Supplementary Material for "Synthetic Tunable Amplifying Buffer Circuit in *E. coli*"

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Contents

List of Figures

List of Tables

1 Additional Materials and Methods

1.1 Gene circuit design

The amplifying buffer circuit is basically a phosphorylation-dephosphorylation based system. The circuit was designed towards constitutive expression of a fixed amount of the nitrogen regulatory transcription factor NRI in a given circuit. The kinase and phosphatase are required as an input and negative feedback, respectively, and hence, they were designed for variable induced expression with two different inducers. The reporter system was constructed to detect the output, which is the phosphorylated substrate. Overall, the design of the gene circuits allowed tuning the input and feedback with a fixed amount of substrate in a given circuit.

The complete circuit was constructed in pACYC184 plasmid vector. All the genes were sequentially cloned in the vector as described below. All the BioBrick DNA parts (Table S1), primers used for cloning (Table S2) and primer for genomic deletions (Table S3) are mentioned in the Supplementary Tables.

1.2 Circuit construction

The *ntr*-based gene circuit was mainly constructed using the BioBrick strategy. Some of the DNA was synthesized or assembled using the Gibson method. The primary genes for the substrate, kinase, and phosphatase were PCR amplified and the respective promoter, ribosome binding site (RBS), and double terminator was added sequentially to each gene. The details of the construction of each gene are given below.

1.2.1 Superfolder GFP reporter system

The DNA of the reporter gene for superfolder GFP (sf-*gfp* gene) (BBa_I746916) was originally synthesized with the native P*glnA* promoter/RBS upstream of the sf-*gfp* gene and with the LVA degradation tag and double terminator downstream of the gene in the pUC57 plasmid (GenScript, Inc., NJ). The complete gene was clone at the *Xba*I-*Hind*III site in pACYC184

plasmid to obtain the reporter construct.

The native RBS in the P*glnA* has very low strength in comparison to the RBSs of the other circuit genes (Table S4) and hence, to make its strength compatible with the other circuit genes in order to avoid any competitive disadvantage to it (with respect to availability of the ribosomes), the native RBS in the P*glnA* promoter was replaced with a strong RBS after forward engineering using the RBS calculator software(*1*). The engineered RBS which has around 100-fold higher protein expression strength (RBS(39,754)) was cloned by Gibson assembly using primers C1 and C2 primers. This new construct with the strong RBS was used as the reporter system in all the circuits.

1.2.2 Repressor system (*tetR* and *lacI* gene)

The *tetR* and *lacI* genes, both with the LVA degradation tag were PCR amplified using template plasmids BBa_C0040 and BBa_C0012 respectively. The individual genes were then cloned downstream of the promoter (BBa_J23114) and RBS34 (BBa_B0034) and then subsequently cloned upstream of the double terminator (BBa_B0015) in the BioBrick plasmid. Later, the *tetR* gene was PCR amplified using the primers C8 and C4. The *lacI* gene was PCR amplified with the primers C3 and C12. The two genes were then cloned in tandem at the *Bam*HI site in the pACYC184 plasmid containing the reporter system.

1.2.3 Kinase (*ntrB*-L16R)

The kinase gene *ntrB*-L16R was initially cloned with the strong RBS (BBa_B0034). The gene was PCR amplified using the primers C13 and C14 from the template plasmid pAP096 (pJLA503/*glnL* (L16R)) obtained from Prof. Alexander J Ninfa (University of Michigan, Ann Arbor). The amplified DNA was cloned downstream of the P*tet* promoter (BBa_R0040) in the BioBrick plasmid. This assembled DNA was then cloned upstream of the double terminator (BBa_B0015) containing BioBrick plasmid to obtain the kinase expressing system. The kinase construct was later modified to replace the strong RBS (BBa_B0034) with a much weaker

RBS (BBa_B0033) and for addition of the LVA degradation tag. The DNA was amplified in three parts: Part-1 (Ptet+RBS(BBa_B0033)) was generated by using primers C3 and C15. Part-2 (RBS(BBa_B0033)+NRII(kinase) with LVA tag) was amplified using primers C16 and C17. Part-3 (NRII(kinase) with LVA tag+double terminator (BBa_B0015)) was amplified using primers C18 and C4. The three parts were assembled by Gibson method and the complete gene was PCR amplified with the primers Prefix (*Sph*I) and Suffix (*Bsp*HI) to obtain the new construct with the restriction enzyme site *Sph*I-*Bsp*HI at both the terminals. The amplicon was cloned at the *Sph*I-*Bsp*HI site in the pACYC184 plasmid containing the reporter and repressor systems.

1.2.4 NRI substrate (*ntr*C)

The *ntr*C gene was PCR amplified from the DH5α strain using the primers C19 and C20 and cloned upstream of the double terminator (BBa_B0015). The amplicon was then sequentially cloned in the plasmids with either strong RBS (BBa_B0034) or weak RBS (BBa_B0032). These two constructs were then cloned with different promoters to give the circuits expressing different concentrations of NRI protein.

For constructing the circuit with very low (constitutive) expression of NRI, the prior construct with RBS (BBa_B0034) was cloned downstream of the promoter BBa_J23113 (to create a combination of P21/RBS34) (Fig. S1). In order to create the circuit with low NRI, the NRI construct with RBS (BBa_B0032) was cloned downstream of the promoter BBa_J23114 (to obtain a combination of P256/RBS32) (Fig. S2). To construct the medium (Fig. S3) and high (Fig. S4) expressing NRI gene, the construct with RBS (BBa_B0034) was cloned downstream of the promoters BBa_J23117 and BBa_J23114 in order to get the combination of P162/RBS34 and P256/RBS34. The comparison of the four combinations of the promoter/RBS is given Table S5.

The respective promoter/RBS combinations of the NRI gene were subsequently cloned at the *Bso*BI site in the pACYC184 plasmid containing the reporter, repressor, and kinase genes

using the primers C7 and C11. In the circuit with high NRI, the NRI gene construct was cloned at the *Sph*I-*Bso*BI site using the primers C5 and C11.

1.2.5 Phosphatase (*ntrB*-H139N)

The phosphatase gene *ntrB*-H139N was originally cloned with the strong RBS (BBa_B0034). The phosphatase gene was PCR amplified using the primers C13 and C14 using the template plasmid pLOP22mRB9132(H139N) obtained from Prof. Alexander J Ninfa (University of Michigan, Ann Arbor). The amplified DNA was cloned downstream of the Plac promoter (BBa_R0011) in the BioBrick plasmid. This assembled DNA was then cloned upstream of the double terminator (BBa_B0015) containing BioBrick plasmid to obtain the phosphatase expressing system. This phosphatase construct was later modified to add the LVA degradation tag. The DNA was amplified in two parts: Part-1 (Plac+RBS(BBa_B0034)+NRII(H139)) was generated by using primers C3 and C17. Part-2 (NRII(H139) with LVA tag and double terminator (BBa_B0015)) was amplified using C18 and C4. The two parts were assembled by Gibson method and the complete gene was PCR amplified with the primers C3 and C11 to obtain the new construct with the restriction enzyme site *Bso*BI at the downstream end. The phosphatase was later cloned in tandem with the substrate gene and cloned in the pACYC184 plasmid containing the reporter, repressors, kinase, and the NRI. In case of the circuit with very high NRI, the phosphatase gene was cloned at the BsoBI site after amplification using the primers C7 and C11. The phosphatase was not cloned in the circuit with very low NRI. It is to be noted that all the circuit parts except the NRI substrate were tagged with C-terminal degradation tag (LVA) for a faster turnover.

At each step, the plasmids were sequenced to confirm the correctness of the DNA of the genes and any circuit with mutation(s) was discarded.

1.2.6 Load DNA

The single stranded complementary DNA sequences of the strong enhancer binding site-2 (located in the P*glnA* enhancer region) were synthesized by annealing the primers C21 and C22 together to form double stranded DNA. Two such DNA binding sites were cloned in tandem in pUC19 plasmid (New England BioLabs Inc., USA) at the *Eco*RI and *Pst*I sites. This high copy number plasmid was used as a DNA load plasmid and was co-transformed along with the circuit plasmid for cells with DNA load. The cells without DNA load were co-transformed with empty pUC19 plasmid.

All the plasmids used in this study are listed in Table S6.

1.3 Bacterial strain construction

1.3.1 Primary strain (3.300LG) and gene deletions

The original bacterial strain selected for studying the circuits was *E. coli* 3.300 *glnL glnG* strain. This strain is the *E. coli* MG1655 strain deleted for the lacI gene and is also a double deletion mutant of the genes for NRI (*ntrC* or *glnG*) and kinase/phosphatase (*ntrB* or *glnL*) required for orthogonality of the circuit. In the process to achieve circuit DNA stability and the requirement for stricter orthogonality, we sequentially deleted various other genes as detailed below.

All the gene deletions were performed by one-step inactivation of chromosomal genes using PCR products produced using the DNA primers for amplifying the kanamycin resistance gene from the plasmid pKD4 (*2*). These primers were designed to have specific DNA sequences homologous to the region flanking the gene to be deleted. The PCR products so obtained were electrotransformed in the *E. coli* strain and were screened for kanamycin resistance. The mutants were confirmed and the antibiotic cassette was later removed from the chromosome by transforming the strain with the pKD20 plasmid. This plasmid was cured after incubating the strain at 42°C overnight and screening for kanamycin sensitive colonies. The details of the

strain construction by the deletions of the chromosomal genes are given below.

1.3.2 *recA* deletion

Since the strain *E. coli* 3.300LG is *recA*$^+$, we noticed that the circuit plasmids transformed in this strain were unstable and showed frequent deletions. In order to obtain a robust strain to allow the plasmid containing various genes to be stable, we deleted the *recA* gene. The DNA primers D1 and D2 were used for amplification of the kanamycin resistance gene from the pKD4 plasmid in order to delete the *recA* gene.

1.3.3 *glnK* and *glnB* deletions

GlnK and GlnB expressed from the *glnK* and *glnB* genes respectively from the chromosome are regulators of the kinase/phosphatase (NtrB), hence, to increase the orthogonal stringency, the *glnK* and *glnB* genes were sequentially deleted. The DNA primers D5 and D6 were used for amplification of the kanamycin resistance gene in order to delete the *recA* gene. The *glnB* gene was deleted in the next step using the primers D9 and D10.

1.3.4 *ackA/pta* deletions

NRI (NtrC) has the ability to be phosphorylated by excess acetyl phosphate in the cells and hence, to reduce any background phosphorylation, the *ackA* (acetate kinase) and *pta* (phosphotransacetylase) genes were deleted. The DNA primers D13 and D14 were used for amplification of the kanamycin resistance gene in order to delete the *recA* gene.

All the cell strains used in this study are listed in Table S7.

1.4 Steady state, dynamic, bimodal, and biphasic system behavior

The effect of DNA load on the steady state diminished with increase in the amount of NRI. The system with very low NRI showed a prominent effect of the DNA load in terms of much reduced steady state levels at all values of kinase. The difference in steady state reduced

with increase in NRI; whereas there was no difference for the systems with medium and high NRI at all kinase levels (Fig. S5). Additionally, the cells without and with DNA load for all amounts of NRI showed a bimodal behavior after induction with the kinase. After induction, the cells showed two types of populations based on fluorescence measurements using the flow cytometer, the non-fluorescent and the fluorescent cells, albeit the cell population with high fluorescence increased with time with corresponding reduction in the non-fluorescent cells. At steady state after induction, most of the cells were observed to be at high fluorescence (Figs. S6-9). Such bimodal behavior is typically found in biological systems due to high cooperative binding of the transcription factor and/or because on the interplay between analog single-cell signaling and protein expression noise (*3*, *4*). The rise-time dynamics showed a prominent slowdown with increasing NRI without and with DNA load. Although with high NRI, the system became marginally faster (Fig. S10). This behavior is explained in Section 3.5.

Interestingly, the system also displayed a biphasic behavior with increasing NRI at high kinase levels (induced with more than 4 nM aTc) (Fig. S11). This phenomenon can be explained due to the increase in retroactivity to the input (kinase) with an increase in substrate NRI in the system in which the unphophorylated NRI can also bind to the DNA (although it is unable to bring activation of the reporter gene).

1.5 Cell growth

1.5.1 Preculture

Individual circuit plasmids were co-transformed either with/without DNA load in pUC19 and were plated on Luria-Bertani agar medium (supplemented with 0.5X W-salts minimal medium). The plates were incubated at 37°C for 18-24 h and individual colonies were selected for preparing a preculture grown in W-salts medium at 30°C for 12 h with shaking at 150 rpm. This preculture was used to inoculate the main culture (1:1,000 to 1:5,000 dilution in fresh W-salts medium) and was incubated at 30°C for 10-12 h in an orbital shaker at 100 rpm. The cells after incubation (OD600nm $\simeq$ 0.01 to 0.02) were used for the assays.

1.5.2 Growth during assay

The growth during the assay was analyzed by incubating the assay plate in the plate reader and recording the absorbance at 600 nm. The growth of the cells without and with increasing concentrations of aTc for a given circuit with/without DNA load was not affected (Figs. S12-15). A marginal decrease in growth rate was observed only for the cells containing high NRI (Fig. S16). This could be presumably due to the higher metabolic load in the cells of this circuit.

1.6 Flow cytometry

Reporter analysis was conducted by measuring fluorescence of the super-folder GFP protein using a flow-cytometer (BD Biosciences, USA; Model: BD Accuri C6). An aliquot of 50 microliters was collected from individual replicates for each assay condition every hour and 25,000 cells were analyzed for their fluorescence profile (channel FL-1 for green fluorescence) in the flow-cytometer. The aliquots were collected in thin-walled PCR tubes (8-well strips) and were placed in the PCR cooler in order to stall the assay before it was analyzed in the cytometer.

1.7 DNA sequences

1.7.1 Circuit with very low NRI

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   1  GAATTCCGGA  TGAGCATTCA  TCAGGCGGGC  AAGAATGTGA  ATAAAGGCCG  GATAAAACTT
  61  GTGCTTATTT  TTCTTTACGG  TCTTTAAAAA  GGCCGTAATA  TCCAGCTGAA  CGGTCTGGTT
 121  ATAGGTACAT  TGAGCAACTG  ACTGAAATGC  CTCAAAATGT  TCTTTACGAT  GCCATTGGGA
 181  TATATCAACG  GTGGTATATC  CAGTGATTTT  TTTCTCCATT  TTAGCTTCCT  TAGCTCCTGA
 241  AAATCTCGAT  AACTCAAAAA  ATACGCCCGG  TAGTGATCTT  ATTTCATTAT  GGTGAAAGTT
 301  GGAACCTCTT  ACGTGCCGAT  CAACGTCTCA  TTTTCGCCAA  AAGTTGGCCC  AGGGCTTCCC
 361  GGTATCAACA  GGGACACCAG  GATTTATTTA  TTCTGCGAAG  TGATCTTCCG  TCACAGGTAT
 421  TTATTCGGCG  CAAAGTGCGT  CGGGTGATGC  TGCCAACTTA  CTGATTTAGT  GTATGATGGT
 481  GTTTTTGAGG  TGCTCCAGTG  GCTTCTGTTT  CTATCAGCTG  TCCCTCCTGT  TCAGCTACTG
 541  ACGGGGTGGT  GCGTAACGGC  AAAAGCACCG  CCGGACATCA  GCGCTAGCGG  AGTGTATACT
 601  GGCTTACTAT  GTTGGCACTG  ATGAGGGTGT  CAGTGAAGTG  CTTCATGTGG  CAGGAGAAAA
 661  AAGGCTGCAC  CGGTGCGTCA  GCAGAATATG  TGATACAGGA  TATATTCCGC  TTCCTCGCTC
 721  ACTGACTCGC  TACGCTCGGT  CGTTCGACTG  CGGCGAGCGG  AAATGGCTTA  CGAACGGGGC
 781  GGAGATTTCC  TGGAAGATGC  CAGGAAGATA  CTTAACAGGG  AAGTGAGAGG  GCCGCGGCAA
 841  AGCCGTTTTT  CCATAGGCTC  CGCCCCCCTG  ACAAGCATCA  CGAAATCTGA  CGCTCAAATC
 901  AGTGGTGGCG  AAACCCGACA  GGACTATAAA  GATACCAGGC  GTTTCCCCCT  GGCGGCTCCC
 961  TCGTGCGCTC  TCCTGTTCCT  GCCTTTCGGT  TTACCGGTGT  CATTCCGCTG  TTATGGCCGC
1021  GTTTGTCTCA  TTCCACGCCT  GACACTCAGT  TCCGGGTAGG  CAGTTCGCTC  CAAGCTGGAC
1081  TGTATGCACG  AACCCCCCGT  TCAGTCCGAC  CGCTGCGCCT  TATCCGGTAA  CTATCGTCTT
1141  GAGTCCAACC  CGGAAAGACA  TGCAAAAGCA  CCACTGGCAG  CAGCCACTGG  TAATTGATTT
1201  AGAGGAGTTA  GTCTTGAAGT  CATGCGCCGG  TTAAGGCTAA  ACTGAAAGGA  CAAGTTTTGG
1261  TGACTGCGCT  CCTCCAAGCC  AGTTACCTCG  GTTCAAAGAG  TTGGTAGCTC  AGAGAACCTT
1321  CGAAAAACCG  CCCTGCAAGG  CGGTTTTTTC  GTTTTCAGAG  CAAGAGATTA  CGCGCAGACC
1381  AAAACGATCT  CAAGAAGATC  ATCTTATTAA  TCAGATAAAA  TATTTCTAGA  GCATCCTCCG
1441  CAAACAAGTA  TTGCAGAGTC  CCTTTGTGAT  CGCTTTCACG  GAGCATAAAA  AGGGTTATCC
1501  AAAGGTCATT  GCACCAACAT  GGTGCTTAAT  GTTTCCATTG  AAGCACTATA  TTGGTGCAAC
1561  ATTCACATCG  TGGTGCAGCC  CTTTTGCACG  ATGGTGCGCA  TGATAACGCC  TTTTAGGGGC
1621  AATTTAAAAG  TTGGCACAGA  TTTCGCTTTA  TCTTTTTTAC  GGCGACACGG  CCAAAATAAT
1681  TGCAGATTTC  GTTACCACGA  CGACCTAACT  TTCAATTCTA  ATAAGGAGGA  AGACTTCAAA
1741  TGCGTAAAGG  CGAAGAGCTG  TTCACTGGTG  TCGTCCCTAT  TCTGGTGGAA  CTGGATGGTG
1801  ATGTCAACGG  TCATAAGTTT  TCCGTGCGTG  GCGAGGGTGA  AGGTGACGCA  ACTAATGGTA
1861  AACTGACGCT  GAAGTTCATC  TGTACTACTG  GTAAACTGCC  GGTACCTTGG  CCGACTCTGG
1921  TAACGACGCT  GACTTATGGT  GTTCAGTGCT  TTGCTCGTTA  TCCGGACCAT  ATGAAGCAGC
1981  ATGACTTCTT  CAAGTCCGCC  ATGCCGGAAG  GCTATGTGCA  GGAACGCACG  ATTTCCTTTA
2041  AGGATGACGG  CACGTACAAA  ACGCGTGCGG  AAGTGAAATT  TGAAGGCGAT  ACCCTGGTAA
2101  ACCGCATTGA  GCTGAAAGGC  ATTGACTTTA  AAGAAGACGG  CAATATCCTG  GGCCATAAGC
2161  TGGAATACAA  TTTTAACAGC  CACAATGTTT  ACATCACCGC  CGATAAACAA  AAAAATGGCA
2221  TTAAAGCGAA  TTTTAAAATT  CGCCACAACG  TGGAGGATGG  CAGCGTGCAG  CTGGCTGATC
2281  ACTACCAGCA  AAACACTCCA  ATCGGTGATG  GTCCTGTTCT  GCTGCCAGAC  AATCACTATC
2341  TGAGCACGCA  AAGCGTTCTG  TCTAAAGATC  CGAACGAGAA  ACGCGATCAT  ATGGTTCTGC
2401  TGGAGTTCGT  AACCGCAGCG  GGCATCACGC  ATGGTATGGA  TGAACTGTAC  AAACAGGCTG
2461  CAAACGACGA  AAACTACGCT  TTAGTAGCTT  GATGACTTAA  GGAGCTCCAA  TTGCCAGGCA
2521  TCAAATAAAA  CGAAAGGCTC  AGTCGAAAGA  CTGGGCCTTT  CGTTTTATCT  GTTGTTTGTC
2581  GGTGAACGCT  CTCTACTAGA  GTCACACTGG  CTCACCTTCG  GGTGGGCCTT  TCTGCGTTTA
2641  TAAAGCTTTA  ATGCGGTAGT  TTATCACAGT  TAAATTGCTA  ACGCAGTCAG  GCACCGTGTA
2701  TGAAATCTAA  CAATGCGCTC  ATCGTCATCC  TCGGCACCGT  CACCCTGGAT  GCTGTAGGCA
2761  TAGGCTTGGT  TATGCCGGTA  CTGCCGGGCC  TCTTGCGGGA  TATCGTCCAT  TCCGACAGCA
2821  TCGCCAGTCA  CTATGGCGTG  CTGCTAGCGC  TATATGCGTT  GATGCAATTT  CTATGCGCAC
2881  CCGTTCTCGG  AGCACTGTCC  GACCGCTTTG  GCCGCCGCCC  AGTCCTGCTC  GCTTCGCTAC
2941  TTGGAGCCAC  TATCGACTAC  GCGATCATGG  CGACCACACC  CGTCCTGTGG  ATCCGAATTC
3001  GCGGCCGCTT  CTAGAGTTTA  TGGCTAGCTC  AGTCCTAGGT  ACAATGCTAG  CTACTAGAGA
3061  AAGAGGAGAA  ATACTAGATG  TCCAGATTAG  ATAAAAGTAA  AGTGATTAAC  AGCGCATTAG
3121  AGCTGCTTAA  TGAGGTCGGA  ATCGAAGGTT  TAACAACCCG  TAAACTCGCC  CAGAAGCTAG
3181  GTGTAGAGCA  GCCTACATTG  TATTGGCATG  TAAAAAATAA  GCGGGCTTTG  CTCGACGCCT
3241  TAGCCATTGA  GATGTTAGAT  AGGCACCATA  CTCACTTTTG  CCCTTTAGAA  GGGGAAAGCT
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3301 GGCAAGATTT TTTACGTAAT AACGCTAAAA GTTTTAGATG TGCTTTACTA AGTCATCGCG
3361 ATGGAGCAAA AGTACATTTA GGTACACGGC CTACAGAAAA ACAGTATGAA ACTCTCGAAA
3421 ATCAATTAGC CTTTTTATGC CAACAAGGTT TTTCACTAGA GAATGCATTA TATGCACTCA
3481 GCGCTGTGGG GCATTTTACT TTAGGTTGCG TATTGGAAGA TCAAGAGCAT CAAGTCGCTA
3541 AAGAAGAAAG GGAAACACCT ACTACTGATA GTATGCCGCC ATTATTACGA CAAGCTATCG
3601 AATTATTTGA TCACCAAGGT GCAGAGCCAG CCTTCTTATT CGGCCTTGAA TTGATCATAT
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3781 GACTGGGCCT TTCGTTTTAT CTGTTGTTTG TCGGTGAACG CTCTCTACTA GAGTCACACT
3841 GGCTCACCTT CGGGTGGGCC TTTCTGCGTT TATATACTAG AGTTTATGGC TAGCTCAGTC
3901 CTAGGTACAA TGCTAGCTAC TAGAGAAAGA GGAGAAATAC TAGATGGTGA ATGTGAAACC
3961 AGTAACGTTA TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG TTTCCCGCGT
4021 GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAG CGGCGATGGC
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4141 GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCCG TCGCAAATTG TCGCGGCGAT
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4561 ATATCTCACT CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT GGAGTGCCAT
4621 GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA CTGCGATGCT
4681 GGTTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT CCGGGCTGCG
4741 CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT CATGTTATAT
4801 CCCGCCGTTA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA GCGTGGACCG
4861 CTTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC CCGTCTCACT
4921 GGTGAAAAGA AAAACCACCC TGGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC
4981 CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC AGGCTGCAAA
5041 CGACGAAAAC TACGCTTTAG TAGCTTAATA ATACTAGAGC CAGGCATCAA ATAAAACGAA
5101 AGGCTCAGTC GAAAGACTGG GCCTTTCGTT TTATCTGTTG TTTGTCGGTG AACGCTCTCT
5161 ACTAGAGTCA CACTGGCTCA CCTTCGGGTG GGCCTTTCTG CGTTTATATA CTAGTAGCGG
5221 CCGCTGCAGG GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG
5281 CGGTTGCTGG CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG
5341 GGCTCATGAC TGCAGCGGCC GCTACTAGTA TATAAACGCA GAAAGGCCCA CCCGAAGGTG
5401 AGCCAGTGTG ACTCTAGTAG AGAGCGTTCA CCGACAAACA ACAGATAAAA CGAAAGGCCC
5461 AGTCTTTCGA CTGAGCCTTT CGTTTTATTT GATGCCTGGC TCTAGTATTA AGCTACTAAA
5521 GCGTAGTTTT CGTCGTTTGC AGCTTTCCTG ATAGGCAGGT AAACCGAGAA CTCGGTATGC
5581 CCTGGCCAAC TGGTAAATTC AATTTTGCCT GAATGCTGAT CAATCAAATT ACGAGCGATG
5641 GATAAGCCAA GCCCGGTGCC ACCTTCGCGG CCGCTGACCA TCGGGTAAAA CAGCGTATCC
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5761 TAGCGCTCGC CGTGTAAGGT CAGTTGAAAC GCGGTGCGGG TACGCAGAAT GATTTCACCG
5821 CCTTCCGGCC CCAGCGCCTG TAGCGCATTG CGCACAATAT TCAGCAAGAC CTGTTCAATT
5881 TGATCCGGGT CGTGCGCCAG TTCCGGTAGG CTGGGATCGT AATCACGAAT CAACCGCACG
5941 TTGTCCGGCA GTTCCATCGA CACCAGCGTT ACCACGCGTT CAGCCACTTT GTGAATACTT
6001 TCGGTAACGC GCGTACCGGG CAGCTGCGGC CCCAACAGAC GGTCGACCAG ATTTCGCAGC
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6121 TTGCTGAGCA GCTGCGCCGC GCCACGTAAA CCGCCAAGCG GATTTTTAAT CTCATGTGCC
6181 AGGCCGCGCA CTAAATCACG GGCAGCAACC TGCTGGGCGT GCTGTAGCTG TTCCTGACTT
6241 AAGCGGCGCT GGTTATCCAT CGGAGCCATC TCCAGCAGGA TCATGCCGTC CGGCATACGC
6301 TGGGCCGTCA CAGAAAGGAT ATGCGAGCGC CCGTCGATGA CCAGCGTCAC TTCGTTATCG
6361 GTAAAACCTT GCCCCGCCTC CAGACTTTCT TGCATCAGCT CGATATTTAA TGAGAAGTAG
6421 CTCAACAGTT CCGGTAACGG TGTACCAAAC AATTTGCGGG AGCTTTGGGC GAGCAGTTGT
6481 TGCGCGGCAG GGTTGGCGTA ATGGATCGCC AGGTTGTCAT CGATTAACAA AATACTGTTA
6541 ATCCGCGAGT TGAGGATCTG CCCAGCATCG GGCTGCGTGC CTGTTGCCAT CTAGTAGTCC
6601 TGTGTGACTC TAGTAGTGCT CAGTATCTCT ATCACTGATA GGGATGTCAA TCTCTATCAC
6661 TGATAGGGAC TCTAGAAGCG GCCGCGAATT CGCATGCACC ATTCCTTGCG GCGGCGGTGC
6721 TCAACGGCCT CAACCTACTA CTGGGCTGCT TCCTAATGCA GGAGTCGCAT AAGGGAGAGC
6781 GTCGACCGAT GCCCTTGAGA GCCTTCAACC CAGTCAGCTC CTTCCGGTGG GCGCGGGGCA
6841 TGACTATCGT CGCCGCACTT ATGACTGTCT TCTTTATCAT GCAACTCGTA GGACAGGTGC

