgtttgtc gtgcaactgc
      121 aaaaaagctt cccgcaattt gaggtagtag cacagcaggt cactccaaat gaccatgcta
      181 atgccagagc attttcgcat ctggccagta aactaatcga gctggaggtt cctaccacag
      241 cgacgatctt ggacataggc agcgcaccgg ctcgtagaat gttttccgag caccagtatc
      301 attgtgtctg ccccatgcgt agtccagaag acccggaccg catgatgaaa tacgccagta
      361 aactggcgga aaaagcgtgc aagattacaa acaagaactt gcatgagaag attaaggatc
      421 tccggaccgt acttgatacg ccggatgctg aaacaccatc gctctgcttt cacaacgatg
      481 ttacctgcaa catgcgtgcc gaatattccg tcatgcagga cgtgtatatc aacgctcccg
      541 gaactatcta tcatcaggct atgaaaggcg tgcggaccct gtactggatt ggcttcgaca
      601 ccacccagtt catgttctcg gctatggcag gttcgtaccc tgcgtacaac accaactggg
      661 ccgacgagaa agtccttgaa gcgcgtaaca tcggactttg cagcacaaag ctgagtgaag
```

```
 721 gtaggacagg aaaattgtcg ataatgagga agaaggagtt gaagcccggg tcgcgggttt
 781 atttctccgt aggatcgaca ctttatccag aacacagagc cagcttgcag agctggcatc
 841 ttccatcggt gttccacttg aatggaaagc agtcgtacac ttgccgctgt gatacagtgg
 901 tgagttgcga aggctacgta gtgaagaaaa tcaccatcag tcccgggatc acgggagaaa
 961 ccgtgggata cgcggttaca cacaatagcg agggcttctt gctatgcaaa gttactgaca
1021 cagtaaaagg agaacgggta tcgttccctg tgtgcacgta catcccggcc accatatgcg
1081 atcagatgac tggtataatg gccacggata tatcacctga cgatgcacaa aaacttctgg
1141 ttgggctcaa ccagcgaatt gtcattaacg gtaggactaa caggaacacc aacaccatgc
1201 aaaattacct tctgccgatc atagcacaag ggttcagcaa atgggctaag gagcgcaagg
1261 atgatcttga taacgagaaa atgctgggta ctagagaacg caagcttacg tatggctgct
1321 tgtgggcgtt tcgcactaag aaagtacatt cgttttatcg cccacctgga acgcagacct
1381 gcgtaaaagt cccagcctct tttagcgctt ttcccatgtc gtccgtatgg acgacctctt
1441 tgcccatgtc gctgaggcag aaattgaaac tggcattgca accaaagaag gaggaaaaac
1501 tgctgcaggt ctcggaggaa ttagtcatgg aggccaaggc tgcttttgag gatgctcagg
1561 aggaagccag agcggagaag ctccgagaag cacttccacc attagtggca gacaaaggca
1621 tcgaggcagc cgcagaagtt gtctgcgaag tggaggggct ccaggcggac atcggagcag
1681 cattagttga aaccccgcgc ggtcacgtaa ggataatacc tcaagcaaat gaccgtatga
1741 tcggacagta tatcgttgtc tcgccaaact ctgtgctgaa gaatgccaaa ctcgcaccag
1801 cgcacccgct agcagatcag gttaagatca taacacactc cggaagatca ggaaggtacg
1861 cggtcgaacc atacgacgct aaagtactga tgccagcagg aggtgccgta ccatggccag
1921 aattcctagc actgagtgag agcgccacgt tagtgtacaa cgaaagagag tttgtgaacc
1981 gcaaactata ccacattgcc atgcatggcc ccgccaagaa tacagaagag gagcagtaca
2041 aggttacaaa ggcagagctt gcagaaacag agtacgtgtt tgacgtggac aagaagcgtt
2101 gcgttaagaa ggaagaagcc tcaggtctgg tcctctcggg agaactgacc aaccctccct