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 6901  CGGCAGCGCT  CTGGGTCATT  TTCGGCGAGG  ACCGCTTTCG  CTGGAGCGCG  ACGATGATCG
 6961  GCCTGTCGCT  TGCGGTATTC  GGAATCTTGC  ACGCCCTCGC  TCAAGCCTTC  GTCACTGGTC
 7021  CCGCCACCAA  ACGTTTCGGC  GAGAAGCAGG  CCATTATCGC  CGGCATGGCG  GCCGACGCGC
 7081  TGGGCTACGT  CTTGCTGGCG  TTCGCGACGC  GAGGCTGGAT  GGCCTTCCCC  ATTATGATTC
 7141  TTCTCGCTTC  CGGCGGCATC  GGGATGCCCG  CGTTGCAGGC  CATGCTGTCC  AGGCAGGTAG
 7201  ATGACGACCA  TCAGGGACAG  CTTCAAGGAT  CGCTCGCGGC  TCTTACCAGC  CTAACTTCGA
 7261  TCATTGGACC  GCTGATCGTC  ACGGCGATTT  ATGCCGCCTC  GGCGAGCACA  TGGAACGGGT
 7321  TGGCATGGAT  TGTAGGCGCC  GCCCTATACC  TTGTCTGCCT  CCCCGCGTTG  CGTCGCGGTG
 7381  CATGGAGCCG  GGCCACCTCG  ACCTGAATGG  AAGCCGGCGG  CACCTCGCTA  ACGGATTCAC
 7441  CACTCCAAGA  ATTGGAGCCA  ATCAATTCTT  GCGGAGAACT  GTGAATGCGC  AAACCAACCC
 7501  TTGGCAGAAC  ATATCCATCG  CGTCCGCCAT  CTCCAGCAGC  CGCACGCGGC  GCATCTCGGG
 7561  GAATTCGCGG  CCGCTTCTAG  AGCTGATGGC  TAGCTCAGTC  CTAGGGATTA  TGCTAGCTAC
 7621  TAGAGAAAGA  GGAGAAATAC  TAGATGCAAC  GAGGGATAGT  CTGGGTAGTC  GATGACGATA
 7681  GTTCCATCCG  TTGGGTGCTT  GAACGTGCGC  TCGCTGGGGC  AGGTTTAACC  TGTACGACGT
 7741  TTGAGAACGG  CGCAGAAGTG  CTGGAGGCGC  TGGCGAGCAA  AACGCCGGAT  GTGCTGCTTT
 7801  CAGATATCCG  TATGCCGGGA  ATGGACGGGC  TGGCGCTGCT  CAAGCAGATT  AAACAGCGCC
 7861  ATCCAATGCT  TCCGGTCATC  ATTATGACCG  CACATTCCGA  TCTGGATGCT  GCCGTCAGCG
 7921  CCTATCAACA  AGGGGCGTTT  GATTATCTGC  CCAAACCGTT  TGATATCGAC  GAAGCAGTGG
 7981  CGCTGGTTGA  GCGCGCTATC  AGTCATTACC  AGGAACAGCA  GCAGCCGCGT  AATGTTCAGC
 8041  TTAACGGCCC  AACGACCGAT  ATCATCGGCG  AAGCGCCAGC  CATGCAGGAC  GTGTTCCGTA
 8101  TTATCGGTCG  GCTTTCGCGT  TCTTCTATTA  GCGTGCTGAT  TAACGGCGAA  TCCGGCACCG
 8161  GTAAAGAACT  GGTCGCTCAT  GCCCTGCATC  GCCACAGTCC  GCGCGCCAAA  GCGCCGTTTA
 8221  TCGCGCTGAA  TATGGCAGCT  ATCCCAAAAG  ATTTGATCGA  ATCAGAACTG  TTTGGCCACG
 8281  AGAAAGGCGC  GTTTACTGGC  GCGAATACCA  TTCGTCAGGG  GCGTTTTGAA  CAGGCCGATG
 8341  GCGGTACATT  ATTCCTCGAC  GAAATTGGTG  ATATGCCGCT  GGATGTGCAG  ACGCGTTTGC
 8401  TGCGCGTGCT  GGCAGACGGT  CAGTTTTACC  GCGTTGGCGG  CTATGCGCCG  GTGAAAGTGG
 8461  ATGTGCGGAT  TATCGCTGCC  ACTCACCAGA  ATCTCGAACA  GCGAGTGCAG  GAAGGTAAGT
 8521  TCCGTGAGGA  TCTGTTCCAC  CGCCTGAACG  TTATCCGCGT  TCATCTGCCG  CCGCTGCGCG
 8581  AACGTCGGGA  AGATATTCCC  CGTCTGGCGC  GCCATTTTTT  ACAGGTTGCC  GCGCGCGAAC
 8641  TGGGCGTAGA  AGCGAAGTTA  CTGCATCCGG  AAACCGAAGC  TGCTCTGACG  CGTCTGGCGT
 8701  GGCCAGGCAA  CGTGCGCCAG  CTGGAAAACA  CCTGCCGCTG  GCTAACGGTG  ATGGCCGCCG
 8761  GGCAGGAAGT  GTTGATTCAG  GATTTGCCCG  GCGAACTGTT  TGAATCAACG  GTTGCGGAGA
 8821  GTACTTCGCA  AATGCAACCG  GACAGCTGGG  CGACGCTTCT  TGCGCAGTGG  GCAGACAGAG
 8881  CGCTGCGTTC  CGGTCATCAA  AATCTGCTTT  CCGAAGCGCA  GCCAGAGCTG  GAGCGGACGT
 8941  TACTGACGAC  CGCGTTGCGA  CATACGCAGG  GGCATAAACA  GGAAGCGGCG  CGGCTACTCG
 9001  GCTGGGGCCG  CAACACCCTG  ACGCGTAAGT  TAAAAGAGCT  GGGGATGGAG  TGATACTAGA
 9061  GCCAGGCATC  AAATAAAACG  AAAGGCTCAG  TCGAAAGACT  GGGCCTTTCG  TTTTATCTGT
 9121  TGTTTGTCGG  TGAACGCTCT  CTACTAGAGT  CACACTGGCT  CACCTTCGGG  TGGGCCTTTC
 9181  TGCGTTTATA  TACTAGTAGC  GGCCGCTGCA  GCTCGGGCAG  CGTTGGGTCC  TGGCCACGGG
 9241  TGCGCATGAT  CGTGCTCCTG  TCGTTGAGGA  CCCGGCTAGG  CTGGCGGGGT  TGCCTTACTG
 9301  GTTAGCAGAA  TGAATCACCG  ATACGCGAGC  GAACGTGAAG  CGACTGCTGC  TGCAAAACGT
 9361  CTGCGACCTG  AGCAACAACA  TGAATGGTCT  TCGGTTTCCG  TGTTTCGTAA  AGTCTGGAAA
 9421  CGCGGAAGTC  CCCTACGTGC  TGCTGAAGTT  GCCCGCAACA  GAGAGTGGAA  CCAACCGGTG
 9481  ATACCACGAT  ACTATGACTG  AGAGTCAACG  CCATGAGCGG  CCTCATTTCT  TATTCTGAGT
 9541  TACAACAGTC  CGCACCGCTG  TCCGGTAGCT  CCTTCCGGTG  GGCGCGGGGC  ATGACTATCG
 9601  TCGCCGCACT  TATGACTGTC  TTCTTTATCA  TGCAACTCGT  AGGACAGGTG  CCGGCAGCGC
 9661  CCAACAGTCC  CCCGGCCACG  GGGCCTGCCA  CCATACCCAC  GCCGAAACAA  GCGCCCTGCA
 9721  CCATTATGTT  CCGGATCTGC  ATCGCAGGAT  GCTGCTGGCT  ACCCTGTGGA  ACACCTACAT
 9781  CTGTATTAAC  GAAGCGCTAA  CCGTTTTTAT  CAGGCTCTGG  GAGGCAGAAT  AAATGATCAT
 9841  ATCGTCAATT  ATTACCTCCA  CGGGGAGAGC  CTGAGCAAAC  TGGCCTCAGG  CATTTGAGAA
 9901  GCACACGGTC  ACACTGCTTC  CGGTAGTCAA  TAAACCGGTA  AACCAGCAAT  AGACATAAGC
 9961  GGCTATTTAA  CGACCCTGCC  CTGAACCGAC  GACCGGGTCG  AATTTGCTTT  CGAATTTCTG
10021  CCATTCATCC  GCTTATTATC  ACTTATTCAG  GCGTAGCACC  AGGCGTTTAA  GGGCACCAAT
10081  AACTGCCTTA  AAAAAATTAC  GCCCCGCCCT  GCCACTCATC  GCAGTACTGT  TGTAATTCAT
10141  TAAGCATTCT  GCCGACATGG  AAGCCATCAC  AGACGGCATG  ATGAACCTGA  ATCGCCAGCG
10201  GCATCAGCAC  CTTGTCGCCT  TGCGTATAAT  ATTTGCCCAT  GGTGAAAACG  GGGGCGAAGA
10261  AGTTGTCCAT  ATTGGCCACG  TTTAAATCAA  AACTGGTGAA  ACTCACCCAG  GGATTGGCTG
10321  AGACGAAAAA  CATATTCTCA  ATAAACCCTT  TAGGGAAATA  GGCCAGGTTT  TCACCGTAAC
10381  ACGCCACATC  TTGCGAATAT  ATGTGTAGAA  ACTGCCGGAA  ATCGTCGTGG  TATTCACTCC
10441  AGAGCGATGA  AAACGTTTCA  GTTTGCTCAT  GGAAAACGGT  GTAACAAGGG  TGAACACTAT
10501  CCCATATCAC  CAGCTCACCG  TCTTTCATTG  CCATACG
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1.7.2 Circuit with low NRI:

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   1  GAATTCCGGA  TGAGCATTCA  TCAGGCGGGC  AAGAATGTGA  ATAAAGGCCG  GATAAAACTT
  61  GTGCTTATTT  TTCTTTACGG  TCTTTAAAAA  GGCCGTAATA  TCCAGCTGAA  CGGTCTGGTT
 121  ATAGGTACAT  TGAGCAACTG  ACTGAAATGC  CTCAAAATGT  TCTTTACGAT  GCCATTGGGA
 181  TATATCAACG  GTGGTATATC  CAGTGATTTT  TTTCTCCATT  TTAGCTTCCT  TAGCTCCTGA
 241  AAATCTCGAT  AACTCAAAAA  ATACGCCCGG  TAGTGATCTT  ATTTCATTAT  GGTGAAAGTT
 301  GGAACCTCTT  ACGTGCCGAT  CAACGTCTCA  TTTTCGCCAA  AAGTTGGCCC  AGGGCTTCCC
 361  GGTATCAACA  GGGACACCAG  GATTTATTTA  TTCTGCGAAG  TGATCTTCCG  TCACAGGTAT
 421  TTATTCGGCG  CAAAGTGCGT  CGGGTGATGC  TGCCAACTTA  CTGATTTAGT  GTATGATGGT
 481  GTTTTTGAGG  TGCTCCAGTG  GCTTCTGTTT  CTATCAGCTG  TCCCTCCTGT  TCAGCTACTG
 541  ACGGGGTGGT  GCGTAACGGC  AAAAGCACCG  CCGGACATCA  GCGCTAGCGG  AGTGTATACT
 601  GGCTTACTAT  GTTGGCACTG  ATGAGGGTGT  CAGTGAAGTG  CTTCATGTGG  CAGGAGAAAA
 661  AAGGCTGCAC  CGGTGCGTCA  GCAGAATATG  TGATACAGGA  TATATTCCGC  TTCCTCGCTC
 721  ACTGACTCGC  TACGCTCGGT  CGTTCGACTG  CGGCGAGCGG  AAATGGCTTA  CGAACGGGGC
 781  GGAGATTTCC  TGGAAGATGC  CAGGAAGATA  CTTAACAGGG  AAGTGAGAGG  GCCGCGGCAA
 841  AGCCGTTTTT  CCATAGGCTC  CGCCCCCCTG  ACAAGCATCA  CGAAATCTGA  CGCTCAAATC
 901  AGTGGTGGCG  AAACCCGACA  GGACTATAAA  GATACCAGGC  GTTTCCCCCT  GGCGGCTCCC
 961  TCGTGCGCTC  TCCTGTTCCT  GCCTTTCGGT  TTACCGGTGT  CATTCCGCTG  TTATGGCCGC
1021  GTTTGTCTCA  TTCCACGCCT  GACACTCAGT  TCCGGGTAGG  CAGTTCGCTC  CAAGCTGGAC
1081  TGTATGCACG  AACCCCCCGT  TCAGTCCGAC  CGCTGCGCCT  TATCCGGTAA  CTATCGTCTT
1141  GAGTCCAACC  CGGAAAGACA  TGCAAAAGCA  CCACTGGCAG  CAGCCACTGG  TAATTGATTT
1201  AGAGGAGTTA  GTCTTGAAGT  CATGCGCCGG  TTAAGGCTAA  ACTGAAAGGA  CAAGTTTTGG
1261  TGACTGCGCT  CCTCCAAGCC  AGTTACCTCG  GTTCAAAGAG  TTGGTAGCTC  AGAGAACCTT
1321  CGAAAAACCG  CCCTGCAAGG  CGGTTTTTTC  GTTTTCAGAG  CAAGAGATTA  CGCGCAGACC
1381  AAAACGATCT  CAAGAAGATC  ATCTTATTAA  TCAGATAAAA  TATTTCTAGA  GCATCCTCCG
1441  CAAACAAGTA  TTGCAGAGTC  CCTTTGTGAT  CGCTTTCACG  GAGCATAAAA  AGGGTTATCC
1501  AAAGGTCATT  GCACCAACAT  GGTGCTTAAT  GTTTCCATTG  AAGCACTATA  TTGGTGCAAC
1561  ATTCACATCG  TGGTGCAGCC  CTTTTGCACG  ATGGTGCGCA  TGATAACGCC  TTTTAGGGGC
1621  AATTTAAAAG  TTGGCACAGA  TTTCGCTTTA  TCTTTTTTAC  GGCGACACGG  CCAAAAATAAT
1681  TGCAGATTTC  GTTACCACGA  CGACCTAACT  TTCAATTCTA  ATAAGGAGGA  AGACTTCAAA
1741  TGCGTAAAGG  CGAAGAGCTG  TTCACTGGTG  TCGTCCCTAT  TCTGGTGGAA  CTGGATGGTG
1801  ATGTCAACGG  TCATAAGTTT  TCCGTGCGTG  GCGAGGGTGA  AGGTGACGCA  ACTAATGGTA
1861  AACTGACGCT  GAAGTTCATC  TGTACTACTG  GTAAACTGCC  GGTACCTTGG  CCGACTCTGG
1921  TAACGACGCT  GACTTATGGT  GTTCAGTGCT  TTGCTCGTTA  TCCGGACCAT  ATGAAGCAGC
1981  ATGACTTCTT  CAAGTCCGCC  ATGCCGGAAG  GCTATGTGCA  GGAACGCACG  ATTTCCTTTA
2041  AGGATGACGG  CACGTACAAA  ACGCGTGCGG  AAGTGAAATT  TGAAGGCGAT  ACCCTGGTAA
2101  ACCGCATTGA  GCTGAAAGGC  ATTGACTTTA  AAGAAGACGG  CAATATCCTG  GGCCATAAGC
2161  TGGAATACAA  TTTTAACAGC  CACAATGTTT  ACATCACCGC  CGATAAACAA  AAAAATGGCA
2221  TTAAAGCGAA  TTTTAAAATT  CGCCACAACG  TGGAGGATGG  CAGCGTGCAG  CTGGCTGATC
2281  ACTACCAGCA  AAACACTCCA  ATCGGTGATG  GTCCTGTTCT  GCTGCCAGAC  AATCACTATC
2341  TGAGCACGCA  AAGCGTTCTG  TCTAAAGATC  CGAACGAGAA  ACGCGATCAT  ATGGTTCTGC
2401  TGGAGTTCGT  AACCGCAGCG  GGCATCACGC  ATGGTATGGA  TGAACTGTAC  AAACAGGCTG
2461  CAAACGACGA  AAACTACGCT  TTAGTAGCTT  GATGACTTAA  GGAGCTCCAA  TTGCCAGGCA
2521  TCAAATAAAA  CGAAAGGCTC  AGTCGAAAGA  CTGGGCCTTT  CGTTTTATCT  GTTGTTTGTC
2581  GGTGAACGCT  CTCTACTAGA  GTCACACTGG  CTCACCTTCG  GGTGGGCCTT  TCTGCGTTTA
2641  TAAAGCTTTA  ATGCGGTAGT  TTATCACAGT  TAAATTGCTA  ACGCAGTCAG  GCACCGTGTA
2701  TGAAATCTAA  CAATGCGCTC  ATCGTCATCC  TCGGCACCGT  CACCCTGGAT  GCTGTAGGCA
2761  TAGGCTTGGT  TATGCCGGTA  CTGCCGGGCC  TCTTGCGGGA  TATCGTCCAT  TCCGACAGCA
2821  TCGCCAGTCA  CTATGGCGTG  CTGCTAGCGC  TATATGCGTT  GATGCAATTT  CTATGCGCAC
2881  CCGTTCTCGG  AGCACTGTCC  GACCGCTTTG  GCCGCCGCCC  AGTCCTGCTC  GCTTCGCTAC
2941  TTGGAGCCAC  TATCGACTAC  GCGATCATGG  CGACCACACC  CGTCCTGTGG  ATCCGAATTC
3001  GCGGCCGCTT  CTAGAGTTTA  TGGCTAGCTC  AGTCCTAGGT  ACAATGCTAG  CTACTAGAGA
3061  AAGAGGAGAA  ATACTAGATG  TCCAGATTAG  ATAAAAGTAA  AGTGATTAAC  AGCGCATTAG
3121  AGCTGCTTAA  TGAGGTCGGA  ATCGAAGGTT  TAACAACCCG  TAAACTCGCC  CAGAAGCTAG
3181  GTGTAGAGCA  GCCTACATTG  TATTGGCATG  TAAAAAATAA  GCGGGCTTTG  CTCGACGCCT
3241  TAGCCATTGA  GATGTTAGAT  AGGCACCATA  CTCACTTTTG  CCCTTTAGAA  GGGGAAAGCT
3301  GGCAAGATTT  TTTACGTAAT  AACGCTAAAA  GTTTTAGATG  TGCTTTACTA  AGTCATCGCG
3361  ATGGAGCAAA  AGTACATTTA  GGTACACGGC  CTACAGAAAA  ACAGTATGAA  ACTCTCGAAA
3421  ATCAATTAGC  CTTTTTATGC  CAACAAGGTT  TTTCACTAGA  GAATGCATTA  TATGCACTCA
3481  GCGCTGTGGG  GCATTTTACT  TTAGGTTGCG  TATTGGAAGA  TCAAGAGCAT  CAAGTCGCTA
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3541   AAGAAGAAAG   GGAAACACCT   ACTACTGATA   GTATGCCGCC   ATTATTACGA   CAAGCTATCG
3601   AATTATTTGA   TCACCAAGGT   GCAGAGCCAG   CCTTCTTATT   CGGCCTTGAA   TTGATCATAT
3661   GCGGATTAGA   AAAACAACTT   AAATGTGAAA   GTGGGTCCGC   TGCAAACGAC   GAAAACTACG
3721   CTTTAGTAGC   TTAATAATAC   TAGAGCCAGG   CATCAAATAA   AACGAAAGGC   TCAGTCGAAA
3781   GACTGGGCCT   TTCGTTTTAT   CTGTTGTTTG   TCGGTGAACG   CTCTCTACTA   GAGTCACACT
3841   GGCTCACCTT   CGGGTGGGCC   TTTCTGCGTT   TATATACTAG   AGTTTATGGC   TAGCTCAGTC
3901   CTAGGTACAA   TGCTAGCTAC   TAGAGAAAGA   GGAGAAATAC   TAGATGGTGA   ATGTGAAACC
3961   AGTAACGTTA   TACGATGTCG   CAGAGTATGC   CGGTGTCTCT   TATCAGACCG   TTTCCCGCGT
4021   GGTGAACCAG   GCCAGCCACG   TTTCTGCGAA   AACGCGGGAA   AAAGTGGAAG   CGGCGATGGC
4081   GGAGCTGAAT   TACATTCCCA   ACCGCGTGGC   ACAACAACTG   GCGGGCAAAC   AGTCGTTGCT
4141   GATTGGCGTT   GCCACCTCCA   GTCTGGCCCT   GCACGCGCCG   TCGCAAATTG   TCGCGGCGAT
4201   TAAATCTCGC   GCCGATCAAC   TGGGTGCCAG   CGTGGTGGTG   TCGATGGTAG   AACGAAGCGG
4261   CGTCGAAGCC   TGTAAAGCGG   CGGTGCACAA   TCTTCTCGCG   CAACGCGTCA   GTGGGCTGAT
4321   CATTAACTAT   CCGCTGGATG   ACCAGGATGC   CATTGCTGTG   GAAGCTGCCT   GCACTAATGT
4381   TCCGGCGTTA   TTTCTTGATG   TCTCTGACCA   GACACCCATC   AACAGTATTA   TTTTCTCCCA
4441   TGAAGACGGT   ACGCGACTGG   GCGTGGAGCA   TCTGGTCGCA   TTGGGTCACC   AGCAAATCGC
4501   GCTGTTAGCG   GGCCCATTAA   GTTCTGTCTC   GGCGCGTCTG   CGTCTGGCTG   GCTGGCATAA
4561   ATATCTCACT   CGCAATCAAA   TTCAGCCGAT   AGCGGAACGG   GAAGGCGACT   GGAGTGCCAT
4621   GTCCGGTTTT   CAACAAACCA   TGCAAATGCT   GAATGAGGGC   ATCGTTCCCA   CTGCGATGCT
4681   GGTTGCCAAC   GATCAGATGG   CGCTGGGCGC   AATGCGCGCC   ATTACCGAGT   CCGGGCTGCG
4741   CGTTGGTGCG   GATATCTCGG   TAGTGGGATA   CGACGATACC   GAAGACAGCT   CATGTTATAT
4801   CCCGCCGTTA   ACCACCATCA   AACAGGATTT   TCGCCTGCTG   GGGCAAACCA   GCGTGGACCG
4861   CTTGCTGCAA   CTCTCTCAGG   GCCAGGCGGT   GAAGGGCAAT   CAGCTGTTGC   CCGTCTCACT
4921   GGTGAAAAGA   AAAACCACCC   TGGCGCCCAA   TACGCAAACC   GCCTCTCCCC   GCGCGTTGGC
4981   CGATTCATTA   ATGCAGCTGG   CACGACAGGT   TTCCCGACTG   GAAAGCGGGC   AGGCTGCAAA
5041   CGACGAAAAC   TACGCTTTAG   TAGCTTAATA   ATACTAGAGC   CAGGCATCAA   ATAAAACGAA
5101   AGGCTCAGTC   GAAAGACTGG   GCCTTTCGTT   TTATCTGTTG   TTTGTCGGTG   AACGCTCTCT
5161   ACTAGAGTCA   CACTGGCTCA   CCTTCGGGTG   GGCCTTTCTG   CGTTTATATA   CTAGTAGCGG
5221   CCGCTGCAGG   GATCCTCTAC   GCCGGACGCA   TCGTGGCCGG   CATCACCGGC   GCCACAGGTG
5281   CGGTTGCTGG   CGCCTATATC   GCCGACATCA   CCGATGGGGA   AGATCGGGCT   CGCCACTTCG
5341   GGCTCATGAC   TGCAGCGGCC   GCTACTAGTA   TATAAACGCA   GAAAGGCCCA   CCCGAAGGTG
5401   AGCCAGTGTG   ACTCTAGTAG   AGAGCGTTCA   CCGACAAACA   ACAGATAAAA   CGAAAGGCCC
5461   AGTCTTTCGA   CTGAGCCTTT   CGTTTTATTT   GATGCCTGGC   TCTAGTATTA   AGCTACTAAA
5521   GCGTAGTTTT   CGTCGTTTGC   AGCTTTCCTG   ATAGGCAGGT   AAACCGAGAA   CTCGGTATGC
5581   CCTGGCCAAC   TGGTAAATTC   AATTTTGCCT   GAATGCTGAT   CAATCAAATT   ACGAGCGATG
5641   GATAAGCCAA   GCCCGGTGCC   ACCTTCGCGG   CCGCTGACCA   TCGGGTAAAA   CAGCGTATCC
5701   TGCAAATGAG   GCGGAATGCC   CGGCCCGTTA   TCTTCCACAT   CAATCCGCGC   CGCCAGCCGG
5761   TAGCGCTCGC   CGTGTAAGGT   CAGTTGAAAC   GCGGTGCGGG   TACGCAGAAT   GATTTCACCG
5821   CCTTCCGGCC   CCAGCGCCTG   TAGCGCATTG   CGCACAATAT   TCAGCAAGAC   CTGTTCAATT
5881   TGATCCGGGT   CGTGCGCCAG   TTCCGGTAGG   CTGGGATCGT   AATCACGAAT   CAACCGCACG
5941   TTGTCCGGCA   GTTCCATCGA   CACCAGCGTT   ACCACGCGTT   CAGCCACTTT   GTGAATACTT
6001   TCGGTAACGC   GCGTACCGGG   CAGCTGCGGC   CCCAACAGAC   GGTCGACCAG   ATTTCGCAGC
6061   CGGTCCGCCT   GTTCGATAAT   CACTTTGGTA   TATTCGAGTA   GTGATGGGTC   AGGTAACGCT
6121   TTGCTGAGCA   GCTGCGCCGC   GCCACGTAAA   CCGCCAAGCG   GATTTTTAAT   CTCATGTGCC
6181   AGGCCGCGCA   CTAAATCACG   GGCAGCAACC   TGCTGGGCGT   GCTGTAGCTG   TTCCTGACTT
6241   AAGCGGCGCT   GGTTATCCAT   CGGAGCCATC   TCCAGCAGGA   TCATGCCGTC   CGGCATACGC
6301   TGGGCCGTCA   CAGAAAGGAT   ATGCGAGCGC   CCGTCGATGA   CCAGCGTCAC   TTCGTTATCG
6361   GTAAAACCTT   GCCCCGCCTC   CAGACTTTCT   TGCATCAGCT   CGATATTTAA   TGAGAAGTAG
6421   CTCAACAGTT   CCGGTAACGG   TGTACCAAAC   AATTTGCGGG   AGCTTTGGGC   GAGCAGTTGT
6481   TGCGCGGCAG   GGTTGGCGTA   ATGGATCGCC   AGGTTGTCAT   CGATTAACAA   AATACTGTTA
6541   ATCCGCGAGT   TGAGGATCTG   CCCAGCATCG   GGCTGCGTGC   CTGTTGCCAT   CTAGTAGTCC
6601   TGTGTGACTC   TAGTAGTGCT   CAGTATCTCT   ATCACTGATA   GGGATGTCAA   TCTCTATCAC
6661   TGATAGGGAC   TCTAGAAGCG   GCCGCGAATT   CGCATGCACC   ATTCCTTGCG   GCGGCGGTGC
6721   TCAACGGCCT   CAACCTACTA   CTGGGCTGCT   TCCTAATGCA   GGAGTCGCAT   AAGGGAGAGC
6781   GTCGACCGAT   GCCCTTGAGA   GCCTTCAACC   CAGTCAGCTC   CTTCCGGTGG   GCGCGGGGCA
6841   TGACTATCGT   CGCCGCACTT   ATGACTGTCT   TCTTTATCAT   GCAACTCGTA   GGACAGGTGC
6901   CGGCAGCGCT   CTGGGTCATT   TTCGGCGAGG   ACCGCTTTCG   CTGGAGCGCG   ACGATGATCG
6961   GCCTGTCGCT   TGCGGTATTC   GGAATCTTGC   ACGCCCTCGC   TCAAGCCTTC   GTCACTGGTC
7021   CCGCCACCAA   ACGTTTCGGC   GAGAAGCAGG   CCATTATCGC   CGGCATGGCG   GCCGACGCGC
7081   TGGGCTACGT   CTTGCTGGCG   TTCGCGACGC   GAGGCTGGAT   GGCCTTCCCC   ATTATGATTC
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 7141  TTCTCGCTTC  CGGCGGCATC  GGGATGCCCG  CGTTGCAGGC  CATGCTGTCC  AGGCAGGTAG
 7201  ATGACGACCA  TCAGGGACAG  CTTCAAGGAT  CGCTCGCGGC  TCTTACCAGC  CTAACTTCGA
 7261  TCATTGGACC  GCTGATCGTC  ACGGCGATTT  ATGCCGCCTC  GGCGAGCACA  TGGAACGGGT
 7321  TGGCATGGAT  TGTAGGCGCC  GCCCTATACC  TTGTCTGCCT  CCCCGCGTTG  CGTCGCGGTG
 7381  CATGGAGCCG  GGCCACCTCG  ACCTGAATGG  AAGCCGGCGG  CACCTCGCTA  ACGGATTCAC
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 7501  TTGGCAGAAC  ATATCCATCG  CGTCCGCCAT  CTCCAGCAGC  CGCACGCGGC  GCATCTCGGG
 7561  GAATTCGCGG  CCGCTTCTAG  AGTTTATGGC  TAGCTCAGTC  CTAGGTACAA  TGCTAGCTAC
 7621  TAGAGTCACA  CAGGAAAGTA  CTAGATGCAA  CGAGGGATAG  TCTGGGTAGT  CGATGACGAT
 7681  AGTTCCATCC  GTTGGGTGCT  TGAACGTGCG  CTCGCTGGGG  CAGGTTTAAC  CTGTACGACG
 7741  TTTGAGAACG  GCGCAGAAGT  GCTGGAGGCG  CTGGCGAGCA  AAACGCCGGA  TGTGCTGCTT
 7801  TCAGATATCC  GTATGCCGGG  AATGGACGGG  CTGGCGCTGC  TCAAGCAGAT  TAAACAGCGC
 7861  CATCCAATGC  TTCCGGTCAT  CATTATGACC  GCACATTCCG  ATCTGGATGC  TGCCGTCAGC
 7921  GCCTATCAAC  AAGGGGCGTT  TGATTATCTG  CCCAAACCGT  TTGATATCGA  CGAAGCAGTG
 7981  GCGCTGGTTG  AGCGCGCTAT  CAGTCATTAC  CAGGAACAGC  AGCAGCCGCG  TAATGTTCAG
 8041  CTTAACGGCC  CAACGACCGA  TATCATCGGC  GAAGCGCCAG  CCATGCAGGA  CGTGTTCCGT
 8101  ATTATCGGTC  GGCTTTCGCG  TTCTTCTATT  AGCGTGCTGA  TTAACGGCGA  ATCCGGCACC
 8161  GGTAAAGAAC  TGGTCGCTCA  TGCCCTGCAT  CGCCACAGTC  CGCGCGCCAA  AGCGCCGTTT
 8221  ATCGCGCTGA  ATATGGCAGC  TATCCCAAAA  GATTTGATCG  AATCAGAACT  GTTTGGCCAC
 8281  GAGAAAGGCG  CGTTTACTGG  CGCGAATACC  ATTCGTCAGG  GGCGTTTTGA  ACAGGCCGAT
 8341  GGCGGTACAT  TATTCCTCGA  CGAAATTGGT  GATATGCCGC  TGGATGTGCA  GACGCGTTTG
 8401  CTGCGCGTGC  TGGCAGACGG  TCAGTTTTAC  CGCGTTGGCG  GCTATGCGCC  GGTGAAAGTG
 8461  GATGTGCGGA  TTATCGCTGC  CACTCACCAG  AATCTCGAAC  AGCGAGTGCA  GGAAGGTAAG
 8521  TTCCGTGAGG  ATCTGTTCCA  CCGCCTGAAC  GTTATCCGCG  TTCATCTGCC  GCCGCTGCGC
 8581  GAACGTCGGG  AAGATATTCC  CCGTCTGGCG  CGCCATTTTT  TACAGGTTGC  CGCGCGCGAA
 8641  CTGGGCGTAG  AAGCGAAGTT  ACTGCATCCG  GAAACCGAAG  CTGCTCTGAC  GCGTCTGGCG
 8701  TGGCCAGGCA  ACGTGCGCCA  GCTGGAAAAC  ACCTGCCGCT  GGCTAACGGT  GATGGCCGCC
 8761  GGGCAGGAAG  TGTTGATTCA  GGATTTGCCC  GGCGAACTGT  TTGAATCAAC  GGTTGCGGAG
 8821  AGTACTTCGC  AAATGCAACC  GGACAGCTGG  GCGACGCTTC  TTGCGCAGTG  GGCAGACAGA
 8881  GCGCTGCGTT  CCGGTCATCA  AAATCTGCTT  TCCGAAGCGC  AGCCAGAGCT  GGAGCGGACG
 8941  TTACTGACGA  CCGCGTTGCG  ACATACGCAG  GGGCATAAAC  AGGAAGCGGC  GCGGCTACTC
 9001  GGCTGGGGCC  GCAACACCCT  GACGCGTAAG  TTAAAAGAGC  TGGGGATGGA  GTGATACTAG
 9061  AGCCAGGCAT  CAAATAAAAC  GAAAGGCTCA  GTCGAAAGAC  TGGGCCTTTC  GTTTTATCTG
 9121  TTGTTTGTCG  GTGAACGCTC  TCTACTAGAG  TCACACTGGC  TCACCTTCGG  GTGGGCCTTT
 9181  CTGCGTTTAT  ATACTAGAGA  ATTGTGAGCG  GATAACAATT  GACATTGTGA  GCGGATAACA
 9241  AGATACTGAG  CACATACTAG  AGAAAGAGGA  GAAATACTAG  ATGGCAACAG  GCACGCAGCC
 9301  CGATGCTGGG  CAGATCCTCA  ACTCGCTGAT  TAACAGTATT  TTGTTAATCG  ATGACAACCT
 9361  GGCGATCCAT  TACGCCAACC  CTGCCGCGCA  ACAACTGCTC  GCCCAAAGCT  CCCGCAAATT
 9421  GTTTGGTACA  CCGTTACCGG  AACTGTTGAG  CTACTTCTCA  TTAAATATCG  AGCTGATGCA
 9481  AGAAAGTCTG  GAGGCGGGGC  AAGGTTTTAC  CGATAACGAA  GTGACGCTGG  TCATCGACGG
 9541  GCGCTCGCAT  ATCCTTTCTG  TGACGGCCCA  GCGTATGCCG  GACGGCATGA  TCCTGCTGGA
 9601  GATGGCTCCG  ATGGATAACC  AGCGCCGCTT  AAGTCAGGAA  CAGCTACAGC  ACGCCCAGCA
 9661  GGTTGCTGCC  CGTGATTTAG  TGCGCGGCCT  GGCAAATGAG  ATTAAAAATC  CGCTTGGCGG
 9721  TTTACGTGGC  GCGGCGCAGC  TGCTCAGCAA  AGCGTTACCT  GACCCATCAC  TACTCGAATA
 9781  TACCAAAGTG  ATTATCGAAC  AGGCGGACCG  GCTGCGAAAT  CTGGTCGACC  GTCTGTTGGG
 9841  GCCGCAGCTG  CCCGGTACGC  GCGTTACCGA  AAGTATTCAC  AAAGTGGCTG  AACGCGTGGT
 9901  AACGCTGGTG  TCGATGGAAC  TGCCGGACAA  CGTGCGGTTG  ATTCGTGATT  ACGATCCCAG
 9961  CCTACCGGAA  CTGGCGCACG  ACCCGGATCA  AATTGAACAG  GTCTTGCTGA  ATATTGTGCG
10021  CAATGCGCTA  CAGGCGCTGG  GGCCGGAAGG  CGGTGAAATC  ATTCTGCGTA  CCCGCACCGC
10081  GTTTCAACTG  ACCTTACACG  GCGAGCGCTA  CCGGCTGGCG  GCGCGGATTG  ATGTGGAAGA
10141  TAACGGGCCG  GGCATTCCGC  CTCATTTGCA  GGATACGCTG  TTTTACCCGA  TGGTCAGCGG
10201  CCGCGAAGGT  GGCACCGGGC  TTGGCTTATC  CATCGCTCGT  AATTTGATTG  ATCAGCATTC
10261  AGGCAAAATT  GAATTTACCA  GTTGGCCAGG  GCATACCGAG  TTCTCGGTTT  ACCTGCCTAT
10321  CAGGAAAGCT  GCAAACGACG  AAAACTACGC  TTTAGTAGCT  TAATGATACT  AGAGCCAGGC
10381  ATCAAATAAA  ACGAAAGGCT  CAGTCGAAAG  ACTGGGCCTT  TCGTTTTATC  TGTTGTTTGT
10441  CGGTGAACGC  TCTCTACTAG  AGTCACACTG  GCTCACCTTC  GGGTGGGCCT  TTCTGCGTTT
10501  ATATACTAGT  AGCGGCCGCT  GCAGCTCGGG  CAGCGTTGGG  TCCTGGCCAC  GGGTGCGCAT
10561  GATCGTGCTC  CTGTCGTTGA  GGACCCGGCT  AGGCTGGCGG  GGTTGCCTTA  CTGGTTAGCA
10621  GAATGAATCA  CCGATACGCG  AGCGAACGTG  AAGCGACTGC  TGCTGCAAAA  CGTCTGCGAC
10681  CTGAGCAACA  ACATGAATGG  TCTTCGGTTT  CCGTGTTTCG  TAAAGTCTGG  AAACGCGGAA
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10741   GTCCCCTACG   TGCTGCTGAA   GTTGCCCGCA   ACAGAGAGTG   GAACCAACCG   GTGATACCAC
10801   GATACTATGA   CTGAGAGTCA   ACGCCATGAG   CGGCCTCATT   TCTTATTCTG   AGTTACAACA
10861   GTCCGCACCG   CTGTCCGGTA   GCTCCTTCCG   GTGGGCGCGG   GGCATGACTA   TCGTCGCCGC
10921   ACTTATGACT   GTCTTCTTTA   TCATGCAACT   CGTAGGACAG   GTGCCGGCAG   CGCCCAACAG
10981   TCCCCCGGCC   ACGGGGCCTG   CCACCATACC   CACGCCGAAA   CAAGCGCCCT   GCACCATTAT
11041   GTTCCGGATC   TGCATCGCAG   GATGCTGCTG   GCTACCCTGT   GGAACACCTA   CATCTGTATT
11101   AACGAAGCGC   TAACCGTTTT   TATCAGGCTC   TGGGAGGCAG   AATAAATGAT   CATATCGTCA
11161   ATTATTACCT   CCACGGGGAG   AGCCTGAGCA   AACTGGCCTC   AGGCATTTGA   GAAGCACACG
11221   GTCACACTGC   TTCCGGTAGT   CAATAAACCG   GTAAACCAGC   AATAGACATA   AGCGGCTATT
11281   TAACGACCCT   GCCCTGAACC   GACGACCGGG   TCGAATTTGC   TTTCGAATTT   CTGCCATTCA
11341   TCCGCTTATT   ATCACTTATT   CAGGCGTAGC   ACCAGGCGTT   TAAGGGCACC   AATAACTGCC
11401   TTAAAAAAAT   TACGCCCCGC   CCTGCCACTC   ATCGCAGTAC   TGTTGTAATT   CATTAAGCAT
11461   TCTGCCGACA   TGGAAGCCAT   CACAGACGGC   ATGATGAACC   TGAATCGCCA   GCGGCATCAG
11521   CACCTTGTCG   CCTTGCGTAT   AATATTTGCC   CATGGTGAAA   ACGGGGGCGA   AGAAGTTGTC
11581   CATATTGGCC   ACGTTTAAAT   CAAAACTGGT   GAAACTCACC   CAGGGATTGG   CTGAGACGAA
11641   AAACATATTC   TCAATAAACC   CTTTAGGGAA   ATAGGCCAGG   TTTTCACCGT   AACACGCCAC
11701   ATCTTGCGAA   TATATGTGTA   GAAACTGCCG   GAAATCGTCG   TGGTATTCAC   TCCAGAGCGA
11761   TGAAAACGTT   TCAGTTTGCT   CATGGAAAAC   GGTGTAACAA   GGGTGAACAC   TATCCCATAT
11821   CACCAGCTCA   CCGTCTTTCA   TTGCCATACG
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1.7.3 Circuit with medium NRI:

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   1  GAATTCCGGA  TGAGCATTCA  TCAGGCGGGC  AAGAATGTGA  ATAAAGGCCG  GATAAAACTT
  61  GTGCTTATTT  TTCTTTACGG  TCTTTAAAAA  GGCCGTAATA  TCCAGCTGAA  CGGTCTGGTT
 121  ATAGGTACAT  TGAGCAACTG  ACTGAAATGC  CTCAAAATGT  TCTTTACGAT  GCCATTGGGA
 181  TATATCAACG  GTGGTATATC  CAGTGATTTT  TTTCTCCATT  TTAGCTTCCT  TAGCTCCTGA
 241  AAATCTCGAT  AACTCAAAAA  ATACGCCCGG  TAGTGATCTT  ATTTCATTAT  GGTGAAAGTT
 301  GGAACCTCTT  ACGTGCCGAT  CAACGTCTCA  TTTTCGCCAA  AAGTTGGCCC  AGGGCTTCCC
 361  GGTATCAACA  GGGACACCAG  GATTTATTTA  TTCTGCGAAG  TGATCTTCCG  TCACAGGTAT
 421  TTATTCGGCG  CAAAGTGCGT  CGGGTGATGC  TGCCAACTTA  CTGATTTAGT  GTATGATGGT
 481  GTTTTTGAGG  TGCTCCAGTG  GCTTCTGTTT  CTATCAGCTG  TCCCTCCTGT  TCAGCTACTG
 541  ACGGGGTGGT  GCGTAACGGC  AAAAGCACCG  CCGGACATCA  GCGCTAGCGG  AGTGTATACT
 601  GGCTTACTAT  GTTGGCACTG  ATGAGGGTGT  CAGTGAAGTG  CTTCATGTGG  CAGGAGAAAA
 661  AAGGCTGCAC  CGGTGCGTCA  GCAGAATATG  TGATACAGGA  TATATTCCGC  TTCCTCGCTC
 721  ACTGACTCGC  TACGCTCGGT  CGTTCGACTG  CGGCGAGCGG  AAATGGCTTA  CGAACGGGGC
 781  GGAGATTTCC  TGGAAGATGC  CAGGAAGATA  CTTAACAGGG  AAGTGAGAGG  GCCGCGGCAA
 841  AGCCGTTTTT  CCATAGGCTC  CGCCCCCCTG  ACAAGCATCA  CGAAATCTGA  CGCTCAAATC
 901  AGTGGTGGCG  AAACCCGACA  GGACTATAAA  GATACCAGGC  GTTTCCCCCT  GGCGGCTCCC
 961  TCGTGCGCTC  TCCTGTTCCT  GCCTTTCGGT  TTACCGGTGT  CATTCCGCTG  TTATGGCCGC
1021  GTTTGTCTCA  TTCCACGCCT  GACACTCAGT  TCCGGGTAGG  CAGTTCGCTC  CAAGCTGGAC
1081  TGTATGCACG  AACCCCCCGT  TCAGTCCGAC  CGCTGCGCCT  TATCCGGTAA  CTATCGTCTT
1141  GAGTCCAACC  CGGAAAGACA  TGCAAAAGCA  CCACTGGCAG  CAGCCACTGG  TAATTGATTT
1201  AGAGGAGTTA  GTCTTGAAGT  CATGCGCCGG  TTAAGGCTAA  ACTGAAAGGA  CAAGTTTTGG
1261  TGACTGCGCT  CCTCCAAGCC  AGTTACCTCG  GTTCAAAGAG  TTGGTAGCTC  AGAGAACCTT
1321  CGAAAAACCG  CCCTGCAAGG  CGGTTTTTTC  GTTTTCAGAG  CAAGAGATTA  CGCGCAGACC
1381  AAAACGATCT  CAAGAAGATC  ATCTTATTAA  TCAGATAAAA  TATTTCTAGA  GCATCCTCCG
1441  CAAACAAGTA  TTGCAGAGTC  CCTTTGTGAT  CGCTTTCACG  GAGCATAAAA  AGGGTTATCC
1501  AAAGGTCATT  GCACCAACAT  GGTGCTTAAT  GTTTCCATTG  AAGCACTATA  TTGGTGCAAC
1561  ATTCACATCG  TGGTGCAGCC  CTTTTGCACG  ATGGTGCGCA  TGATAACGCC  TTTTAGGGGC
1621  AATTTAAAAG  TTGGCACAGA  TTTCGCTTTA  TCTTTTTTAC  GGCGACACGG  CCAAAATAAT
1681  TGCAGATTTC  GTTACCACGA  CGACCTAACT  TTCAATTCTA  ATAAGGAGGA  AGACTTCAAA
1741  TGCGTAAAGG  CGAAGAGCTG  TTCACTGGTG  TCGTCCCTAT  TCTGGTGGAA  CTGGATGGTG
1801  ATGTCAACGG  TCATAAGTTT  TCCGTGCGTG  GCGAGGGTGA  AGGTGACGCA  ACTAATGGTA
1861  AACTGACGCT  GAAGTTCATC  TGTACTACTG  GTAAACTGCC  GGTACCTTGG  CCGACTCTGG
1921  TAACGACGCT  GACTTATGGT  GTTCAGTGCT  TTGCTCGTTA  TCCGGACCAT  ATGAAGCAGC
1981  ATGACTTCTT  CAAGTCCGCC  ATGCCGGAAG  GCTATGTGCA  GGAACGCACG  ATTTCCTTTA
2041  AGGATGACGG  CACGTACAAA  ACGCGTGCGG  AAGTGAAATT  TGAAGGCGAT  ACCCTGGTAA
2101  ACCGCATTGA  GCTGAAAGGC  ATTGACTTTA  AAGAAGACGG  CAATATCCTG  GGCCATAAGC
2161  TGGAATACAA  TTTTAACAGC  CACAATGTTT  ACATCACCGC  CGATAAACAA  AAAAATGGCA
2221  TTAAAGCGAA  TTTTAAAATT  CGCCACAACG  TGGAGGATGG  CAGCGTGCAG  CTGGCTGATC
2281  ACTACCAGCA  AAACACTCCA  ATCGGTGATG  GTCCTGTTCT  GCTGCCAGAC  AATCACTATC
2341  TGAGCACGCA  AAGCGTTCTG  TCTAAAGATC  CGAACGAGAA  ACGCGATCAT  ATGGTTCTGC
2401  TGGAGTTCGT  AACCGCAGCG  GGCATCACGC  ATGGTATGGA  TGAACTGTAC  AAACAGGCTG
2461  CAAACGACGA  AAACTACGCT  TTAGTAGCTT  GATGACTTAA  GGAGCTCCAA  TTGCCAGGCA
2521  TCAAATAAAA  CGAAAGGCTC  AGTCGAAAGA  CTGGGCCTTT  CGTTTTATCT  GTTGTTTGTC
2581  GGTGAACGCT  CTCTACTAGA  GTCACACTGG  CTCACCTTCG  GGTGGGCCTT  TCTGCGTTTA
2641  TAAAGCTTTA  ATGCGGTAGT  TTATCACAGT  TAAATTGCTA  ACGCAGTCAG  GCACCGTGTA
2701  TGAAATCTAA  CAATGCGCTC  ATCGTCATCC  TCGGCACCGT  CACCCTGGAT  GCTGTAGGCA
2761  TAGGCTTGGT  TATGCCGGTA  CTGCCGGGCC  TCTTGCGGGA  TATCGTCCAT  TCCGACAGCA
2821  TCGCCAGTCA  CTATGGCGTG  CTGCTAGCGC  TATATGCGTT  GATGCAATTT  CTATGCGCAC
2881  CCGTTCTCGG  AGCACTGTCC  GACCGCTTTG  GCCGCCGCCC  AGTCCTGCTC  GCTTCGCTAC
2941  TTGGAGCCAC  TATCGACTAC  GCGATCATGG  CGACCACACC  CGTCCTGTGG  ATCCGAATTC
3001  GCGGCCGCTT  CTAGAGTTTA  TGGCTAGCTC  AGTCCTAGGT  ACAATGCTAG  CTACTAGAGA
3061  AAGAGGAGAA  ATACTAGATG  TCCAGATTAG  ATAAAAGTAA  AGTGATTAAC  AGCGCATTAG
3121  AGCTGCTTAA  TGAGGTCGGA  ATCGAAGGTT  TAACAACCCG  TAAACTCGCC  CAGAAGCTAG
3181  GTGTAGAGCA  GCCTACATTG  TATTGGCATG  TAAAAAATAA  GCGGGCTTTG  CTCGACGCCT
3241  TAGCCATTGA  GATGTTAGAT  AGGCACCATA  CTCACTTTTG  CCCTTTAGAA  GGGGAAAGCT
3301  GGCAAGATTT  TTTACGTAAT  AACGCTAAAA  GTTTTAGATG  TGCTTTACTA  AGTCATCGCG
3361  ATGGAGCAAA  AGTACATTTA  GGTACACGGC  CTACAGAAAA  ACAGTATGAA  ACTCTCGAAA
3421  ATCAATTAGC  CTTTTTATGC  CAACAAGGTT  TTTCACTAGA  GAATGCATTA  TATGCACTCA
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3481   GCGCTGTGGG   GCATTTTACT   TTAGGTTGCG   TATTGGAAGA   TCAAGAGCAT   CAAGTCGCTA
3541   AAGAAGAAAG   GGAAACACCT   ACTACTGATA   GTATGCCGCC   ATTATTACGA   CAAGCTATCG
3601   AATTATTTGA   TCACCAAGGT   GCAGAGCCAG   CCTTCTTATT   CGGCCTTGAA   TTGATCATAT
3661   GCGGATTAGA   AAAACAACTT   AAATGTGAAA   GTGGGTCCGC   TGCAAACGAC   GAAAACTACG
3721   CTTTAGTAGC   TTAATAATAC   TAGAGCCAGG   CATCAAATAA   AACGAAAGGC   TCAGTCGAAA
3781   GACTGGGCCT   TTCGTTTTAT   CTGTTGTTTG   TCGGTGAACG   CTCTCTACTA   GAGTCACACT
3841   GGCTCACCTT   CGGGTGGGCC   TTTCTGCGTT   TATATACTAG   AGTTTATGGC   TAGCTCAGTC
3901   CTAGGTACAA   TGCTAGCTAC   TAGAGAAAGA   GGAGAAATAC   TAGATGGTGA   ATGTGAAACC
3961   AGTAACGTTA   TACGATGTCG   CAGAGTATGC   CGGTGTCTCT   TATCAGACCG   TTTCCCGCGT
4021   GGTGAACCAG   GCCAGCCACG   TTTCTGCGAA   AACGCGGGAA   AAAGTGGAAG   CGGCGATGGC
4081   GGAGCTGAAT   TACATTCCCA   ACCGCGTGGC   ACAACAACTG   GCGGGCAAAC   AGTCGTTGCT
4141   GATTGGCGTT   GCCACCTCCA   GTCTGGCCCT   GCACGCGCCG   TCGCAAATTG   TCGCGGCGAT
4201   TAAATCTCGC   GCCGATCAAC   TGGGTGCCAG   CGTGGTGGTG   TCGATGGTAG   AACGAAGCGG
4261   CGTCGAAGCC   TGTAAAGCGG   CGGTGCACAA   TCTTCTCGCG   CAACGCGTCA   GTGGGCTGAT
4321   CATTAACTAT   CCGCTGGATG   ACCAGGATGC   CATTGCTGTG   GAAGCTGCCT   GCACTAATGT
4381   TCCGGCGTTA   TTTCTTGATG   TCTCTGACCA   GACACCCATC   AACAGTATTA   TTTTCTCCCA
4441   TGAAGACGGT   ACGCGACTGG   GCGTGGAGCA   TCTGGTCGCA   TTGGGTCACC   AGCAAATCGC
4501   GCTGTTAGCG   GGCCCATTAA   GTTCTGTCTC   GGCGCGTCTG   CGTCTGGCTG   GCTGGCATAA
4561   ATATCTCACT   CGCAATCAAA   TTCAGCCGAT   AGCGGAACGG   GAAGGCGACT   GGAGTGCCAT
4621   GTCCGGTTTT   CAACAAACCA   TGCAAATGCT   GAATGAGGGC   ATCGTTCCCA   CTGCGATGCT
4681   GGTTGCCAAC   GATCAGATGG   CGCTGGGCGC   AATGCGCGCC   ATTACCGAGT   CCGGGCTGCG
4741   CGTTGGTGCG   GATATCTCGG   TAGTGGGATA   CGACGATACC   GAAGACAGCT   CATGTTATAT
4801   CCCGCCGTTA   ACCACCATCA   AACAGGATTT   TCGCCTGCTG   GGGCAAACCA   GCGTGGACCG
4861   CTTGCTGCAA   CTCTCTCAGG   GCCAGGCGGT   GAAGGGCAAT   CAGCTGTTGC   CCGTCTCACT
4921   GGTGAAAAGA   AAAACCACCC   TGGCGCCCAA   TACGCAAACC   GCCTCTCCCC   GCGCGTTGGC
4981   CGATTCATTA   ATGCAGCTGG   CACGACAGGT   TTCCCGACTG   GAAAGCGGGC   AGGCTGCAAA
5041   CGACGAAAAC   TACGCTTTAG   TAGCTTAATA   ATACTAGAGC   CAGGCATCAA   ATAAAACGAA
5101   AGGCTCAGTC   GAAAGACTGG   GCCTTTCGTT   TTATCTGTTG   TTTGTCGGTG   AACGCTCTCT
5161   ACTAGAGTCA   CACTGGCTCA   CCTTCGGGTG   GGCCTTTCTG   CGTTTATATA   CTAGTAGCGG
5221   CCGCTGCAGG   GATCCTCTAC   GCCGGACGCA   TCGTGGCCGG   CATCACCGGC   GCCACAGGTG
5281   CGGTTGCTGG   CGCCTATATC   GCCGACATCA   CCGATGGGGA   AGATCGGGCT   CGCCACTTCG
5341   GGCTCATGAC   TGCAGCGGCC   GCTACTAGTA   TATAAACGCA   GAAAGGCCCA   CCCGAAGGTG
5401   AGCCAGTGTG   ACTCTAGTAG   AGAGCGTTCA   CCGACAAACA   ACAGATAAAA   CGAAAGGCCC
5461   AGTCTTTCGA   CTGAGCCTTT   CGTTTTATTT   GATGCCTGGC   TCTAGTATTA   AGCTACTAAA
5521   GCGTAGTTTT   CGTCGTTTGC   AGCTTTCCTG   ATAGGCAGGT   AAACCGAGAA   CTCGGTATGC
5581   CCTGGCCAAC   TGGTAAATTC   AATTTTGCCT   GAATGCTGAT   CAATCAAATT   ACGAGCGATG
5641   GATAAGCCAA   GCCCGGTGCC   ACCTTCGCGG   CCGCTGACCA   TCGGGTAAAA   CAGCGTATCC
5701   TGCAAATGAG   GCGGAATGCC   CGGCCCGTTA   TCTTCCACAT   CAATCCGCGC   CGCCAGCCGG
5761   TAGCGCTCGC   CGTGTAAGGT   CAGTTGAAAC   GCGGTGCGGG   TACGCAGAAT   GATTTCACCG
5821   CCTTCCGGCC   CCAGCGCCTG   TAGCGCATTG   CGCACAATAT   TCAGCAAGAC   CTGTTCAATT
5881   TGATCCGGGT   CGTGCGCCAG   TTCCGGTAGG   CTGGGATCGT   AATCACGAAT   CAACCGCACG
5941   TTGTCCGGCA   GTTCCATCGA   CACCAGCGTT   ACCACGCGTT   CAGCCACTTT   GTGAATACTT
6001   TCGGTAACGC   GCGTACCGGG   CAGCTGCGGC   CCCAACAGAC   GGTCGACCAG   ATTTCGCAGC
6061   CGGTCCGCCT   GTTCGATAAT   CACTTTGGTA   TATTCGAGTA   GTGATGGGTC   AGGTAACGCT
6121   TTGCTGAGCA   GCTGCGCCGC   GCCACGTAAA   CCGCCAAGCG   GATTTTTAAT   CTCATGTGCC
6181   AGGCCGCGCA   CTAAATCACG   GGCAGCAACC   TGCTGGGCGT   GCTGTAGCTG   TTCCTGACTT
6241   AAGCGGCGCT   GGTTATCCAT   CGGAGCCATC   TCCAGCAGGA   TCATGCCGTC   CGGCATACGC
6301   TGGGCCGTCA   CAGAAAGGAT   ATGCGAGCGC   CCGTCGATGA   CCAGCGTCAC   TTCGTTATCG
6361   GTAAAACCTT   GCCCCGCCTC   CAGACTTTCT   TGCATCAGCT   CGATATTTAA   TGAGAAGTAG
6421   CTCAACAGTT   CCGGTAACGG   TGTACCAAAC   AATTTGCGGG   AGCTTTGGGC   GAGCAGTTGT
6481   TGCGCGGCAG   GGTTGGCGTA   ATGGATCGCC   AGGTTGTCAT   CGATTAACAA   AATACTGTTA
6541   ATCCGCGAGT   TGAGGATCTG   CCCAGCATCG   GGCTGCGTGC   CTGTTGCCAT   CTAGTAGTCC
6601   TGTGTGACTC   TAGTAGTGCT   CAGTATCTCT   ATCACTGATA   GGGATGTCAA   TCTCTATCAC
6661   TGATAGGGAC   TCTAGAAGCG   GCCGCGAATT   CGCATGCACC   ATTCCTTGCG   GCGGCGGTGC
6721   TCAACGGCCT   CAACCTACTA   CTGGGCTGCT   TCCTAATGCA   GGAGTCGCAT   AAGGGAGAGC
6781   GTCGACCGAT   GCCCTTGAGA   GCCTTCAACC   CAGTCAGCTC   CTTCCGGTGG   GCGCGGGGCA
6841   TGACTATCGT   CGCCGCACTT   ATGACTGTCT   TCTTTATCAT   GCAACTCGTA   GGACAGGTGC
6901   CGGCAGCGCT   CTGGGTCATT   TTCGGCGAGG   ACCGCTTTCG   CTGGAGCGCG   ACGATGATCG
6961   GCCTGTCGCT   TGCGGTATTC   GGAATCTTGC   ACGCCCTCGC   TCAAGCCTTC   GTCACTGGTC
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 7021  CCGCCACCAA  ACGTTTCGGC  GAGAAGCAGG  CCATTATCGC  CGGCATGGCG  GCCGACGCGC
 7081  TGGGCTACGT  CTTGCTGGCG  TTCGCGACGC  GAGGCTGGAT  GGCCTTCCCC  ATTATGATTC
 7141  TTCTCGCTTC  CGGCGGCATC  GGGATGCCCG  CGTTGCAGGC  CATGCTGTCC  AGGCAGGTAG
 7201  ATGACGACCA  TCAGGGACAG  CTTCAAGGAT  CGCTCGCGGC  TCTTACCAGC  CTAACTTCGA
 7261  TCATTGGACC  GCTGATCGTC  ACGGCGATTT  ATGCCGCCTC  GGCGAGCACA  TGGAACGGGT
 7321  TGGCATGGAT  TGTAGGCGCC  GCCCTATACC  TTGTCTGCCT  CCCCGCGTTG  CGTCGCGGTG
 7381  CATGGAGCCG  GGCCACCTCG  ACCTGAATGG  AAGCCGGCGG  CACCTCGCTA  ACGGATTCAC
 7441  CACTCCAAGA  ATTGGAGCCA  ATCAATTCTT  GCGGAGAACT  GTGAATGCGC  AAACCAACCC
 7501  TTGGCAGAAC  ATATCCATCG  CGTCCGCCAT  CTCCAGCAGC  CGCACGCGGC  GCATCTCGGG
 7561  GAATTCGCGG  CCGCTTCTAG  AGGTTGACAG  CTAGCTCAGT  CCTAGGGATT  GTGCTAGCTA
 7621  CTAGAGAAAG  AGGAGAAATA  CTAGATGCAA  CGAGGGATAG  TCTGGGTAGT  CGATGACGAT
 7681  AGTTCCATCC  GTTGGGTGCT  TGAACGTGCG  CTCGCTGGGG  CAGGTTTAAC  CTGTACGACG
 7741  TTTGAGAACG  GCGCAGAAGT  GCTGGAGGCG  CTGGCGAGCA  AAACGCCGGA  TGTGCTGCTT
 7801  TCAGATATCC  GTATGCCGGG  AATGGACGGG  CTGGCGCTGC  TCAAGCAGAT  TAAACAGCGC
 7861  CATCCAATGC  TTCCGGTCAT  CATTATGACC  GCACATTCCG  ATCTGGATGC  TGCCGTCAGC
 7921  GCCTATCAAC  AAGGGGCGTT  TGATTATCTG  CCCAAACCGT  TTGATATCGA  CGAAGCAGTG
 7981  GCGCTGGTTG  AGCGCGCTAT  CAGTCATTAC  CAGGAACAGC  AGCAGCCGCG  TAATGTTCAG
 8041  CTTAACGGCC  CAACGACCGA  TATCATCGGC  GAAGCGCCAG  CCATGCAGGA  CGTGTTCCGT
 8101  ATTATCGGTC  GGCTTTCGCG  TTCTTCTATT  AGCGTGCTGA  TTAACGGCGA  ATCCGGCACC
 8161  GGTAAAGAAC  TGGTCGCTCA  TGCCCTGCAT  CGCCACAGTC  CGCGCGCCAA  AGCGCCGTTT
 8221  ATCGCGCTGA  ATATGGCAGC  TATCCCAAAA  GATTTGATCG  AATCAGAACT  GTTTGGCCAC
 8281  GAGAAAGGCG  CGTTTACTGG  CGCGAATACC  ATTCGTCAGG  GGCGTTTTGA  ACAGGCCGAT
 8341  GGCGGTACAT  TATTCCTCGA  CGAAATTGGT  GATATGCCGC  TGGATGTGCA  GACGCGTTTG
 8401  CTGCGCGTGC  TGGCAGACGG  TCAGTTTTAC  CGCGTTGGCG  GCTATGCGCC  GGTGAAAGTG
 8461  GATGTGCGGA  TTATCGCTGC  CACTCACCAG  AATCTCGAAC  AGCGAGTGCA  GGAAGGTAAG
 8521  TTCCGTGAGG  ATCTGTTCCA  CCGCCTGAAC  GTTATCCGCG  TTCATCTGCC  GCCGCTGCGC
 8581  GAACGTCGGG  AAGATATTCC  CCGTCTGGCG  CGCCATTTTT  TACAGGTTGC  CGCGCGCGAA
 8641  CTGGGCGTAG  AAGCGAAGTT  ACTGCATCCG  GAAACCGAAG  CTGCTCTGAC  GCGTCTGGCG
 8701  TGGCCAGGCA  ACGTGCGCCA  GCTGGAAAAC  ACCTGCCGCT  GGCTAACGGT  GATGGCCGCC
 8761  GGGCAGGAAG  TGTTGATTCA  GGATTTGCCC  GGCGAACTGT  TTGAATCAAC  GGTTGCGGAG
 8821  AGTACTTCGC  AAATGCAACC  GGACAGCTGG  GCGACGCTTC  TTGCGCAGTG  GGCAGACAGA
 8881  GCGCTGCGTT  CCGGTCATCA  AAATCTGCTT  TCCGAAGCGC  AGCCAGAGCT  GGAGCGGACG
 8941  TTACTGACGA  CCGCGTTGCG  ACATACGCAG  GGGCATAAAC  AGGAAGCGGC  GCGGCTACTC
 9001  GGCTGGGGCC  GCAACACCCT  GACGCGTAAG  TTAAAAGAGC  TGGGGATGGA  GTGATACTAG
 9061  AGCCAGGCAT  CAAATAAAAC  GAAAGGCTCA  GTCGAAAGAC  TGGGCCTTTC  GTTTTATCTG
 9121  TTGTTTGTCG  GTGAACGCTC  TCTACTAGAG  TCACACTGGC  TCACCTTCGG  GTGGGCCTTT
 9181  CTGCGTTTAT  ATACTAGAGA  ATTGTGAGCG  GATAACAATT  GACATTGTGA  GCGGATAACA
 9241  AGATACTGAG  CACATACTAG  AGAAAGAGGA  GAAATACTAG  ATGGCAACAG  GCACGCAGCC
 9301  CGATGCTGGG  CAGATCCTCA  ACTCGCTGAT  TAACAGTATT  TTGTTAATCG  ATGACAACCT
 9361  GGCGATCCAT  TACGCCAACC  CTGCCGCGCA  ACAACTGCTC  GCCCAAAGCT  CCCGCAAATT
 9421  GTTTGGTACA  CCGTTACCGG  AACTGTTGAG  CTACTTCTCA  TTAAATATCG  AGCTGATGCA
 9481  AGAAAGTCTG  GAGGCGGGGC  AAGGTTTTAC  CGATAACGAA  GTGACGCTGG  TCATCGACGG
 9541  GCGCTCGCAT  ATCCTTTCTG  TGACGGCCCA  GCGTATGCCG  GACGGCATGA  TCCTGCTGGA
 9601  GATGGCTCCG  ATGGATAACC  AGCGCCGCTT  AAGTCAGGAA  CAGCTACAGC  ACGCCCAGCA
 9661  GGTTGCTGCC  CGTGATTTAG  TGCGCGGCCT  GGCAAATGAG  ATTAAAAATC  CGCTTGGCGG
 9721  TTTACGTGGC  GCGGCGCAGC  TGCTCAGCAA  AGCGTTACCT  GACCCATCAC  TACTCGAATA
 9781  TACCAAAGTG  ATTATCGAAC  AGGCGGACCG  GCTGCGAAAT  CTGGTCGACC  GTCTGTTGGG
 9841  GCCGCAGCTG  CCCGGTACGC  GCGTTACCGA  AAGTATTCAC  AAAGTGGCTG  AACGCGTGGT
 9901  AACGCTGGTG  TCGATGGAAC  TGCCGGACAA  CGTGCGGTTG  ATTCGTGATT  ACGATCCCAG
 9961  CCTACCGGAA  CTGGCGCACG  ACCCGGATCA  AATTGAACAG  GTCTTGCTGA  ATATTGTGCG
10021  CAATGCGCTA  CAGGCGCTGG  GGCCGGAAGG  CGGTGAAATC  ATTCTGCGTA  CCCGCACCGC
10081  GTTTCAACTG  ACCTTACACG  GCGAGCGCTA  CCGGCTGGCG  GCGCGGATTG  ATGTGGAAGA
10141  TAACGGGCCG  GGCATTCCGC  CTCATTTGCA  GGATACGCTG  TTTTACCCGA  TGGTCAGCGG
10201  CCGCGAAGGT  GGCACCGGGC  TTGGCTTATC  CATCGCTCGT  AATTTGATTG  ATCAGCATTC
10261  AGGCAAAATT  GAATTTACCA  GTTGGCCAGG  GCATACCGAG  TTCTCGGTTT  ACCTGCCTAT
10321  CAGGAAAGCT  GCAAACGACG  AAAACTACGC  TTTAGTAGCT  TAATGATACT  AGAGCCAGGC
10381  ATCAAATAAA  ACGAAAGGCT  CAGTCGAAAG  ACTGGGCCTT  TCGTTTTATC  TGTTGTTTGT
10441  CGGTGAACGC  TCTCTACTAG  AGTCACACTG  GCTCACCTTC  GGGTGGGCCT  TTCTGCGTTT
10501  ATATACTAGT  AGCGGCCGCT  GCAGCTCGGG  CAGCGTTGGG  TCCTGGCCAC  GGGTGCGCAT
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10561   GATCGTGCTC   CTGTCGTTGA   GGACCCGGCT   AGGCTGGCGG   GGTTGCCTTA   CTGGTTAGCA
10621   GAATGAATCA   CCGATACGCG   AGCGAACGTG   AAGCGACTGC   TGCTGCAAAA   CGTCTGCGAC
10681   CTGAGCAACA   ACATGAATGG   TCTTCGGTTT   CCGTGTTTCG   TAAAGTCTGG   AAACGCGGAA
10741   GTCCCCTACG   TGCTGCTGAA   GTTGCCCGCA   ACAGAGAGTG   GAACCAACCG   GTGATACCAC
10801   GATACTATGA   CTGAGAGTCA   ACGCCATGAG   CGGCCTCATT   TCTTATTCTG   AGTTACAACA
10861   GTCCGCACCG   CTGTCCGGTA   GCTCCTTCCG   GTGGGCGCGG   GGCATGACTA   TCGTCGCCGC
10921   ACTTATGACT   GTCTTCTTTA   TCATGCAACT   CGTAGGACAG   GTGCCGGCAG   CGCCCAACAG
10981   TCCCCCGGCC   ACGGGGCCTG   CCACCATACC   CACGCCGAAA   CAAGCGCCCT   GCACCATTAT
11041   GTTCCGGATC   TGCATCGCAG   GATGCTGCTG   GCTACCCTGT   GGAACACCTA   CATCTGTATT
11101   AACGAAGCGC   TAACCGTTTT   TATCAGGCTC   TGGGAGGCAG   AATAAATGAT   CATATCGTCA
11161   ATTATTACCT   CCACGGGGAG   AGCCTGAGCA   AACTGGCCTC   AGGCATTTGA   GAAGCACACG
11221   GTCACACTGC   TTCCGGTAGT   CAATAAACCG   GTAAACCAGC   AATAGACATA   AGCGGCTATT
11281   TAACGACCCT   GCCCTGAACC   GACGACCGGG   TCGAATTTGC   TTTCGAATTT   CTGCCATTCA
11341   TCCGCTTATT   ATCACTTATT   CAGGCGTAGC   ACCAGGCGTT   TAAGGGCACC   AATAACTGCC
11401   TTAAAAAAAT   TACGCCCCGC   CCTGCCACTC   ATCGCAGTAC   TGTTGTAATT   CATTAAGCAT
11461   TCTGCCGACA   TGGAAGCCAT   CACAGACGGC   ATGATGAACC   TGAATCGCCA   GCGGCATCAG
11521   CACCTTGTCG   CCTTGCGTAT   AATATTTGCC   CATGGTGAAA   ACGGGGGCGA   AGAAGTTGTC
11581   CATATTGGCC   ACGTTTAAAT   CAAAACTGGT   GAAACTCACC   CAGGGATTGG   CTGAGACGAA
11641   AAACATATTC   TCAATAAACC   CTTTAGGGAA   ATAGGCCAGG   TTTTCACCGT   AACACGCCAC
11701   ATCTTGCGAA   TATATGTGTA   GAAACTGCCG   GAAATCGTCG   TGGTATTCAC   TCCAGAGCGA
11761   TGAAAACGTT   TCAGTTTGCT   CATGGAAAAC   GGTGTAACAA   GGGTGAACAC   TATCCCATAT
11821   CACCAGCTCA   CCGTCTTTCA   TTGCCATACG
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1.7.4 Circuit with high NRI:

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   1  GAATTCCGGA  TGAGCATTCA  TCAGGCGGGC  AAGAATGTGA  ATAAAGGCCG  GATAAAACTT
  61  GTGCTTATTT  TTCTTTACGG  TCTTTAAAAA  GGCCGTAATA  TCCAGCTGAA  CGGTCTGGTT
 121  ATAGGTACAT  TGAGCAACTG  ACTGAAATGC  CTCAAAATGT  TCTTTACGAT  GCCATTGGGA
 181  TATATCAACG  GTGGTATATC  CAGTGATTTT  TTTCTCCATT  TTAGCTTCCT  TAGCTCCTGA
 241  AAATCTCGAT  AACTCAAAAA  ATACGCCCGG  TAGTGATCTT  ATTTCATTAT  GGTGAAAGTT
 301  GGAACCTCTT  ACGTGCCGAT  CAACGTCTCA  TTTTCGCCAA  AAGTTGGCCC  AGGGCTTCCC
 361  GGTATCAACA  GGGACACCAG  GATTTATTTA  TTCTGCGAAG  TGATCTTCCG  TCACAGGTAT
 421  TTATTCGGCG  CAAAGTGCGT  CGGGTGATGC  TGCCAACTTA  CTGATTTAGT  GTATGATGGT
 481  GTTTTTGAGG  TGCTCCAGTG  GCTTCTGTTT  CTATCAGCTG  TCCCTCCTGT  TCAGCTACTG
 541  ACGGGGTGGT  GCGTAACGGC  AAAAGCACCG  CCGGACATCA  GCGCTAGCGG  AGTGTATACT
 601  GGCTTACTAT  GTTGGCACTG  ATGAGGGTGT  CAGTGAAGTG  CTTCATGTGG  CAGGAGAAAA
 661  AAGGCTGCAC  CGGTGCGTCA  GCAGAATATG  TGATACAGGA  TATATTCCGC  TTCCTCGCTC
 721  ACTGACTCGC  TACGCTCGGT  CGTTCGACTG  CGGCGAGCGG  AAATGGCTTA  CGAACGGGGC
 781  GGAGATTTCC  TGGAAGATGC  CAGGAAGATA  CTTAACAGGG  AAGTGAGAGG  GCCGCGGCAA
 841  AGCCGTTTTT  CCATAGGCTC  CGCCCCCCTG  ACAAGCATCA  CGAAATCTGA  CGCTCAAATC
 901  AGTGGTGGCG  AAACCCGACA  GGACTATAAA  GATACCAGGC  GTTTCCCCCT  GGCGGCTCCC
 961  TCGTGCGCTC  TCCTGTTCCT  GCCTTTCGGT  TTACCGGTGT  CATTCCGCTG  TTATGGCCGC
1021  GTTTGTCTCA  TTCCACGCCT  GACACTCAGT  TCCGGGTAGG  CAGTTCGCTC  CAAGCTGGAC
1081  TGTATGCACG  AACCCCCCGT  TCAGTCCGAC  CGCTGCGCCT  TATCCGGTAA  CTATCGTCTT
1141  GAGTCCAACC  CGGAAAGACA  TGCAAAAGCA  CCACTGGCAG  CAGCCACTGG  TAATTGATTT
1201  AGAGGAGTTA  GTCTTGAAGT  CATGCGCCGG  TTAAGGCTAA  ACTGAAAGGA  CAAGTTTTGG
1261  TGACTGCGCT  CCTCCAAGCC  AGTTACCTCG  GTTCAAAGAG  TTGGTAGCTC  AGAGAACCTT
1321  CGAAAAACCG  CCCTGCAAGG  CGGTTTTTTC  GTTTTCAGAG  CAAGAGATTA  CGCGCAGACC
1381  AAAACGATCT  CAAGAAGATC  ATCTTATTAA  TCAGATAAAA  TATTTCTAGA  GCATCCTCCG
1441  CAAACAAGTA  TTGCAGAGTC  CCTTTGTGAT  CGCTTTCACG  GAGCATAAAA  AGGGTTATCC
1501  AAAGGTCATT  GCACCAACAT  GGTGCTTAAT  GTTTCCATTG  AAGCACTATA  TTGGTGCAAC
1561  ATTCACATCG  TGGTGCAGCC  CTTTTGCACG  ATGGTGCGCA  TGATAACGCC  TTTTAGGGGC
1621  AATTTAAAAG  TTGGCACAGA  TTTCGCTTTA  TCTTTTTTAC  GGCGACACGG  CCAAAATAAT
1681  TGCAGATTTC  GTTACCACGA  CGACCTAACT  TTCAATTCTA  ATAAGGAGGA  AGACTTCAAA
1741  TGCGTAAAGG  CGAAGAGCTG  TTCACTGGTG  TCGTCCCTAT  TCTGGTGGAA  CTGGATGGTG
1801  ATGTCAACGG  TCATAAGTTT  TCCGTGCGTG  GCGAGGGTGA  AGGTGACGCA  ACTAATGGTA
1861  AACTGACGCT  GAAGTTCATC  TGTACTACTG  GTAAACTGCC  GGTACCTTGG  CCGACTCTGG
1921  TAACGACGCT  GACTTATGGT  GTTCAGTGCT  TTGCTCGTTA  TCCGGACCAT  ATGAAGCAGC
1981  ATGACTTCTT  CAAGTCCGCC  ATGCCGGAAG  GCTATGTGCA  GGAACGCACG  ATTTCCTTTA
2041  AGGATGACGG  CACGTACAAA  ACGCGTGCGG  AAGTGAAATT  TGAAGGCGAT  ACCCTGGTAA
2101  ACCGCATTGA  GCTGAAAGGC  ATTGACTTTA  AAGAAGACGG  CAATATCCTG  GGCCATAAGC
2161  TGGAATACAA  TTTTAACAGC  CACAATGTTT  ACATCACCGC  CGATAAACAA  AAAAATGGCA
2221  TTAAAGCGAA  TTTTAAAATT  CGCCACAACG  TGGAGGATGG  CAGCGTGCAG  CTGGCTGATC
2281  ACTACCAGCA  AAACACTCCA  ATCGGTGATG  GTCCTGTTCT  GCTGCCAGAC  AATCACTATC
2341  TGAGCACGCA  AAGCGTTCTG  TCTAAAGATC  CGAACGAGAA  ACGCGATCAT  ATGGTTCTGC
2401  TGGAGTTCGT  AACCGCAGCG  GGCATCACGC  ATGGTATGGA  TGAACTGTAC  AAACAGGCTG
2461  CAAACGACGA  AAACTACGCT  TTAGTAGCTT  GATGACTTAA  GGAGCTCCAA  TTGCCAGGCA
2521  TCAAATAAAA  CGAAAGGCTC  AGTCGAAAGA  CTGGGCCTTT  CGTTTTATCT  GTTGTTTGTC
2581  GGTGAACGCT  CTCTACTAGA  GTCACACTGG  CTCACCTTCG  GGTGGGCCTT  TCTGCGTTTA
2641  TAAAGCTTTA  ATGCGGTAGT  TTATCACAGT  TAAATTGCTA  ACGCAGTCAG  GCACCGTGTA
2701  TGAAATCTAA  CAATGCGCTC  ATCGTCATCC  TCGGCACCGT  CACCCTGGAT  GCTGTAGGCA
2761  TAGGCTTGGT  TATGCCGGTA  CTGCCGGGCC  TCTTGCGGGA  TATCGTCCAT  TCCGACAGCA
2821  TCGCCAGTCA  CTATGGCGTG  CTGCTAGCGC  TATATGCGTT  GATGCAATTT  CTATGCGCAC
2881  CCGTTCTCGG  AGCACTGTCC  GACCGCTTTG  GCCGCCGCCC  AGTCCTGCTC  GCTTCGCTAC
2941  TTGGAGCCAC  TATCGACTAC  GCGATCATGG  CGACCACACC  CGTCCTGTGG  ATCCGAATTC
3001  GCGGCCGCTT  CTAGAGTTTA  TGGCTAGCTC  AGTCCTAGGT  ACAATGCTAG  CTACTAGAGA
3061  AAGAGGAGAA  ATACTAGATG  TCCAGATTAG  ATAAAAGTAA  AGTGATTAAC  AGCGCATTAG
3121  AGCTGCTTAA  TGAGGTCGGA  ATCGAAGGTT  TAACAACCCG  TAAACTCGCC  CAGAAGCTAG
3181  GTGTAGAGCA  GCCTACATTG  TATTGGCATG  TAAAAAATAA  GCGGGCTTTG  CTCGACGCCT
3241  TAGCCATTGA  GATGTTAGAT  AGGCACCATA  CTCACTTTTG  CCCTTTAGAA  GGGGAAAGCT
3301  GGCAAGATTT  TTTACGTAAT  AACGCTAAAA  GTTTTAGATG  TGCTTTACTA  AGTCATCGCG
3361  ATGGAGCAAA  AGTACATTTA  GGTACACGGC  CTACAGAAAA  ACAGTATGAA  ACTCTCGAAA
3421  ATCAATTAGC  CTTTTTATGC  CAACAAGGTT  TTTCACTAGA  GAATGCATTA  TATGCACTCA
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3481   GCGCTGTGGG   GCATTTTACT   TTAGGTTGCG   TATTGGAAGA   TCAAGAGCAT   CAAGTCGCTA
3541   AAGAAGAAAG   GGAAACACCT   ACTACTGATA   GTATGCCGCC   ATTATTACGA   CAAGCTATCG
3601   AATTATTTGA   TCACCAAGGT   GCAGAGCCAG   CCTTCTTATT   CGGCCTTGAA   TTGATCATAT
3661   GCGGATTAGA   AAAACAACTT   AAATGTGAAA   GTGGGTCCGC   TGCAAACGAC   GAAAACTACG
3721   CTTTAGTAGC   TTAATAATAC   TAGAGCCAGG   CATCAAATAA   AACGAAAGGC   TCAGTCGAAA
3781   GACTGGGCCT   TTCGTTTTAT   CTGTTGTTTG   TCGGTGAACG   CTCTCTACTA   GAGTCACACT
3841   GGCTCACCTT   CGGGTGGGCC   TTTCTGCGTT   TATATACTAG   AGTTTATGGC   TAGCTCAGTC
3901   CTAGGTACAA   TGCTAGCTAC   TAGAGAAAGA   GGAGAAATAC   TAGATGGTGA   ATGTGAAACC
3961   AGTAACGTTA   TACGATGTCG   CAGAGTATGC   CGGTGTCTCT   TATCAGACCG   TTTCCCGCGT
4021   GGTGAACCAG   GCCAGCCACG   TTTCTGCGAA   AACGCGGGAA   AAAGTGGAAG   CGGCGATGGC
4081   GGAGCTGAAT   TACATTCCCA   ACCGCGTGGC   ACAACAACTG   GCGGGCAAAC   AGTCGTTGCT
4141   GATTGGCGTT   GCCACCTCCA   GTCTGGCCCT   GCACGCGCCG   TCGCAAATTG   TCGCGGCGAT
4201   TAAATCTCGC   GCCGATCAAC   TGGGTGCCAG   CGTGGTGGTG   TCGATGGTAG   AACGAAGCGG
4261   CGTCGAAGCC   TGTAAAGCGG   CGGTGCACAA   TCTTCTCGCG   CAACGCGTCA   GTGGGCTGAT
4321   CATTAACTAT   CCGCTGGATG   ACCAGGATGC   CATTGCTGTG   GAAGCTGCCT   GCACTAATGT
4381   TCCGGCGTTA   TTTCTTGATG   TCTCTGACCA   GACACCCATC   AACAGTATTA   TTTTCTCCCA
4441   TGAAGACGGT   ACGCGACTGG   GCGTGGAGCA   TCTGGTCGCA   TTGGGTCACC   AGCAAATCGC
4501   GCTGTTAGCG   GGCCCATTAA   GTTCTGTCTC   GGCGCGTCTG   CGTCTGGCTG   GCTGGCATAA
4561   ATATCTCACT   CGCAATCAAA   TTCAGCCGAT   AGCGGAACGG   GAAGGCGACT   GGAGTGCCAT
4621   GTCCGGTTTT   CAACAAACCA   TGCAAATGCT   GAATGAGGGC   ATCGTTCCCA   CTGCGATGCT
4681   GGTTGCCAAC   GATCAGATGG   CGCTGGGCGC   AATGCGCGCC   ATTACCGAGT   CCGGGCTGCG
4741   CGTTGGTGCG   GATATCTCGG   TAGTGGGATA   CGACGATACC   GAAGACAGCT   CATGTTATAT
4801   CCCGCCGTTA   ACCACCATCA   AACAGGATTT   TCGCCTGCTG   GGGCAAACCA   GCGTGGACCG
4861   CTTGCTGCAA   CTCTCTCAGG   GCCAGGCGGT   GAAGGGCAAT   CAGCTGTTGC   CCGTCTCACT
4921   GGTGAAAAGA   AAAACCACCC   TGGCGCCCAA   TACGCAAACC   GCCTCTCCCC   GCGCGTTGGC
4981   CGATTCATTA   ATGCAGCTGG   CACGACAGGT   TTCCCGACTG   GAAAGCGGGC   AGGCTGCAAA
5041   CGACGAAAAC   TACGCTTTAG   TAGCTTAATA   ATACTAGAGC   CAGGCATCAA   ATAAAACGAA
5101   AGGCTCAGTC   GAAAGACTGG   GCCTTTCGTT   TTATCTGTTG   TTTGTCGGTG   AACGCTCTCT
5161   ACTAGAGTCA   CACTGGCTCA   CCTTCGGGTG   GGCCTTTCTG   CGTTTATATA   CTAGTAGCGG
5221   CCGCTGCAGG   GATCCTCTAC   GCCGGACGCA   TCGTGGCCGG   CATCACCGGC   GCCACAGGTG
5281   CGGTTGCTGG   CGCCTATATC   GCCGACATCA   CCGATGGGGA   AGATCGGGCT   CGCCACTTCG
5341   GGCTCATGAC   TGCAGCGGCC   GCTACTAGTA   TATAAACGCA   GAAAGGCCCA   CCCGAAGGTG
5401   AGCCAGTGTG   ACTCTAGTAG   AGAGCGTTCA   CCGACAAACA   ACAGATAAAA   CGAAAGGCCC
5461   AGTCTTTCGA   CTGAGCCTTT   CGTTTTATTT   GATGCCTGGC   TCTAGTATTA   AGCTACTAAA
5521   GCGTAGTTTT   CGTCGTTTGC   AGCTTTCCTG   ATAGGCAGGT   AAACCGAGAA   CTCGGTATGC
5581   CCTGGCCAAC   TGGTAAATTC   AATTTTGCCT   GAATGCTGAT   CAATCAAATT   ACGAGCGATG
5641   GATAAGCCAA   GCCCGGTGCC   ACCTTCGCGG   CCGCTGACCA   TCGGGTAAAA   CAGCGTATCC
5701   TGCAAATGAG   GCGGAATGCC   CGGCCCGTTA   TCTTCCACAT   CAATCCGCGC   CGCCAGCCGG
5761   TAGCGCTCGC   CGTGTAAGGT   CAGTTGAAAC   GCGGTGCGGG   TACGCAGAAT   GATTTCACCG
5821   CCTTCCGGCC   CCAGCGCCTG   TAGCGCATTG   CGCACAATAT   TCAGCAAGAC   CTGTTCAATT
5881   TGATCCGGGT   CGTGCGCCAG   TTCCGGTAGG   CTGGGATCGT   AATCACGAAT   CAACCGCACG
5941   TTGTCCGGCA   GTTCCATCGA   CACCAGCGTT   ACCACGCGTT   CAGCCACTTT   GTGAATACTT
6001   TCGGTAACGC   GCGTACCGGG   CAGCTGCGGC   CCCAACAGAC   GGTCGACCAG   ATTTCGCAGC
6061   CGGTCCGCCT   GTTCGATAAT   CACTTTGGTA   TATTCGAGTA   GTGATGGGTC   AGGTAACGCT
6121   TTGCTGAGCA   GCTGCGCCGC   GCCACGTAAA   CCGCCAAGCG   GATTTTTAAT   CTCATGTGCC
6181   AGGCCGCGCA   CTAAATCACG   GGCAGCAACC   TGCTGGGCGT   GCTGTAGCTG   TTCCTGACTT
6241   AAGCGGCGCT   GGTTATCCAT   CGGAGCCATC   TCCAGCAGGA   TCATGCCGTC   CGGCATACGC
6301   TGGGCCGTCA   CAGAAAGGAT   ATGCGAGCGC   CCGTCGATGA   CCAGCGTCAC   TTCGTTATCG
6361   GTAAAACCTT   GCCCCGCCTC   CAGACTTTCT   TGCATCAGCT   CGATATTTAA   TGAGAAGTAG
6421   CTCAACAGTT   CCGGTAACGG   TGTACCAAAC   AATTTGCGGG   AGCTTTGGGC   GAGCAGTTGT
6481   TGCGCGGCAG   GGTTGGCGTA   ATGGATCGCC   AGGTTGTCAT   CGATTAACAA   AATACTGTTA
6541   ATCCGCGAGT   TGAGGATCTG   CCCAGCATCG   GGCTGCGTGC   CTGTTGCCAT   CTAGTAGTCC
6601   TGTGTGACTC   TAGTAGTGCT   CAGTATCTCT   ATCACTGATA   GGGATGTCAA   TCTCTATCAC
6661   TGATAGGGAC   TCTAGAAGCG   GCCGCGAATT   CGCATGCGAA   TTCGCGGCCG   CTTCTAGAGT
6721   TTATGGCTAG   CTCAGTCCTA   GGTACAATGC   TAGCTACTAG   AGAAAGAGGA   GAAATACTAG
6781   ATGCAACGAG   GGATAGTCTG   GGTAGTCGAT   GACGATAGTT   CCATCCGTTG   GGTGCTTGAA
6841   CGTGCGCTCG   CTGGGGCAGG   TTTAACCTGT   ACGACGTTTG   AGAACGGCGC   AGAAGTGCTG
6901   GAGGCGCTGG   CGAGCAAAAC   GCCGGATGTG   CTGCTTTCAG   ATATCCGTAT   GCCGGGAATG
6961   GACGGGCTGG   CGCTGCTCAA   GCAGATTAAA   CAGCGCCATC   CAATGCTTCC   GGTCATCATT
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 7021  ATGACCGCAC  ATTCCGATCT  GGATGCTGCC  GTCAGCGCCT  ATCAACAAGG  GGCGTTTGAT
 7081  TATCTGCCCA  AACCGTTTGA  TATCGACGAA  GCAGTGGCGC  TGGTTGAGCG  CGCTATCAGT
 7141  CATTACCAGG  AACAGCAGCA  GCCGCGTAAT  GTTCAGCTTA  ACGGCCCAAC  GACCGATATC
 7201  ATCGGCGAAG  CGCCAGCCAT  GCAGGACGTG  TTCCGTATTA  TCGGTCGGCT  TTCGCGTTCT
 7261  TCTATTAGCG  TGCTGATTAA  CGGCGAATCC  GGCACCGGTA  AAGAACTGGT  CGCTCATGCC
 7321  CTGCATCGCC  ACAGTCCGCG  CGCCAAAGCG  CCGTTTATCG  CGCTGAATAT  GGCAGCTATC
 7381  CCAAAAGATT  TGATCGAATC  AGAACTGTTT  GGCCACGAGA  AAGGCGCGTT  TACTGGCGCG
 7441  AATACCATTC  GTCAGGGGCG  TTTTGAACAG  GCCGATGGCG  GTACATTATT  CCTCGACGAA
 7501  ATTGGTGATA  TGCCGCTGGA  TGTGCAGACG  CGTTTGCTGC  GCGTGCTGGC  AGACGGTCAG
 7561  TTTTACCGCG  TTGGCGGCTA  TGCGCCGGTG  AAAGTGGATG  TGCGGATTAT  CGCTGCCACT
 7621  CACCAGAATC  TCGAACAGCG  AGTGCAGGAA  GGTAAGTTCC  GTGAGGATCT  GTTCCACCGC
 7681  CTGAACGTTA  TCCGCGTTCA  TCTGCCGCCG  CTGCGCGAAC  GTCGGGAAGA  TATTCCCCGT
 7741  CTGGCGCGCC  ATTTTTTACA  GGTTGCCGCG  CGCGAACTGG  GCGTAGAAGC  GAAGTTACTG
 7801  CATCCGGAAA  CCGAAGCTGC  TCTGACGCGT  CTGGCGTGGC  CAGGCAACGT  GCGCCAGCTG
 7861  GAAAACACCT  GCCGCTGGCT  AACGGTGATG  GCCGCCGGGC  AGGAAGTGTT  GATTCAGGAT
 7921  TTGCCCGGCG  AACTGTTTGA  ATCAACGGTT  GCGGAGAGTA  CTTCGCAAAT  GCAACCGGAC
 7981  AGCTGGGCGA  CGCTTCTTGC  GCAGTGGGCA  GACAGAGCGC  TGCGTTCCGG  TCATCAAAAT
 8041  CTGCTTTCCG  AAGCGCAGCC  AGAGCTGGAG  CGGACGTTAC  TGACGACCGC  GTTGCGACAT
 8101  ACGCAGGGGC  ATAAACAGGA  AGCGGCGCGG  CTACTCGGCT  GGGGCCGCAA  CACCCTGACG
 8161  CGTAAGTTAA  AAGAGCTGGG  GATGGAGTGA  TACTAGAGCC  AGGCATCAAA  TAAAACGAAA
 8221  GGCTCAGTCG  AAAGACTGGG  CCTTTCGTTT  TATCTGTTGT  TTGTCGGTGA  ACGCTCTCTA
 8281  CTAGAGTCAC  ACTGGCTCAC  CTTCGGGTGG  GCCTTTCTGC  GTTTATATAC  TAGTAGCGGC
 8341  CGCTGCAGCT  CGGGGAATTC  GCGGCCGCTT  CTAGAGAATT  GTGAGCGGAT  AACAATTGAC
 8401  ATTGTGAGCG  GATAACAAGA  TACTGAGCAC  ATACTAGAGA  AAGAGGAGAA  ATACTAGATG
 8461  GCAACAGGCA  CGCAGCCCGA  TGCTGGGCAG  ATCCTCAACT  CGCTGATTAA  CAGTATTTTG
 8521  TTAATCGATG  ACAACCTGGC  GATCCATTAC  GCCAACCCTG  CCGCGCAACA  ACTGCTCGCC
 8581  CAAAGCTCCC  GCAAATTGTT  TGGTACACCG  TTACCGGAAC  TGTTGAGCTA  CTTCTCATTA
 8641  AATATCGAGC  TGATGCAAGA  AAGTCTGGAG  GCGGGGCAAG  GTTTTACCGA  TAACGAAGTG
 8701  ACGCTGGTCA  TCGACGGGCG  CTCGCATATC  CTTTCTGTGA  CGGCCCAGCG  TATGCCGGAC
 8761  GGCATGATCC  TGCTGGAGAT  GGCTCCGATG  GATAACCAGC  GCCGCTTAAG  TCAGGAACAG
 8821  CTACAGCACG  CCCAGCAGGT  TGCTGCCCGT  GATTTAGTGC  GCGGCCTGGC  AAATGAGATT
 8881  AAAAATCCGC  TTGGCGGTTT  ACGTGGCGCG  GCGCAGCTGC  TCAGCAAAGC  GTTACCTGAC
 8941  CCATCACTAC  TCGAATATAC  CAAAGTGATT  ATCGAACAGG  CGGACCGGCT  GCGAAATCTG
 9001  GTCGACCGTC  TGTTGGGGCC  GCAGCTGCCC  GGTACGCGCG  TTACCGAAAG  TATTCACAAA
 9061  GTGGCTGAAC  GCGTGGTAAC  GCTGGTGTCG  ATGGAACTGC  CGGACAACGT  GCGGTTGATT
 9121  CGTGATTACG  ATCCCAGCCT  ACCGGAACTG  GCGCACGACC  CGGATCAAAT  TGAACAGGTC
 9181  TTGCTGAATA  TTGTGCGCAA  TGCGCTACAG  GCGCTGGGGC  CGGAAGGCGG  TGAAATCATT
 9241  CTGCGTACCC  GCACCGCGTT  TCAACTGACC  TTACACGGCG  AGCGCTACCG  GCTGGCGGCG
 9301  CGGATTGATG  TGGAAGATAA  CGGGCCGGGC  ATTCCGCCTC  ATTTGCAGGA  TACGCTGTTT
 9361  TACCCGATGG  TCAGCGGCCG  CGAAGGTGGC  ACCGGGCTTG  GCTTATCCAT  CGCTCGTAAT
 9421  TTGATTGATC  AGCATTCAGG  CAAAATTGAA  TTTACCAGTT  GGCCAGGGCA  TACCGAGTTC
 9481  TCGGTTTACC  TGCCTATCAG  GAAAGCTGCA  AACGACGAAA  ACTACGCTTT  AGTAGCTTAA
 9541  TGATACTAGA  GCCAGGCATC  AAATAAAACG  AAAGGCTCAG  TCGAAAGACT  GGGCCTTTCG
 9601  TTTTATCTGT  TGTTTGTCGG  TGAACGCTCT  CTACTAGAGT  CACACTGGCT  CACCTTCGGG
 9661  TGGGCCTTTC  TGCGTTTATA  TACTAGTAGC  GGCCGCTGCA  GCTCGGGCAG  CGTTGGGTCC
 9721  TGGCCACGGG  TGCGCATGAT  CGTGCTCCTG  TCGTTGAGGA  CCCGGCTAGG  CTGGCGGGGT
 9781  TGCCTTACTG  GTTAGCAGAA  TGAATCACCG  ATACGCGAGC  GAACGTGAAG  CGACTGCTGC
 9841  TGCAAAACGT  CTGCGACCTG  AGCAACAACA  TGAATGGTCT  TCGGTTTCCG  TGTTTCGTAA
 9901  AGTCTGGAAA  CGCGGAAGTC  CCCTACGTGC  TGCTGAAGTT  GCCCGCAACA  GAGAGTGGAA
 9961  CCAACCGGTG  ATACCACGAT  ACTATGACTG  AGAGTCAACG  CCATGAGCGG  CCTCATTTCT
10021  TATTCTGAGT  TACAACAGTC  CGCACCGCTG  TCCGGTAGCT  CCTTCCGGTG  GGCGCGGGGC
10081  ATGACTATCG  TCGCCGCACT  TATGACTGTC  TTCTTTATCA  TGCAACTCGT  AGGACAGGTG
10141  CCGGCAGCGC  CCAACAGTCC  CCCGGCCACG  GGGCCTGCCA  CCATACCCAC  GCCGAAACAA
10201  GCGCCCTGCA  CCATTATGTT  CCGGATCTGC  ATCGCAGGAT  GCTGCTGGCT  ACCCTGTGGA
10261  ACACCTACAT  CTGTATTAAC  GAAGCGCTAA  CCGTTTTTAT  CAGGCTCTGG  GAGGCAGAAT
10321  AAATGATCAT  ATCGTCAATT  ATTACCTCCA  CGGGGAGAGC  CTGAGCAAAC  TGGCCTCAGG
10381  CATTTGAGAA  GCACACGGTC  ACACTGCTTC  CGGTAGTCAA  TAAACCGGTA  AACCAGCAAT
10441  AGACATAAGC  GGCTATTTAA  CGACCCTGCC  CTGAACCGAC  GACCGGGTCG  AATTTGCTTT
10501  CGAATTTCTG  CCATTCATCC  GCTTATTATC  ACTTATTCAG  GCGTAGCACC  AGGCGTTTAA