2161 atcatgagct agctctggag ggactgaaga cccgacctgc ggtcccgtac aaggtcgaaa
2221 caataggagt gataggcaca ccggggtcgg gcaagtcagc tattatcaag tcaactgtca
2281 cggcacgaga tcttgttacc agcggaaaga aagaaaattg tcgcgaaatt gaggccgacg
2341 tgctaagact gaggggtatg cagattacgt cgaagacagt agattcggtt atgctcaacg
2401 gatgccacaa agccgtagaa gtgctgtacg ttgacgaagc gttcgcgtgc cacgcaggag
2461 cactacttgc cttgattgct atcgtcaggc cccgcaagaa ggtagtacta tgcggagacc
2521 ccatgcaatg cggattcttc aacatgatgc aactaaaggt acatttcaat caccctgaaa
2581 aagacatatg caccaagaca ttctacaagt atatctcccg gcgttgcaca cagccagtta
2641 cagctattgt atcgacactg cattacgatg gaaagatgaa aaccacgaac ccgtgcaaga
2701 agaacattga aatcgatatt acaggggcca caaagccgaa gccaggggat atcatcctga
2761 catgtttccg cgggtgggtt aagcaattgc aaatcgacta tcccggacat gaagtaatga
2821 cagccgcggc ctcacaaggg ctaaccagaa aaggagtgta tgccgtccgg caaaaagtca
2881 atgaaaaccc actgtacgcg atcacatcag agcatgtgaa cgtgttgctc acccgcactg
2941 aggacaggct agtgtggaaa accttgcagg gcgacccatg gattaagcag ctcactaaca
3001 tacctaaagg aaactttcag gctactatag aggactggga agctgaacac aagggaataa
3061 ttgctgcaat aaacagcccc actccccgtg ccaatccgtt cagctgcaag accaacgttt
3121 gctgggcgaa agcattggaa ccgatactag ccacggccgg tatcgtactt accggttgcc
3181 agtggagcga actgttccca cagtttgcgg atgacaaacc acattcggcc atttacgcct
3241 tagacgtaat ttgcattaag tttttcggca tggacttgac aagcggactg ttttctaaac
3301 agagcatccc actaacgtac catcccgccg attcagcgag gccggtagct cattgggaca
3361 acagcccagg aacccgcaag tatgggtacg atcacgccat tgccgccgaa ctctcccgta
3421 gatttccggt gttccagcta gctgggaagg gcacacaact tgatttgcag acggggagaa
3481 ccagagttat ctctgcacag cataacctgg tcccggtgaa ccgcaatctt cctcacgcct
3541 tagtccccga gtacaaggag aagcaacccg gcccggtcga aaaattcttg aaccagttca
3601 aacaccactc agtacttgtg gtatcagagg aaaaaattga agctccccgt aagagaatcg
3661 aatggatcgc cccgattggc atagccggtg cagataagaa ctacaacctg gctttcgggt
3721 ttccgccgca ggcacggtac gacctggtgt tcatcaacat tggaactaaa tacagaaacc
3781 accactttca gcagtgcgaa gaccatgcgg cgaccttaaa aacccttcg cgttcggccc
3841 tgaattgcct taactcagga ggcaccctcg tggtgaagtc ctatggctac gccgaccgca
3901 acagtgagga cgtagtcacc gctcttgcca gaaagtttgt cagggtgtct gcagcgagac
3961 cagattgtgt ctcaagcaat acagaaatgt acctgatttt ccgacaacta gacaacagcc
4021 gtacacggca attcaccccg caccatctga attgcgtgat ttcgtccgtg tatgagggta
4081 caagagatgg agttggagcc gcgccgtcat accgcaccaa aagggagaat attgctgact
4141 gtcaagagga agcagttgtc aacgcagcca atccgctggg tagaccaggc gaaggagtct
4201 gccgtgccat ctataaacgt tggccgacca gttttaccga ttcagccacg gagacaggca