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10561   GGGCACCAAT   AACTGCCTTA   AAAAAATTAC   GCCCCGCCCT   GCCACTCATC   GCAGTACTGT
10621   TGTAATTCAT   TAAGCATTCT   GCCGACATGG   AAGCCATCAC   AGACGGCATG   ATGAACCTGA
10681   ATCGCCAGCG   GCATCAGCAC   CTTGTCGCCT   TGCGTATAAT   ATTTGCCCAT   GGTGAAAACG
10741   GGGGCGAAGA   AGTTGTCCAT   ATTGGCCACG   TTTAAATCAA   AACTGGTGAA   ACTCACCCAG
10801   GGATTGGCTG   AGACGAAAAA   CATATTCTCA   ATAAACCCTT   TAGGGAAATA   GGCCAGGTTT
10861   TCACCGTAAC   ACGCCACATC   TTGCGAATAT   ATGTGTAGAA   ACTGCCGGAA   ATCGTCGTGG
10921   TATTCACTCC   AGAGCGATGA   AAACGTTTCA   GTTTGCTCAT   GGAAAACGGT   GTAACAAGGG
10981   TGAACACTAT   CCCATATCAC   CAGCTCACCG   TCTTTCATTG    CCATACG
```

2 Simple Model

In this section, we employ simple models to illustrate the essence of the mechanisms that control the load attenuation ability of the phosphorylation cycle and the biphasic regulation of the target genes. In the next section, we provide simulation results using a detailed model that includes all known reactions and has parameter values taken from the literature.

2.1 Load attenuation property

To explain how the phosphorylation cycle attenuates the effect of the load for sufficiently high amounts of substrate and phosphatase, we consider a simple model that uses a one-step reaction for the forward and backward enzymatic processes. Referring to Figure 1 in the main text, we have:

$$\mathrm{Z} + \mathrm{X} \xrightarrow{k_1} \mathrm{Z} + \mathrm{X}^*, \qquad \mathrm{X}^* + \mathrm{Y} \xrightarrow{k_2} \mathrm{X} + \mathrm{Y},$$

along with the binding of X^* to the DNA load:

$$\mathrm{X}^* + \mathrm{p} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{C}, \qquad p + C = p_{\mathrm{tot}},$$

and conservation of the X concentration $X_{\mathrm{tot}} = X + X^* + C$. The resulting ODE model is given by

$$\frac{dX^*}{dt} = k_1 Z(X_{\mathrm{tot}} - X^* - C) - k_2 Y_{\mathrm{tot}} X^* - k_{\mathrm{on}} X^*(p_{\mathrm{tot}} - C) + k_{\mathrm{off}} C \tag{1}$$

$$\frac{dC}{dt} = k_{\mathrm{on}} X^*(p_{\mathrm{tot}} - C) - k_{\mathrm{off}} C. \tag{2}$$

Solving for the steady state when $p_{\mathrm{tot}} = 0$ (unloaded system), we obtain

$$X^* = \frac{k_1 X_{\mathrm{tot}} Z}{k_1 Z + k_2 Y_{\mathrm{tot}}}, \tag{3}$$

while when $p_{\text{tot}} \neq 0$, letting $K_d = k_{\text{off}}/k_{\text{on}}$ be the dissociation constant of the binding of X* with the DNA binding sites, we obtain

$$X^* = \frac{\sqrt{A^2 + 4k_1 X_{\text{tot}} Z K_d (k_1 Z + k_2 Y_{\text{tot}})} + A}{2(k_1 Z + k_2 Y_{\text{tot}})},$$

in which $A = (k_1 X_{\text{tot}} Z - (k_1 Z(K_d + p_{\text{tot}}) + k_2 Y_{\text{tot}} K_d))$. By letting $X_{\text{tot}} = \alpha Y_{\text{tot}}$ and Y_{tot} sufficiently large, we obtain that

$$X^* \approx \frac{Y_{\text{tot}}}{2(k_1 Z + k_2 Y_{\text{tot}})} \left(\sqrt{(k_1 Z\alpha - k_2 K_d)^2 + 4k_1 Z\alpha k_2 K_d} + (k_1 Z\alpha - k_2 K_d) \right),$$

which leads to

$$X^* \approx \frac{Y_{\text{tot}} k_1 Z\alpha}{k_1 Z + k_2 Y_{\text{tot}}},$$

which, considering that $\alpha = X_{\text{tot}}/Y_{\text{tot}}$ leads to the same expression of the unloaded system (3).

Therefore, as X_{tot} and Y_{tot} increase, the effect of the DNA load on the steady state response of the cycle becomes negligible.

2.2 Biphasic response of gene expression

In this section, referring to Fig. S17, we demonstrate that when expression of a protein G is controlled by a phosphorylated transcription factor X, we can have a biphasic response of the expression of such a protein to increasing total concentration X_{tot} of transcription factor. This biphasic response can be obtained by a simple model in which

(1) the kinase Z can be saturated by the substrate X when this is in excess;

(2) the substrate can bind to DNA promoter sites even when not phosphorylated and, in this case, it leads to a low basal expression of the protein G.

To this end, we model the forward enzymatic reaction through a two-step reaction

$$\mathrm{Z} + \mathrm{X} \underset{d}{\overset{a}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_1} Z + X^*, \qquad X_{\mathrm{tot}} = X + X^* + C_1, \qquad Z_{\mathrm{tot}} = Z + C_1,$$

and the backward enzymatic reaction through a one-step reaction as the details of this reaction are not crucial for our current analysis:

$$\mathrm{X}^* + \mathrm{Y} \xrightarrow{k_2} \mathrm{X} + \mathrm{Y}.$$

The ODE model of the system dynamics is given by

$$\frac{dC_1}{dt} = a(X_{\mathrm{tot}} - X^* - C_1)(Z_{\mathrm{tot}} - C_1) - (d + k_1)C_1 \tag{4}$$

$$\frac{dX^*}{dt} = k_1 C_1 - k_2 Y_{\mathrm{tot}} X^* \tag{5}$$

$$\frac{dG}{dt} = \frac{\alpha(X^*/K_d) + \alpha_0(X/K_d)}{1 + (X^*/K_d) + (X/K_d)} - \gamma G, \tag{6}$$

in which we have taken the standard Hill function form for the expression of G, which can be obtained assuming that X and X* bind to the same site exclusively with the same binding affinity and that when X is bound we can have some basal expression rate $\alpha_0 < \alpha$. For simplicity of exposition we have assumed here that the cooperativity of binding of X is equal to 1. Similar results can be obtained by assuming cooperative binding of n molecules of X. We have also assumed that the dissociation constants of the binding to DNA is the same for X and X*, which is the case for the specific protein used in the experiments (NRI) (*5*). However, this assumption is not crucial and similar results can be obtained if the binding affinities are different.

We solve the above system for the steady state value of G as a function of X_{tot}. Assuming that there is an excess of substrate compared to the kinase, we have that $C_1 \ll X_{\mathrm{tot}}$. Under this condition, we obtain the steady state relations:

$$X^* = y\frac{X}{X + K_m}, \qquad K_m = \frac{d + k_1}{a}, \qquad y = \frac{k_1 Z_{\mathrm{tot}}}{k_2 Y_{\mathrm{tot}}}.$$

When $X_{\text{tot}} \ll K_m$, we obtain that

$$X^* \approx X_{\text{tot}} \frac{y/K_m}{1 + y/K_m}, \qquad X \approx \frac{X_{\text{tot}}}{1 + y/K_m},$$

so that the steady state value of G as function of X_{tot} is well approximated by

$$G \approx \frac{\alpha X_{\text{tot}}(y/K_m) + \alpha_0}{\gamma(1 + X_{\text{tot}})(1 + y/K_m)},$$

which is a monotonically increasing function of X_{tot}. When instead $X_{\text{tot}} \gg K_m$, we obtain that $X^* \approx y$ and $X \approx X_{\text{tot}} - y$, so that

$$G \approx \frac{y(\alpha - \alpha_0) + \alpha_0 X_{\text{tot}}}{\gamma(1 + X_{\text{tot}})}.$$

By computing the derivative of this expression with respect to X_{tot}, we can see that it is an increasing function of X_{tot} whenever $y < \alpha_0/(\alpha - \alpha_0)$, which can be satisfied when the concentration Z_{tot} of kinase is sufficiently low, while it is a decreasing function of X_{tot} when $y > \alpha_0/(\alpha - \alpha_0)$, which can be obtained when the concentration of kinase is sufficiently high. This is the pattern observed in the data (Figure S11).

Therefore, when the concentration of kinase is low, the expression level of G increases monotonically with X_{tot}, but when the concentration of kinase is high, then the expression level of G displays a biphasic behavior, increasing for low concentration X_{tot} and decreasing for high concentration X_{tot}.

From the analysis above, it is also apparent that if $\alpha_0 = 0$, that is, X can bind to the promoter but transcription cannot occur when X is bound, then the response of G to X_{tot} is biphasic independent of the amount of kinase Z. In the opposite case in which $\alpha_0 = \alpha$, that is, X is as good an activator as X*, the response of G to X_{tot} cannot be biphasic. The response cannot be biphasic also if X cannot bind to the promoter as, in this case, G is a monotonically increasing function of X^*.

Finally, if the kinase did not become saturated with the substrate, we would have the steady state expression $X^* = k_1 X_{\text{tot}} Z_{\text{tot}}/(k_1 Z_{\text{tot}} + k_2 Y_{\text{tot}})$ and $X = X_{\text{tot}} - X^*$, so that G would be a monotonically increasing function of X_{tot}.This study demonstrates that necessary conditions for having a biphasic response are: (1) saturation of the kinase Z by the substrate X and (2) binding of the unphosphorylated substrate X to the promoter leading to lower expression rate than when X* is bound.

In this simple model, we have assumed for simplifying the mathematical expressions that unphosphorylated X can lead to expression of G directly activating gene expression. However, other reactions such as the cross-reactivity of X by other kinases were not considered. Also, X can lead to expression of G when bound to the promoter also indirectly, by first being phoshorylated when promoter bound, and then leading to gene expression. The reactions capturing these mechanisms are included in the detailed model in the next section.

3 Detailed Model

3.1 Reactions

The reactions considered include the phosphorylation of NRI by NRII(L16R) and cross-reactivity by other kinases, the spontaneous dephosphorylation of NRI, the binding of both phosphorylated and unphosphorylated forms of NRI to the P_{glnA} promoter sites (*5*), the phosphorylation of the NRI/P_{glnA} complex by NRII(L16R) and cross-reactivity by other kinases (*6*), the autophosphatase activity of the phosphorylated NRI/P_{glnA} complex, the dilution of all species and degradation/dilution of NRII(L16R). It was also considered that only the oligomerization of the phosphorylated NRI/P_{glnA} complex leads to GFP expression (*5*, *7*).

When the NRII(H139N) phosphatase is added to the system, we additionally considered the dephosphorylation of NRI by NRII(H139N) and the desphosphorylation of the phosphorylated NRI/P_{glnA} complex by NRII(H139N).

Consider the underscript ($_L$) for species concerning the load and asterisk notation (*) for phosphorylated species. We define C_1 as the NRI/NRII(L16R) complex, C_2 as the NRI/P_{glnA}/NRII(L16R) complex, C_3 as the NRI*/NRII(H139N) complex and C_4 as the C*/NRII(H139N) complex. Letting $f_H(\cdot)$ denote a production Hill function, we have the following reactions. The production and decay of the NRII(L16R) kinase

$$\emptyset \xrightarrow{f_H(\text{aTc})} \text{NRII(L16R)} \tag{7}$$

$$\text{NRII(L16R)} \xrightarrow{\delta} \emptyset \tag{8}$$

$$\text{NRII(L16R)} \xrightarrow{\delta_d} \emptyset; \tag{9}$$

production and decay of the NRI substrate

$$\emptyset \xrightarrow{k_N} \text{NRI} \tag{10}$$

$$\text{NRI} \xrightarrow{\delta} \emptyset; \tag{11}$$

production and decay of the NRII(H139N) phosphatase

$$\emptyset \xrightarrow{k_y} \text{NRII(H139N)} \tag{12}$$

$$\text{NRII(H139N)} \xrightarrow{\delta} \emptyset \tag{13}$$

$$\text{NRII(H139N)} \xrightarrow{\delta_d} \emptyset; \tag{14}$$

NRI substrate phosphorylation and protein decay

$$\text{NRI} + \text{NRII(L16R)} \underset{\beta_1}{\overset{\alpha_1}{\rightleftharpoons}} C_1 \xrightarrow{k_1} \text{NRII(L16R)} + \text{NRI}^* \tag{15}$$

$$\text{NRI} \xrightarrow{k_s} \text{NRI}^* \tag{16}$$

$$\text{C}_1 \xrightarrow{\delta_d} \text{NRI} \tag{17}$$

$$\text{C}_1 \xrightarrow{\delta} \emptyset; \tag{18}$$

NRI substrate dephosphorylation and protein decay

$$\text{NRI}^* + \text{NRII(H139N)} \underset{\beta_1}{\overset{\alpha_1}{\rightleftharpoons}} \text{C}_3 \xrightarrow{k_1} \text{NRI} + \text{NRII(H139N)} \tag{19}$$

$$\text{C}_3 \xrightarrow{\delta} \emptyset \tag{20}$$

$$\text{C}_3 \xrightarrow{\delta_d} \emptyset \tag{21}$$

$$\text{NRI}^* \xrightarrow{k_2} \text{NRI} \tag{22}$$

$$\text{NRI}^* \xrightarrow{\delta} \emptyset; \tag{23}$$

NRI binding to DNA sites and complex decay

$$\text{NRI} + \text{p} \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} \text{C} \tag{24}$$

$$\text{NRI} + \text{p}_L \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} \text{C}_L \tag{25}$$

$$\text{NRI}^* + \text{p} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{C}^* \tag{26}$$

$$\mathrm{NRI}^* + \mathrm{p}_L \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{C}_L^* \tag{27}$$

$$\mathrm{C} \xrightarrow{\delta} \mathrm{p} + \mathrm{NRI} \tag{28}$$

$$\mathrm{C}_L \xrightarrow{\delta} \mathrm{p}_L + \mathrm{NRI} \tag{29}$$

$$\mathrm{C}^* \xrightarrow{\delta} \mathrm{p} + \mathrm{NRI}^* \tag{30}$$

$$\mathrm{C}_L^* \xrightarrow{\delta} \mathrm{p}_L + \mathrm{NRI}^* \tag{31}$$

DNA complex phosphorylation and decay

$$\mathrm{C} + \mathrm{NRII(L16R)} \underset{\beta_3}{\overset{\alpha_3}{\rightleftharpoons}} \mathrm{C}_2 \xrightarrow{k_3} \mathrm{C}^* + \mathrm{NRII(L16R)} \tag{32}$$

$$\mathrm{C} \xrightarrow{k_s} \mathrm{C}^* \tag{33}$$

$$\mathrm{C}_2 \xrightarrow{\delta_d} \mathrm{C} \tag{34}$$

$$\mathrm{C}_2 \xrightarrow{\delta} \mathrm{p} + \mathrm{NRII(L16R)} \tag{35}$$

$$\mathrm{C}_L + \mathrm{NRII(L16R)} \underset{\beta_3}{\overset{\alpha_3}{\rightleftharpoons}} \mathrm{C}_{2L} \xrightarrow{k_3} \mathrm{C}_L^* + \mathrm{NRII(L16R)} \tag{36}$$

$$\mathrm{C}_L \xrightarrow{k_s} \mathrm{C}_L^* \tag{37}$$

$$\mathrm{C}_{2L} \xrightarrow{\delta_d} \mathrm{C}_L \tag{38}$$

$$\mathrm{C}_{2L} \xrightarrow{\delta} \mathrm{p}_L + \mathrm{NRII(L16R)}; \tag{39}$$

DNA complex dephosphorylation and decay

$$\mathrm{C}^* + \mathrm{NRII(H139N)} \underset{\beta_3}{\overset{\alpha_3}{\rightleftharpoons}} \mathrm{C}_4 \xrightarrow{k_3} \mathrm{C} + \mathrm{NRII(H139N)} \tag{40}$$

$$\mathrm{C}_4 \xrightarrow{\delta_d} \mathrm{C}^* \tag{41}$$

$$\mathrm{C}_4 \xrightarrow{\delta} \mathrm{C}^* + \mathrm{NRII(H139N)} \tag{42}$$

$$\mathrm{C}^* \xrightarrow{k_4} \mathrm{C} \tag{43}$$

$$\mathrm{C}_L^* + \mathrm{NRII(H139N)} \underset{\beta_3}{\overset{\alpha_3}{\rightleftharpoons}} \mathrm{C}_{4L} \xrightarrow{k_3} \mathrm{C}_L + \mathrm{NRII(H139N)} \tag{44}$$

$$C_{4L} \xrightarrow{\delta_d} C_L^* \tag{45}$$

$$C_{4L} \xrightarrow{\delta} p_L + \text{NRII(H139N)} \tag{46}$$

$$C_L^* \xrightarrow{k_4} C_L; \tag{47}$$

C^* complex hexamerization and decay

$$3 \cdot C^* \underset{k_{d6}}{\overset{k_{a6}}{\rightleftharpoons}} C_6^* \tag{48}$$