4261 ccgcaagaat gactgtgtgc ctaggaaaga aagtgatcca cgcggtcggc cctgatttcc
4321 ggaagcaccc agaagcagaa gccttgaaat tgctacaaaa cgcctaccat gcagtggcag
4381 acttagtaaa tgaacataac atcaagtctg tcgccattcc actgctatct acaggcattt
4441 acgcagccgg aaaagaccgc cttgaagtat cacttaactg cttgacaacc gcgctagaca
4501 gaactgacgc ggacgtaacc atctattgcc tggataagaa gtggaaggaa agaatcgacg
4561 cggcactcca acttaaggag tctgtaacag agctgaagga tgaagatatg gagatcgacg
4621 atgagttagt atggatccat ccagacagtt gcttgaaggg aagaaaggga ttcagtacta
4681 caaaaggaaa attgtattcg tacttcgaag gcaccaaatt ccatcaagca gcaaaagaca
4741 tggcggagat aaaggtcctg ttccctaatg accaggaaag taatgaacaa ctgtgtgcct
4801 acatattggg tgagaccatg gaagcaatcc gcgaaaagtg cccggtcgac cataacccgt
4861 cgtctagccc gcccaaaacg ttgccgtgcc tttgcatgta tgccatgacg ccagaaaggg
4921 tccacagact tagaagcaat aacgtcaaag aagttacagt atgctcctcc acccccctcc
4981 ctaagcacaa aattaagaat gttcagaagg ttcagtgcac gaaagtagtc ctgtttaatc
5041 cgcacactcc cgcattcgtt cccgcccgta agtacataga agtgccagaa cagcctaccg
5101 ctcctcctgc acaggccgag gaggcccccg aagttgtagc gacaccgtca ccatctacag
5161 ctgataacac ctcgcttgat gtcacagaca tctcactgga tatggatgac agtagcgaag
5221 gctcactttt ttcgagcttt agcggatcgg acaactctat tactagtatg gacagttggt
5281 cgtcaggacc tagttcacta gagatagtag accgaaggca ggtggtggtg gctgacgttc
```

5341 atgccgtcca agagcctgcc cctattccac cgccaaggct aaagaagatg gcccgcctgg
5401 cagcggcaag aaaagagccc actccaccgg caagcaatag ctctgagtcc ctccacctct
5461 cttttggtgg ggtatccatg tccctcggat caattttcga cggagagacg gcccgccagg
5521 cagcggtaca acccctggca acaggcccca cggatgtgcc tatgtctttc ggatcgtttt
5581 ccgacggaga gattgatgag ctgagccgca gagtaactga gtccgaaccc gtcctgtttg
5641 gatcatttga accgggcgaa gtgaactcaa ttatatcgtc ccgatcagcc gtatcttttc
5701 cactacgcaa gcagagacgt agacgcagga gcaggaggac tgaatactga ctaaccgggg
5761 taggtgggta catattttcg acggacacag gccctgggca cttgcaaaag aagtccgttc
5821 tgcagaacca gcttacagaa ccgaccttgg agcgcaatgt cctggaaaga attcatgccc
5881 cggtgctcga cacgtcgaaa gaggaacaac tcaaactcag gtaccagatg atgcccaccg
5941 aagccaacaa aagtaggtac cagtctcgta aagtagaaaa tcagaaagcc ataaccactg
6001 agcgactact gtcaggacta cgactgtata actctgccac agatcagcca gaatgctata
6061 agatcaccta tccgaaacca ttgtactcca gtagcgtacc ggcgaactac tccgatccac
6121 agttcgctgt agctgtctgt aacaactatc tgcatgagaa ctatccgaca gtagcatctt
6181 atcagattac tgacgagtac gatgcttact tggatatggt agacgggaca gtcgcctgcc
6241 tggatactgc aaccttctgc cccgctaagc ttagaagtta cccgaaaaaa catgagtata
6301 gagccccgaa tatccgcagt gcggttccat cagcgatgca gaacacgcta caaaatgtgc
6361 tcattgccgc aactaaaaga aattgcaacg tcacgcagat gcgtgaactg ccaacactgg
6421 actcagcgac attcaatgtc gaatgctttc gaaaatatgc atgtaatgac gagtattggg
6481 aggagttcgc tcggaagcca attaggatta ccactgagtt tgtcaccgca tatgtagcta
6541 gactgaaagg ccctaaggcc gccgcactat ttgcaaagac gtataatttg gtcccattgc
6601 aagaagtgcc tatggataga ttcgtcatgg acatgaaaag agacgtgaaa gttacaccag
6661 gcacgaaaca cacagaagaa agaccgaaag tacaagtgat acaagccgca gaacccctgg
6721 cgactgctta cttatgcggg attcaccggg aattagtgcg taggcttacg gccgtcttgc
6781 ttccaaacat tcacacgctt tttgacatgt cggcggagga ttttgatgca atcatagcag
6841 aacacttcaa gcaaggcgac ccggtactgg agacggatat cgcatcattc gacaaaagcc
6901 aagacgacgc tatggcgtta accggtctga tgatcttgga ggacctgggt gtggatcaac
6961 cactactcga cttgatcgag tgcgcctttg gagaaatatc atccacccat ctacctacgg
7021 gtactcgttt taaattcggg gcgatgatga aatccggaat gttcctcaca ctttttgtca
7081 acacagtttt gaatgtcgtt atcgccagca gagtactaga agagcggctt aaaacgtcca
7141 gatgtgcagc gttcattggc gacgacaaca tcatacatgg agtagtatct gacaaagaaa
7201 tggctgagag gtgcgccacc tggctcaaca tggaggttaa gatcatcgac gcagtcatcg
7261 gtgagagacc accttacttc tgcggcggat ttatcttgca agattcggtt acttccacag
7321 cgtgccgcgt ggcggatccc ctgaaaaggc tgtttaagtt gggtaaaccg ctcccagccg
7381 acgacgagca agacgaagac agaagacgcg ctctgctaga tgaaacaaag gcgtggttta
7441 gagtaggtat aacaggcact ttagcagtgg ccgtgacgac ccggtatgag gtagacaata
7501 ttacacctgt cctactggca ttgagaactt ttgcccagag caaaagagca ttccaagcca
7561 tcagagggga aataaagcat ctctacggtg gtcctaaata gtcagcatag tacatttcat
7621 ctgactaata ctacaacacc accacccgta cgatggtgag caagggcgag gagctgttca
7681 ccggggtggt gcccatcctg gtcgagctgg acggcgacgt aaacggccac aagttcagcg
7741 tgtccggcga gggcgagggc gatgccacct acggcaagct gaccctgaag ctgatctgca
7801 ccaccggcaa gctgcccgtg ccctggccca ccctcgtgac caccctgggc tacggcctgc
7861 agtgcttcgc ccgctacccc gaccacatga agcagcacga cttcttcaag tccgccatgc
7921 ccgaaggcta cgtccaggag cgcaccatct tcttcaagga cgacggcaac tacaagaccc
7981 gcgccgaggt gaagttcgag ggcgacaccc tggtgaaccg catcgagctg aagggcatcg
8041 acttcaagga ggacggcaac atcctggggc acaagctgga gtacaactac aacagccaca
8101 acgtctatat caccgccgac aagcagaaga acggcatcaa ggccaacttc aagatccgcc
8161 acaacatcga ggacggcggc gtgcagctcg ccgaccacta ccagcagaac accccatcg
8221 gcgacggccc cgtgctgctg cccgacaacc actacctgag ctaccagtcc aagctgagca
8281 aagaccccaa cgagaagcgc gatcacatgg tcctgctgga gttcgtgacc gccgccggga
8341 tcactctcgg catggacgag ctgtacaagt aactcgagga agcgcagtac ataatgctgc
8401 gcagtgttgc cacataacca ctatattaac catttatcta gcggacgcca aaaactcaat
8461 gtatttctga ggaagcgtgg tgcataatgc cacgcagcgt ctgcataact tttattattt