$$C_6^* \xrightarrow{\delta} 3 \cdot C^*; \tag{49}$$

and GFP production and decay

$$C_6^* \xrightarrow{k_g} \text{GFP} \tag{50}$$

$$\text{GFP} \xrightarrow{\delta} \emptyset \tag{51}$$

$$\text{GFP} \xrightarrow{\delta_d} \emptyset. \tag{52}$$

Reactions with parameter δ involve dilution, δ_d involve degradation, k_s is a phosphorylation rate due to cross-reactivity with other kinases, k_2 and k_4 are autophosphatase activity rates, and k_N and k_y are the NRI substrate and NRII phosphatase production rates (respectively) to balance out dilution due to cell growth.

3.2 Model Assumptions

To generate an ODE model, the following assumptions were made:

1. Well-mixed reaction compartment.

2. aTc is in saturating conditions and reach a rapid equilibrium level between the cytoplasm and the outside media. The aTc membrane diffusion and degradation can be neglected.

3. NRI dimerizes immediately after expressed (*7*).

4. The oligomerization of the phosphorylated NRI/P_{glnA} complex into a hexamer is required for P_{glnA} activation and is the only process leading to GFP expression (*5*, *7*).

5. The functions governing production of NRI (denoted $f_H(\cdot)$) can be modeled by a Hill function that captures mRNA transcription and translation.

6. All species dilute and/or are degraded.

7. DNA hinders the ability of NRII(L16R) and NRII(H139N) to access DNA bound NRI species (steric hindrance (*6*)), thus it was assumed that $\alpha_1 \leq \alpha_3$.

8. Conservation of mass on the total amount concentration of P_{glnA} promoter sites of the reporter and load. This leads to the conservation laws $p_T = p + C + C^* + C_2 + C_4 + C_6^*$ and $p_{TL} = p_L + C_L + C_L^* + C_{2L} + C_{4L}$.

3.3 ODE Model

The notation considered for the system species is: K represents kinase NRII(L16R), N represents NRI substrate dimers, p represents the free p_{glnA} promoter binding sites, Y represents the phosphatase NRII(H139N), and G represents GFP. These lead to the following set of ordinary differential equations:

$$\dot{K} = k_a \frac{aTc^2}{K_D^{aTc} + aTc^2} - (\delta_d + \delta)K \underbrace{-\alpha_1 NK + (\beta_1 + k_1)C_1 - \alpha_3 CK + (\beta_3 + k_3 + \delta)C_2 - \alpha_3 C_L K + (\beta_3 + k_3 + \delta)C_{2L}}_{r}, \tag{53}$$

$$\dot{N} = k_N - \delta N - \alpha_1 NK + (\beta_1 + \delta_d)C_1 + k_2 N^* - k_s N - k_{\text{on}} Np + (k_{\text{off}} + \delta)C \underbrace{-k_{\text{on}} Np_L + (k_{\text{off}} + \delta)C_L}_{s_1}, \tag{54}$$

$$\dot{N}^* = k_1 C_1 - (k_2 + \delta)N^* - \alpha_3 N^* Y + (\beta_3 + \delta_d)C_3 + k_s N - k_{\text{on}} N^* p + (k_{\text{off}} + \delta)C^*$$

$$\underbrace{-k_{\text{on}}N^* p_L + (k_{\text{off}} + \delta)C_L^*}_{s_2}, \tag{55}$$

$$\dot{Y} = k_y - (\delta + \delta_d)Y_T - \alpha_1 N^* Y + (\beta_1 + k_1)C_3 - \alpha_3 C^* Y + (\beta_3 + k_3 + \delta)C_4$$
$$- \alpha_3 C_L^* Y + (\beta_3 + k_3 + \delta)C_{4L}, \tag{56}$$

$$\dot{C} = k_{\text{on}}Np - (k_{\text{off}} + \delta)C - \alpha_3 CK + (\beta_3 + \delta_d + \delta)C_2 + k_4 C^* + k_3 C_4 - k_s C, \tag{57}$$

$$\dot{C}_L = k_{\text{on}}Np_L - (k_{\text{off}} + \delta)C_L - \alpha_3 C_L K + (\beta_3 + \delta_d + \delta)C_{2L} + k_4 C_L^* + k_3 C_{4L} - k_s C_L, \tag{58}$$

$$\dot{C}^* = k_{\text{on}}N^* p - (k_{\text{off}} + \delta)C^* - 3k_{a6}C^{*3} + 3(k_{d6} + \delta)C_6^* + k_3 C_2 - k_4 C^* - \alpha_3 C^* Y$$
$$+ (\beta_3 + \delta_d + \delta)C_4 + k_s C, \tag{59}$$

$$\dot{C}_L^* = k_{\text{on}}N^* p_L - (k_{\text{off}} + \delta)C_L^* + k_3 * C_{2L} - k_4 * C_L^* - \alpha_3 C_L^* Y + (\beta_3 + \delta_d + \delta)C_{4L} + k_s C_L, \tag{60}$$

$$\dot{C}_6^* = k_{a6}C^{*3} - k_{d6}C_6^* - \delta C_6^*, \tag{61}$$

$$\dot{C}_1 = \alpha_1 NK - (\beta_1 + k_1)C_1 - (\delta_d + \delta)C_1, \tag{62}$$

$$\dot{C}_2 = \alpha_3 CK - (\beta_3 + k_3)C_2 - (\delta_d + \delta)C_2, \tag{63}$$

$$\dot{C}_{2L} = \alpha_3 C_L K - (\beta_3 + k_3)C_{2L} - (\delta_d + \delta)C_{2L}, \tag{64}$$

$$\dot{C}_3 = \alpha_1 N^* Y - (\beta_1 + k_1)C_3 - (\delta_d + \delta)C_3, \tag{65}$$

$$\dot{C}_4 = \alpha_3 C^* Y - (\beta_3 + k_3)C_4 - (\delta_d + \delta)C_4, \tag{66}$$

$$\dot{C}_{4L} = \alpha_3 C_L^* Y - (\beta_3 + k_3)C_{4L} - (\delta_d + \delta)C_{4L}, \tag{67}$$

$$\dot{G} = k_g C_6^* - (\delta_d + \delta)G, \tag{68}$$

where $p = p_T - C - C^* - C_2 - C_4 - C_6^*$ and $p_L = p_{TL} - C_L - C_L^* - C_{2L} - C_{4L}$, since both p_T and p_{TL} are conserved. The terms over brace r represent the retroactivity to the input, while the terms over brace s_i represent the retroactivity to the output. We will call system (53)-(68) the Loaded System since it is assumed $p_{TL} \neq 0$. Let us define now a system where the load is not present, i.e. $p_{TL} = 0$:

$$\dot{K} = k_a \frac{aTc^2}{K_D^{aTc} + aTc^2} - (\delta_d + \delta)K \underbrace{-\alpha_1 NK + (\beta_1 + k_1)C_1 - \alpha_3 CK + (\beta_3 + k_3 + \delta)C_2}_{r_I}, \tag{69}$$

$$\dot{N} = k_N - \delta N - \alpha_1 NK + (\beta_1 + \delta_d)C_1 + k_2 N^* - k_s N - k_{\text{on}} Np + (k_{\text{off}} + \delta)C, \tag{70}$$

$$\dot{N}^* = k_1 C_1 - (k_2 + \delta)N^* - \alpha_3 * N^* Y + (\beta_3 + \delta_d)C_3 + k_s N - k_{\text{on}} N^* p + (k_{\text{off}} + \delta)C^*, \tag{71}$$

$$\dot{Y} = k_y - (\delta_d + \delta)Y_T - \alpha_1 N^* Y + (\beta_1 + k_1)C_3 - \alpha_3 C^* Y + (\beta_3 + k_3 + \delta)C_4, \tag{72}$$

$$\dot{C} = k_{\text{on}} Np - (k_{\text{off}} + \delta)C - \alpha_3 CK + (\beta_3 + \delta_d + \delta)C_2 + k_4 C^* + k_3 C_4 - k_s C, \tag{73}$$

$$\dot{C}^* = k_{\text{on}} N^* p - (k_{\text{off}} + \delta)C^* - 3k_{a6} C^{*3} + 3(k_{d6} + \delta)C_6^* + k_3 C_2 - k_4 C^* - \alpha_3 C^* Y + (\beta_3 + \delta_d + \delta)C_4 + k_s C, \tag{74}$$

$$\dot{C}_6^* = k_{a6} C^{*3} - k_{d6} C_6^* - \delta C_6^*, \tag{75}$$

$$\dot{C}_1 = \alpha_1 NK - (\beta_1 + k_1)C_1 - (\delta_d + \delta)C_1, \tag{76}$$

$$\dot{C}_2 = \alpha_3 CK - (\beta_3 + k_3)C_2 - (\delta_d + \delta)C_2, \tag{77}$$

$$\dot{C}_3 = \alpha_1 N^* Y - (\beta_1 + k_1)C_3 - (\delta_d + \delta)C_3, \tag{78}$$

$$\dot{C}_4 = \alpha_3 C^* Y - (\beta_3 + k_3)C_4 - (\delta_d + \delta)C_4, \tag{79}$$

$$\dot{G} = k_g C_6^* - (\delta_d + \delta)G. \tag{80}$$

where $p = p_T - C - C^* - C_2 - C_4 - C_6^*$ since p_T is conserved. We will call system (70)-(80) the unloaded system. Also note that the retroactivity to the input term r_I does not include the reactions involving the phosphorylation of the load complexes. All kinetic rates used in reactions (7)-(52) were constrained inside their allowable physical range and selected as given in Table S8

3.4 Steady state analysis

3.4.1 Steady state plots for varying NRI and aTc concentrations

The loaded system (53)-(68) and the unloaded system (69)-(80) were simulated for varying levels of NRI substrate and aTc inducer. In Fig. S18, we can see that the steady state level for the loaded system is lower than that of the unloaded system for low values of NRI, while the steady state level becomes the same at higher values of NRI. This effect is due to the

retroactivity to the output, given by the terms over brace s_i in (54) and (55), which is the only difference between the loaded system (53)-(68) and unloaded system (69)-(80).

We can also see that this steady state behavior is the same for all values of aTc, as depicted in Fig. S19. Here we have that for four different levels of NRI, the aTc concentration was varied leading to a difference in steady state for lower values of NRI and no difference in steady state for higher values of NRI. Also note in Fig. S18 that the steady state curve initially increases for increasing values of NRI, then decreases. We have termed this phenomena a biphasic behavior and will be explained in the next section.

3.4.2 Biphasic Behavior

Since GFP reports the concentration of complex C_6^*, we will analyze the concentration of this complex under various concentrations of substrate N := k_N/δ for a constant kinase induction of 20 nM aTc. As we can see in Fig. S20, for low values of N the steady state level of C_6^* increases with N, then starts decreasing after $N = 0.17\mu$M.

This behavior is due to the retroactivity to the input of the system, given by terms over brace r_I in (69)-(80), which causes the steady state reduction of K for increasing amounts of N. Since K is a kinase leading to N^* expression, a decrease in K to a low saturation level reflects in a saturation level for N^*. Since both N and N^* compete for the same load promoter sites p, the saturation of N^* lets the increasing amounts of N occupy all free p promoter sites. This effect leads to a decrease in the concentration of C^* and subsequently C_6^*. Simulating system (69)-(80) with the terms over brace $r_I = 0$ in (53), we have the steady state concentration of K remains constant and the concentration of C_6^* increases until it occupies all the free promoter sites of the reporter, as it is illustrated in Fig. S21. The original system given by the $r_I \neq 0$ in system (69)-(80), shows the decrease in steady state of K and the biphasic behavior in C_6^*.

3.5 Dynamic Analysis

3.5.1 Dynamic plots for different NRI concentrations

The time varying fluorescence value of GFP was recorded after 20nM aTc inductions at different concentrations of NRI. To address the relative speed of GFP inductions for the different values of NRI, the data was normalized to the highest level of GFP fluorescence reached. We can see in Fig. S22, that the GFP response becomes slower with increasing amounts of NRI, until the highest value at which it becomes faster. This behavior was captured by the loaded system model (53)-(68) and unloaded system model (69)-(80), as can be seen Fig. S22. This change in dynamics is due to the retroactivity to the input term r in (53)-(68) and r_I in (69)-(80) and will be explained in the next section.

3.5.2 Dynamic analysis

Let us first observe that GFP activation is due to C^* formation. Taking a closer look at the C^* dynamics and neglecting reactions involving phosphatase Y, oligomerization into C_6^*, and phosphorylation from other kinases (assumed to be small, see Table S8), we have:

$$\dot{C}^* = k_{\text{on}} N^* p - (k_{\text{off}} + \delta) C^* + k_3 C_2 - k_4 C^*.$$

Terms in blue involve the C^* formation due to NRI^* binding, and the terms in red involve the C^* formation due phosphorylation. We can view the dynamics of C^* as a result of two fluxes, one due to the binding and unbinding reactions where N^* is an input, and one due to complex phosphorylation where the kinase K leading to C_2 expression is an input. Thus, we can first explore in simulation which flux dominates the dynamics of C^* by looking at the dynamics of inputs N^* and K individually, for increasing values of NRI.

We can see in Fig. S23 that the dynamics of N^* become slower with increasing amounts of NRI, while the dynamics of K have a similar profile as in Fig. S22. The dynamics of K become slower with increasing values of NRI until the highest concentration for which it

becomes faster. Since the GFP dynamics follow the same profile, this implies that the K driven reactions are the ones producing this dynamic behavior, and it is the retroactivity to the input affecting the K dynamics the responsable for the speedup in the K response at high values of NRI.

To better understand how the retroactivity to the input affects the kinase K dynamics, we built and analyzed a simple model of the kinase dynamics to identify the mechanism giving this change in behavior with NRI. We propose the following simplified model:

$$\begin{aligned}
\dot{K} =& k(t) - \delta K - \alpha_1 N K + (\beta_1 + k_1) C_1 \\
\dot{C}_1 =& \alpha_1 N K - (\beta_1 + k_1) C_1 - \delta C_1 \\
\dot{N}^* =& k_1 C_1 - (k_2 + \delta) N^* \\
\dot{N}_T =& k_N - \delta N_T
\end{aligned} \tag{81}$$

in which $N = N_T - N^* - C_1$. Since N_T can have initial conditions at its steady state value k_N/δ, we will consider for the rest of the analysis N_T to be constant, and increases in N_T steady state concentrations as equivalent to increases in k_N. Note that the production term $k(t)$ denoting transcriptional activation and all terms involving dilution δ evolve in the timescale of hours, while all other terms involve phosphorylation/dephosphorylation which evolve in a timescale of minutes or even seconds as it can be seen in Table S8. This difference in timescale allows us to perform a system order reduction through singular perturbation to have a simple expression for the K dynamics. To this end we can first write the system (81) in terms of non-dimensional time by defining the singular perturbation parameter $\epsilon := \delta/\beta_1$, where $\epsilon \ll 1$ since $\delta \ll \beta_1$ as can be seen form the values in Table S8. Defining parameters not depending on ϵ: $a_1 = \alpha_1/\beta_1$, $b_1 = (k_1 + \delta)/\beta_1$, $b_2 = (k_2 + \delta)/\beta_1$ and by performing the change of variables $y = K + C_1$, we can write the system in standard singular perturbation form (Khalil (*8*)):

$$
\begin{aligned}
\dot{y} =& k(t) - \delta y \\
\epsilon \dot{C}_1 =& a_1 \delta N K - (1 + b_1)\delta C_1 \\
\epsilon \dot{N}^* =& b_1 \delta C_1 - b_2 \delta N^*.
\end{aligned}
\tag{82}
$$

Assuming the slow manifold of this system is locally exponentially stable (*8*), we can approximate the dynamics of K by setting $\epsilon = 0$. Defining $k_{d1} := (\beta_1 + k_1 + \delta)/\alpha_1$ and $k_x := k_1/(k_2 + \delta)$, we have that on the slow manifold

$$
N^*(K) = k_x C_1(K), \quad C_1(K) = N_T \frac{K}{k_{d1} + (1 + k_x)K}.
$$

Differentiating the slow variable y in time we have that $\dot{y} = \dot{K} + \frac{\partial C_1}{\partial K}\dot{K}$. From (82), we can write the reduced model for the kinase dynamics as

$$
\begin{aligned}
\dot{K} =& \frac{1}{1 + \frac{\partial C_1}{\partial K}} \{k(t) - \delta[K + C_1(K)]\} \\
=& \frac{1}{1 + \frac{N_T}{[k_{d1} + (1 + k_x)K]^2}} \left\{ k(t) - \delta \left[K + \frac{N_T K}{k_{d1} + (1 + k_x)K} \right] \right\}.
\end{aligned}
\tag{83}
$$

In this expression the term

$$
\frac{1}{1 + \frac{N_T}{[k_{d1} + (1 + k_x)K]^2}}