8521 cttttattaa tcaacaaaat tttgttttta acatttcaaa aaaaaaaaaa aaaaaaaaaa
8581 aaaaaaaaaa aaaggggaat tcgagctcgg gaattaattc ttgaagacga aagggcctcg
8641 tgatacgcct atttttatag gttaatgtca tgataataat ggtttcttag acgtcaggtg
8701 gcacttttcg gggaaatgtg cgcggaaccc ctatttgttt atttttctaa atacattcaa
8761 atatgtatcc gctcatgaga caataaccct gataaatgct tcaataatat tgaaaaagga
8821 agagtatgag tattcaacat ttccgtgtcg cccttattcc ctttttgcgg gcattttgcc
8881 ttcctgtttt tgctcaccca gaaacgctgg tgaaagtaaa agatgctgaa gatcagttgg
8941 gtgcacgagt gggttacatc gaactggatc tcaacagcgg taagatcctt gagagttttc
9001 gccccgaaga acgttttcca atgatgagca cttttaaagt tctgctatgt ggcgcggtat
9061 tatcccgtgt tgacgccggg caagagcaac tcggtcgccg catacactat tctcagaatg
9121 acttggttga gtactcacca gtcacagaaa agcatcttac ggatggcatg acagtaagag
9181 aattatgcag tgctgccata accatgagtg ataacactgc ggccaacttа cttctgacaa
9241 cgatcggagg accgaaggag ctaaccgctt ttttgcacaa catgggggat catgtaactc
9301 gccttgatcg ttgggaaccg gagctgaatg aagccatacc aaacgacgag cgtgacacca
9361 cgatgcctgt agcaatggca acaacgttgc gcaaactatt aactggcgaa ctacttactc
9421 tagcttcccg gcaacaatta atagactgga tggaggcgga taaagttgca ggaccacttc
9481 tgcgctcggc ccttccggct ggctggttta ttgctgataa atctggagcc ggtgagcgtg
9541 ggtctcgcgg tatcattgca gcactggggc cagatggtaa gccctcccgt atcgtagtta
9601 tctacacgac ggggagtcag gcaactatgg atgaacgaaa tagacagatc gctgagatag
9661 gtgcctcact gattaagcat tggtaactgt cagaccaagt ttactcatat atactttaga
9721 ttgatttaaa acttcatttt taatttaaaa ggatctaggt gaagatcctt tttgataatc
9781 tcatgaccaa aatcccttaa cgtgagtttt cgttccactg agcgtcagac cccgtagaaa
9841 agatcaaagg atcttcttga gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa
9901 aaaaaccacc gctaccagcg gtggtttgtt tgccggatca agagctacca actcttttc

```
     9961 cgaaggtaac tggcttcagc agagcgcaga taccaaatac tgtccttcta gtgtagccgt
    10021 agttaggcca ccacttcaag aactctgtag caccgcctac atacctcgct ctgctaatcc
    10081 tgttaccagt ggctgctgcc agtggcgata agtcgtgtct taccgggttg gactcaagac
    10141 gatagttacc ggataaggcg cagcggtcgg gctgaacggg gggttcgtgc acacagccca
    10201 gcttggagcg aacgacctac accgaactga gatacctaca gcgtgagcat tgagaaagcg
    10261 ccacgcttcc cgaagggaga aaggcggaca ggtatccggt aagcggcagg gtcggaacag
    10321 gagagcgcac gagggagctt ccagggggaa acgcctggta tctttatagt cctgtcgggt
    10381 ttcgccacct ctgacttgag cgtcgatttt tgtgatgctc gtcaggggggg cggagcctat
    10441 ggaaaaacgc cagcaacgcg agctcgtatg gacatattgt cgttagaacg cggctacaat
    10501 taatacataa ccttatgtat catacacata cgatttaggt gacactatag
//
```