$$

decreases monotonically as N_T increases making the K dynamic's slower. This is the same as the retroactivity to the input term provided in Del Vecchio *et. al* (*9*). By contrast, the term

$$
-\delta C_1(K) = -\delta \left[\frac{N_T K}{k_{d1} + (1 + k_x)K} \right]
$$

is a negative feedback term that becomes more negative for higher values of N_T, and is responsible for the speedup in the K dynamics at high leves of N_T. The interplay between the $\frac{1}{1+\frac{\partial C_1}{\partial K}}$ term and the $-\delta C_1(K)$ term makes the dynamics of K become initially slow for increasing values of N_T then faster when the effect of the $-\delta C_1(K)$ term becomes predominant. The dynamic response of system (83) is provided in Fig. S24, where we see that the dynamics slow down

for increasing values of NRI, then speeds up for high values of NRI.

4 Tables

Table S1 BioBrick DNA used for cloning.

BioBrick part*	**DNA function**
BBa_I746916	Superfolder GFP coding sequence
BBa_B0015	Double terminator
BBa_B0034	Ribosome binding site with efficiency 1.0
BBa_B0033	Ribosome binding site with efficiency 0.01
BBa_B0032	Ribosome binding site with efficiency 0.3
BBa_C0040	*tetR*+LVA; *tetR* repressor with the LVA degradation tag
BBa_C0012	*lacI*+LVA; *lacI* repressor with the LVA degradation tag
BBa_R0040	*Ptet*; Promoter for *tet*
BBa_R0011	P*lac*; Promoter for lac
BBa_ J23113	Constitutive promoter (with a strength of 21 au RFP expression)
BBa_J23117	Constitutive promoter (with a strength of 162 au RFP expression)
BBa_J23114	Constitutive promoter (with a strength of 256 au RFP expression)

*All the BioBrick parts can be found on http://parts.igem.org/Main_Page

Table S2 DNA primers used for cloning.

Primer	DNA sequence (5' to 3')
sf-*gfp*LVA with RBS(39,754) For	CTAACTTTCAATTCTAATAAGGAGGAAGACTTCAAATGCGTAAAG GCGAAGAGCTGTTC
P*glnA* with RBS(39,754) Reverse	TTGAAGTCTTCCTCCTTATTAGAATTGAAAGTTAGGTCGTCGTGGT AACGAAATCTGC
BB-Prefix	GAATTCGCGGCCGCTTCTAG
BB-Suffix	CTGCAGCGGCCGCTACTAGTA
Prefix (*Sph*I)	ATGCGCATGCGAATTCGCGGCCGCTTCTAG
Prefix (*Bsp*HI)	ATGCTCATGAGAATTCGCGGCCGCTTCTAG
Prefix (*Bso*BI)	ATGCCTCGGGGAATTCGCGGCCGCTTCTAG
Prefix (*Bam*HI)	ATGCGGATCCGAATTCGCGGCCGCTTCTAG
Suffix (*Sph*I)	ATGCGCATGCCTGCAGCGGCCGCTACTAGTA
Suffix (*Bsp*HI)	ATGCTCATGACTGCAGCGGCCGCTACTAGTA
Suffix (*Bso*BI)	ATGCCCCGAGCTGCAGCGGCCGCTACTAGTA
Suffix (*Bam*HI)	ATGCGGATCCCTGCAGCGGCCGCTACTAGTA
NRIIFor(EX-RBS-NRII)	GAATTCGCGGCCGCTTCTAGAAAGAGGAGAAATACTAGATGGCAA CAGGCACGCAG
NRIIRev(NRII-SP)	CTGCAGCGGCCGCTACTAGTATTATTTCCTGATAGGCAGGTAAACC G
RBS(33)+P*tet*(end) Rev	CTAGTAGTCCTGTGTGACTCTAGTAGTGCTCAGTATCTCTATCAC
P*tet*(end)+RBS(33)+NRII For	ACTACTAGAGTCACACAGGACTACTAGATGGCAACAGGCACGCAG
LVA+NRII Rev	TTAAGCTACTAAAGCGTAGTTTTCGTCGTTTGCAGCTTTCCTGATAG GCAGGTAAAC
LVA+D.Ter For	GCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAATGATACTAG
NRIFor(EX-NRI)	GAATTCGCGGCCGCTTCTAGATGCAACGAGGGATAGTCTG
NRIRev(SP-NRI)	CTGCAGCGGCCGCTACTAGTATCACTCCATCCCCAGCTC
P*glnA* site-2 (strong)-For	ATGCGAATTCGCGGCCGCTTCTAGAGGAAGCACTATATTGGTGCAA CTACTAGTAGCGGCCGCTGCAGATGC
P*glnA* site-2 (strong)-Rev	GCATCTGCAGCGGCCGCTACTAGTAGTTGCACCAATATAGTGCTTC CTCTAGAAGCGGCCGCGAATTCGCAT

Table S3 DNA primers for deleting genes and confirmation of deletions.

Primer	DNA sequence (5' to 3')
recA deletion For	CAACAGAACATATTGACTATCCGGTATTACCCGGCATGACAGGA GTAAAAGTGTAGGCTGGAGCTGCTTC
recA deletion Rev	AAAAAAGCAAAAGGGCCGCAGATGCGACCCTTGTGTATCAAACA AGACGACATATGAATATCCTCCTTAGTTCC
recA deletion confirmation For	ATGGCTATCGACGAAAACAAACAGAAAG
recA deletion confirmation Rev	TTAAAAATCTTCGTTAGTTTCTGCTACG
glnK deletion-For	CAACTTGCGGGCGAAGAGCTGGCAGCCAGCGTGCGTGAAGAGG AATCATTGAGCGCCTGAGTGTAGGCTGGAGCTGCTTC
glnK deletion-Rev	CAAGCCCAGTTTTTATCGTCGCTATCTTCATTTTTCGTTCCTGTTG CTGTGTGCCAGAGACATATGAATATCCTCCTTAGTTCC
glnK deletion confirmation-For	CAACTTGCGGGCGAAGAGCTGGCAGCCAGCGTGCGTGAAGAGG AATCATTG
glnK deletion confirmation-Rev	CAAGCCCAGTTTTTATCGTCGCTATCTTCATTTTTCGTTCCTGTTG CTGTG
glnB deletion For	GTTACGTTTAGCAGATCAAAAGACAGGCGACCTTTTCAAGGAAT AGCGTGTAGGCTGGAGCTGCTTC
glnB deletion Rev	CATTCATTACGAATGCTTTGGCCCGCATAAGGTGCTGTAATTTGA TGCATATGAATATCCTCCTTAGTTCC
glnB deletion confirmation For	GTTACGTTTAGCAGATCAAAAGACAGGCGACCTTTTCAAGGAAT AGC
glnB deletion confirmation Rev	CATTCATTACGAATGCTTTGGCCCGCATAAGGTGCTGTAATTTGA TG
ackA/pta deletion For	GCTGAAAATTACGCAAAATGGCATAGACTCAAGATATTTCTTCC GTGTAGGCTGGAGCTGCTTC
ackA/pta deletion Rev	CGGTTCAGATATCCGCAGCGCAAAGCTGCGGATGATGACGAGAC ATATGAATATCCTCCTTAGTTCC
ackA/pta deletion confirm For	GCTGAAAATTACGCAAAATGGCATAGACTCAAGATATTTCTTCC
ackA/pta deletion confirm Rev	CGGTTCAGATATCCGCAGCGCAAAGCTGCGGATGATGACGAGA

Table S4 Comparison of RBS strengths of the circuit parts.

Circuit part	**RBS**	**RBS strength**[*]	**Fold increase over native RBS of P*glnA***[†]
Reporter with native RBS of P*glnA*	Native	392	1
Reporter with engineered RBS for P*glnA*	Engineered[#]	39,754	100
*tetR*LVA	BBa_B0034	66,884	170
*lacI*LVA	BBa_B0034	35,780	91
NRI	BBa_B0034	17,336	44
Kinase	BBa_B0034	46,872	119
Phosphatase	BBa_B0034	12,708	32

[*] RBS strength calculated by RBS calculator (*1*).

[#] The RBS was designed to increase the reporter expression by 100-fold.

[†] Calculated based on the RBS calculator.

Table S5 Comparison of promoter/RBS upstream of NRI gene.

NRI amount	**Promoter (P) and RBS(R) combination**	**Relative promoter strength**	**Relative RBS strength**	**Comparative production protein**	**Relative amount of NRI protein (approx.)**
Very low	P(21)RBS(34)	0.082	1	0.082	1.0X
Low	P(256)RBS(32)	1	0.33	0.33	4.0X
Medium	P(162)RBS(34)	0.63	1	0.63	7.7X
High	P(256)RBS(34)	1	1	1	12.2X

Table S6 Plasmids used in this study.

Plasmid	Characteristics	Source/Reference
pJLA503/glnL (L16R)	This plasmid contains the gene for NRII (L16R) kinase.	Prof. Alexander Ninfa, University of Michigan
pLOP22mRB9132(H139N)	This plasmid contains the gene for NRII (H139N) phosphatase.	Prof. Alexander Ninfa, University of Michigan
pACYC184	Medium copy number plasmid (20-30 copies/cell) used for cloning the circuit genes.	NEB (New England BioLabs Inc., USA)
pUC19	High copy number plasmid (<100 copies/cell) used for construction of the pLoad plasmid.	NEB (New England BioLabs Inc., USA)
pKD4	Plasmid with kanamycin resistance gene.	Datsenko *et. al*(*2*)
pKD20	Plasmid used for deletion of antibiotic resistance cassette from the chromosome.	Datsenko *et. al* (*2*)
pCircuit1X	Circuit plasmid expressing very low NRI.	This study
pCircuit4X	Circuit plasmid expressing low NRI.	This study
pCircuit8X	Circuit plasmid expressing medium NRI.	This study
pCircuit12X	Circuit plasmid expressing high NRI.	This study
pLoad	Load plasmid containing strong enhancer binding site-2.	This study

Table S7 Bacterial strains used.

Strain	Description	Source/Reference
NEB 5-alpha	*fhuA2*, Δ(*argF-lacZ*)*U169*, *phoA*, *glnV44*, *Φ80*Δ(*lacZ*)*M15*, *gyrA96, recA1, relA1, endA1, thi-1, hsdR17*	NEB (New England BioLabs Inc., USA)
E. coli 3.300LG	*glnL, glnG, lacI22, λ-, e14-, relA1, spoT1, thiE1*	Atkinson *et. al* (*10*)
E. coli 3.300LGR	*E. coli* 3.300LG, Δ*recA*	This study
E. coli 3.300LGRK	*E. coli* 3.300LGR, Δ*glnK*	This study
E. coli 3.300LGRKAP	*E. coli* 3.300LGRK, Δ*ackA,* Δ*pta*	This study
E. coli 3.300LGRKAPB	*E. coli* 3.300LGRKAP, Δ*glnB*	This study

Table S8 Set of values for parameters

Parameter	Description	Value	Unit	Reference
δ	Dilution rate	0.0058	min^{-1}	Experimentally determined
δ_d	NRII(L16R) and NRII(H139N) degradation rate	0.0058	min^{-1}	Assumed same as dilution[a]
K_D^{aTc}	K_D for aTc induction	0.5000	nM	Analytically determined[b]
α_1	NRI/NRII(L16R) and NRI/NRII(H139N) association rate	6.000e3	$[\mu M\ min]^{-1}$	Keener(*11*), Chen(*12*)
β_1	NRI/NRII(L16R) and NRI/NRII(H139N) dissociation rate	6.000	$[\mu M\ min]^{-1}$	Chen (*12*)
k_1	NRI/NRII(L16R) complex and NRI/NRII(H139N) complex catalytic rate	10.000	min^{-1}	Surette(*13*)
k_{6a}	NRI/p_{glnA} complex hexamer formation	0.0220	min^{-1}	Ninfa (*7*)
k_{6d}	NRI/p_{glnA} complex hexamer dissociation	0.0423	min^{-1}	Ninfa (*7*)
k_2	NRI spontaneous dephosphorylation	0.1733	min^{-1}	Keener (*11*)
α_3	[NRI p_{glnA}]/NRII(L16R) and [NRI p_{glnA}]/NRII(H139N) association rate	60.000	$[\mu M\ min]^{-1}$	Keener(*11*), Chen(*12*)
β_3	[NRI p_{glnA}]/NRII(L16R) and [NRI p_{glnA}]/NRII(H139N) dissociation rate	6.000	$[\mu M\ min]^{-1}$	Chen (*12*)
k_3	[NRI p_{glnA}]/NRII(L16R) complex and [NRI p_{glnA}]/NRII(H139N) complex catalytic rate	10.000	min^{-1}	Surette(*13*)
k_4	NRI spontaneous dephosphorylation	0.1733	min^{-1}	Keener(*11*)
k_{on}	NRI-DNA binding	6.0000	$[\mu M\ min]^{-1}$	Schlosshauer(*14*)
k_{off}	NRI-DNA unbinding	8.400e-05	$[\mu M\ min]^{-1}$	Sevenich(*15*)
p_T	Total reporter promoters	0.0498	μM	Experimentally determined
p_{TL}	Total load promoters	0.1495	μM	Experimentally determined
k_a	Maximum velocity for aTc induction	3.1098e-04	μM/min	Analytically determined[c]
k_y	NRII(H139N) production rate	1.4500e-06	μM/min	Analytically determined[c]
k_g	GFP expression	100	μM/min	Analytically determined[c]
k_s	Phosphorylation from other kinases	0.1000	μM/min	Analytically determined[d]
k_N	NRI production rate	{5.8e-05,0.058}	μM/min	Analytically determined[e]

[a] Decay rate δ_d accounts for the degradation due to proteases.

[b] Based on the level of half induction in Fig. S5.

[c] Constrained such that $k_i/\delta < 10\mu$M (*16*).

[d] Constrained such that $k_s < k_3/K_M$ where $K_M = (\beta_3 + k_3)/\alpha 3 = 0.2667\mu$M.

[e] The NRI production rate k_N was determined such that $k_N/\delta \in \{0.01, 10\}\mu$M.

5 Figures

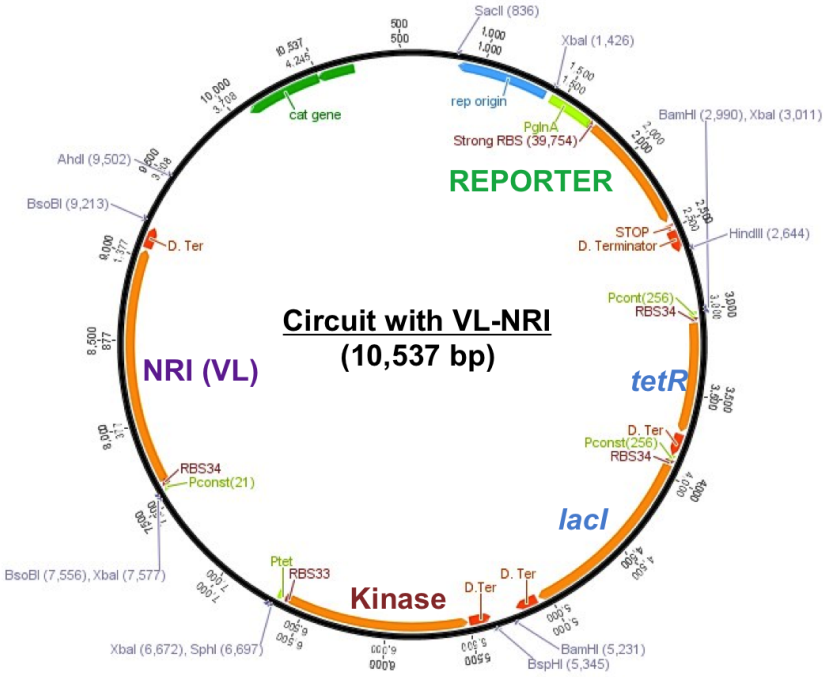


Fig. S1 Circuit plasmid-1 (with very low NRI - VL). The plasmid map of the gene circuit at various restriction sites containing the sf-*gfp* gene (*Xba*I-*Hind*III); *tetR* and *lacI* genes (*Bam*HI); kinase gene, NRII(L16R) (*Sph*I-*Bsp*HI); and NRI gene for very low constitutive expression of NRI (*Bso*BI).

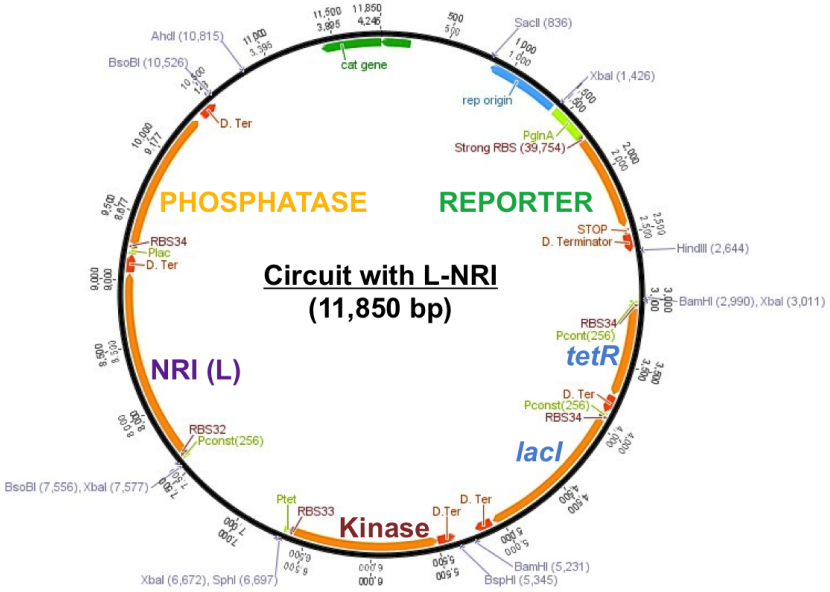


Fig. S2 Circuit plasmid-2 (with low NRI - L). The plasmid map of the gene circuit at various restriction sites containing the sf-*gfp* gene (*Xba*I-*Hind*III); *tetR* and *lacI* genes (*Bam*HI); kinase gene, NRII(L16R) (*Sph*I-*Bsp*HI); and NRI gene for low constitutive expression of NRI and phosphatase gene, NRI(H139N) (*Bso*BI).

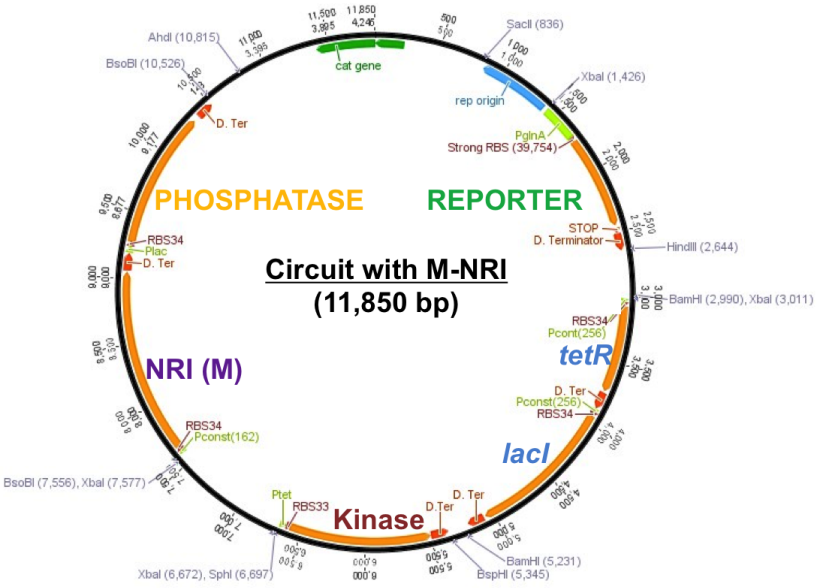


Fig. S3 Circuit plasmid-3 (with medium NRI - M). The plasmid map of the gene circuit at various restriction sites containing the sf-*gfp* gene (*Xba*I-*Hind*III); *tetR* and *lacI* genes (*Bam*HI); kinase gene, NRII(L16R) (*Sph*I-*Bsp*HI); and NRI gene for medium constitutive expression of NRI and phosphatase gene, NRI(H139N) (*Bso*BI).

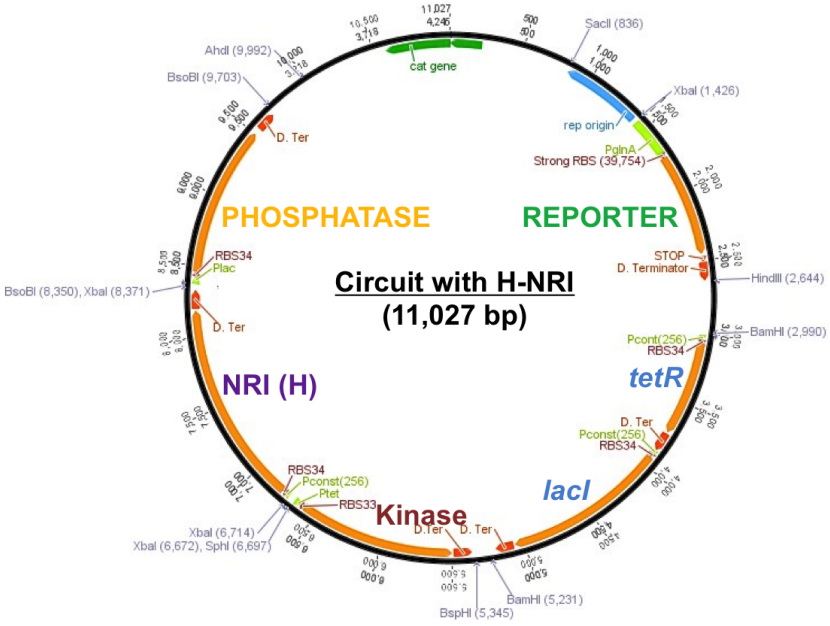


Fig. S4 Circuit plasmid-4 (with high NRI - H). The plasmid map of the gene circuit at various restriction sites containing the sf-*gfp* gene (*Xba*I-*Hind*III); *tetR* and *lacI* genes (*Bam*HI); kinase gene, NRII(L16R) (*Sph*I-*Bsp*HI); and NRI gene for high constitutive expression of NRI (*Sph*I-*Bso*BI) and phosphatase gene, NRI(H139N) (*Bso*BI).

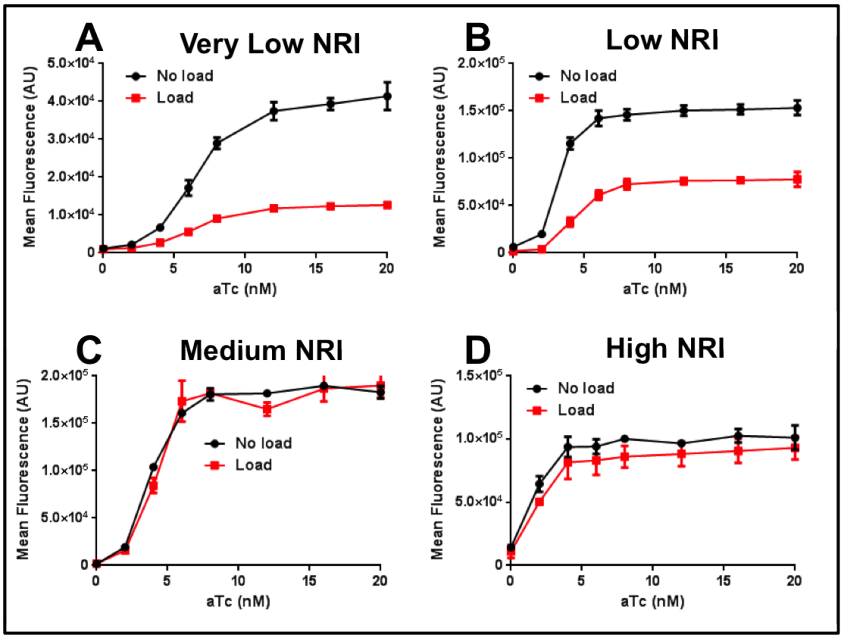


Fig. S5 Steady state behavior of all the four circuits. The steady states of the gene circuits with varying amounts of kinase induced using 2-20 nM aTc, without (black) and with (red) DNA load, and constitutively expressing four different amounts of NRI protein: (A) very low, (B) low, (C) medium, and (D) high NRI.

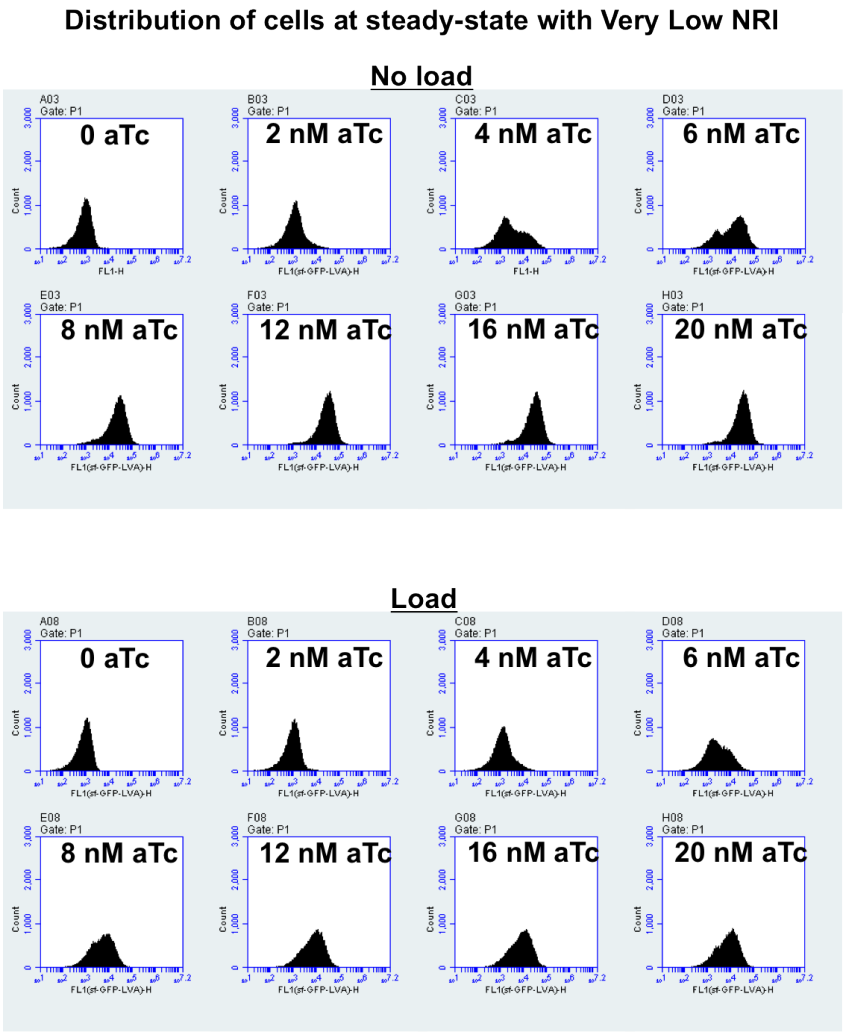


Fig. S6 Distribution of cells at steady-state (NRI-VL). Measurement of fluorescence of cells and distribution of cells by flow cytometer at respective steady states reached after induction of kinase with different concentrations of aTc (2-20 nM) in the circuit expressing very low NRI.

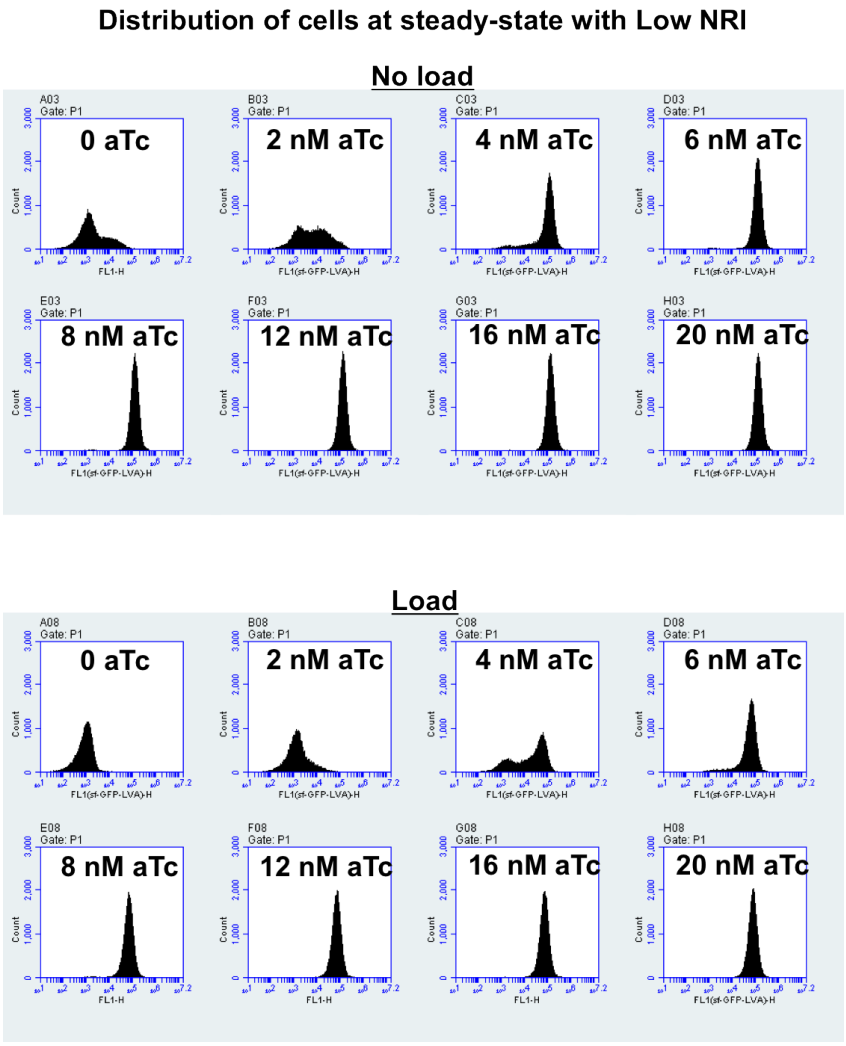


Fig. S7 Distribution of cells at steady-state (NRI-L). Measurement of fluorescence of cells and distribution of cells by flow cytometer at respective steady states reached after induction of kinase with different concentrations of aTc (2-20 nM) in the circuit expressing low NRI.

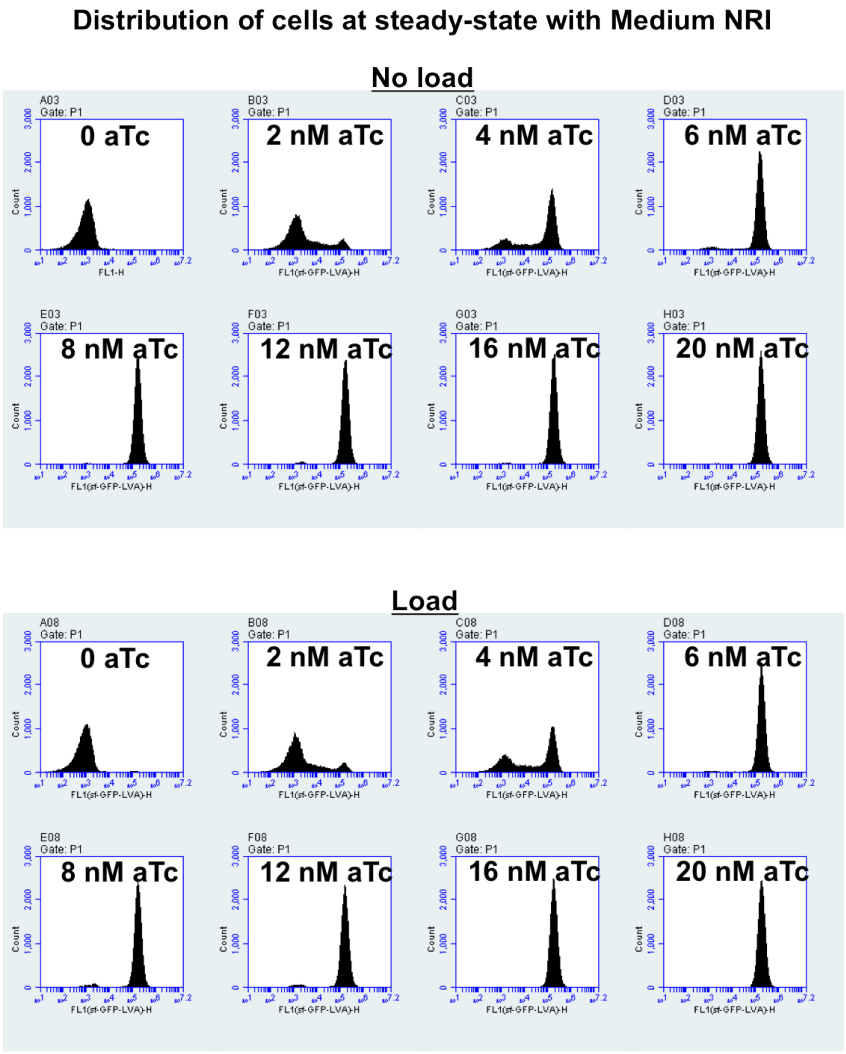


Fig. S8 Distribution of cells at steady-state (NRI-M). Measurement of fluorescence of cells and distribution of cells by flow cytometer at respective steady states reached after induction of kinase with different concentrations of aTc (2-20 nM) in the circuit expressing medium NRI.

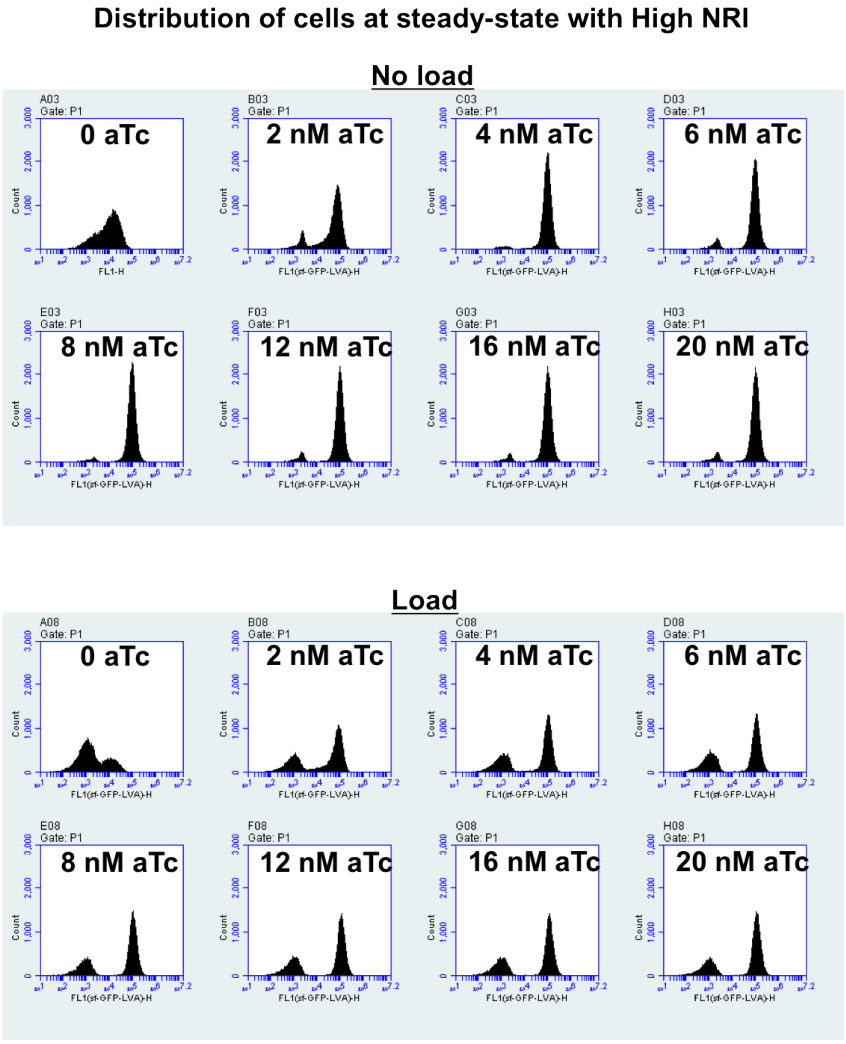


Fig. S9 Distribution of cells at steady-state (NRI-H). Measurement of fluorescence of cells and distribution of cells by flow cytometer at respective steady states reached after induction of kinase with different concentrations of aTc (2-20 nM) in the circuit expressing high NRI.

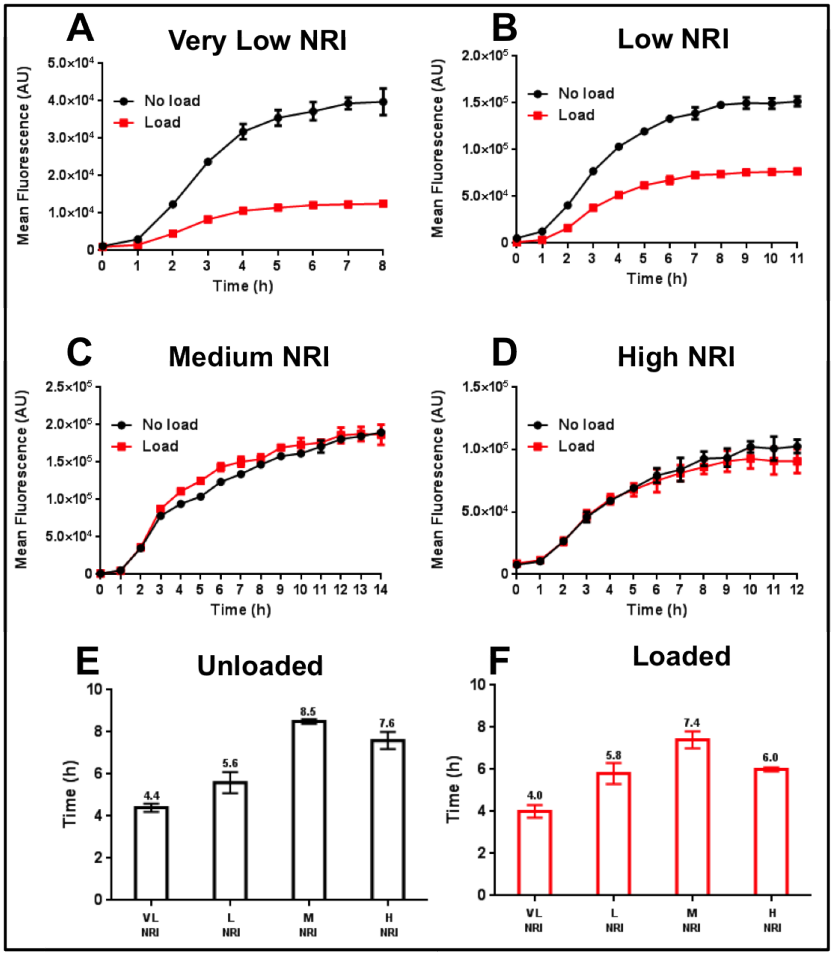


Fig. S10 Dynamics with different amounts of NRI and constant kinase (16 nM aTC). The dynamics of the gene circuits induced for a constant amount of kinase (16 nM aTc), without (black) and with (red) DNA load, and constitutively expressing four different amounts of NRI protein: (A) very low, (B) low, (C) medium, and (D) high NRI. The dynamics of the rise-time (10-90% increase) after induction with 16 nM aTc is shown (E) unloaded and (F) loaded circuits with different amounts of NRI, respectively.

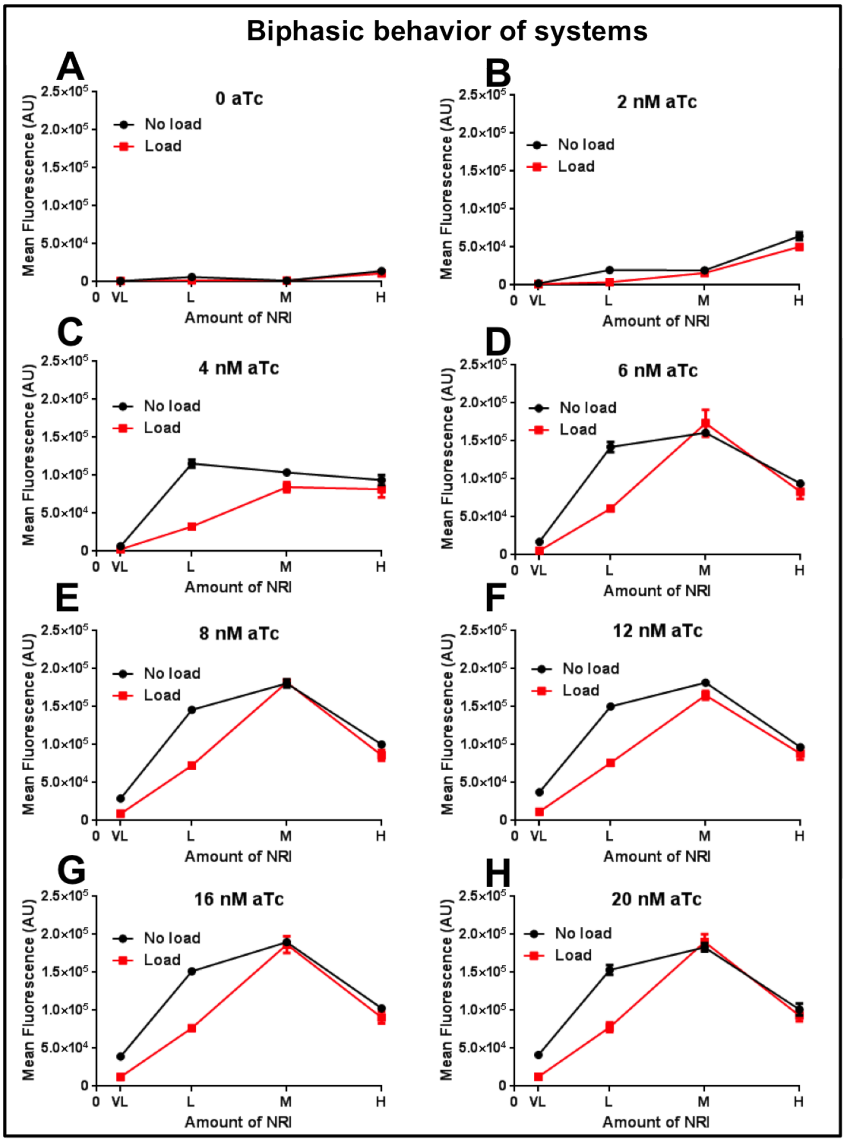


Fig. S11 Biphasic behavior of systems with increasing NRI. Comparison of the steady states of the circuits with different amounts of NRI for each value of kinase induced for the unloaded (black) and DNA loaded (red) systems. A linear relationship for increasing NRI was observed only at very low kinase levels (2 nM aTc); whereas, a biphasic behavior was displayed for higher kinase input levels (≥4 nM aTc).

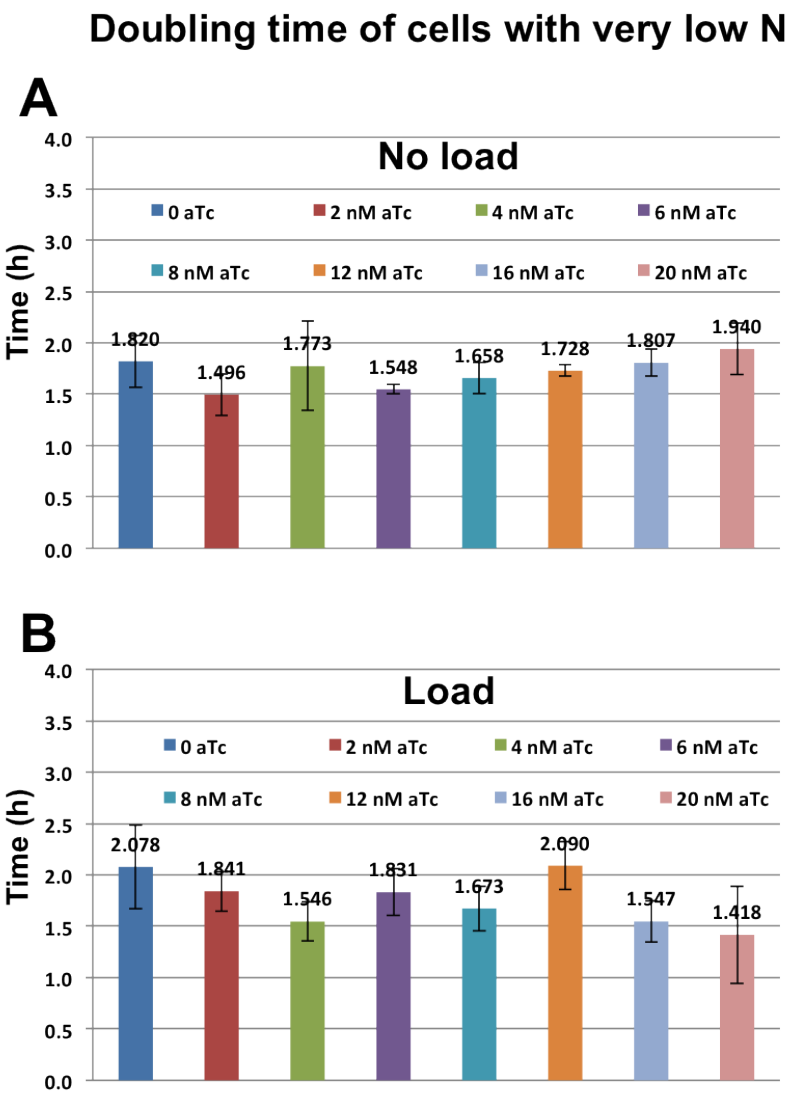


Fig. S12 Doubling time of cells with very low NRI (VL). The doubling time of the cells containing the gene circuit constitutively expressing very low NRI and induced for varying kinase levels (2-20 nM aTc) during the assay. The bars indicate mean doubling time of the cells from three replicates and the error bars indicate standard deviation.

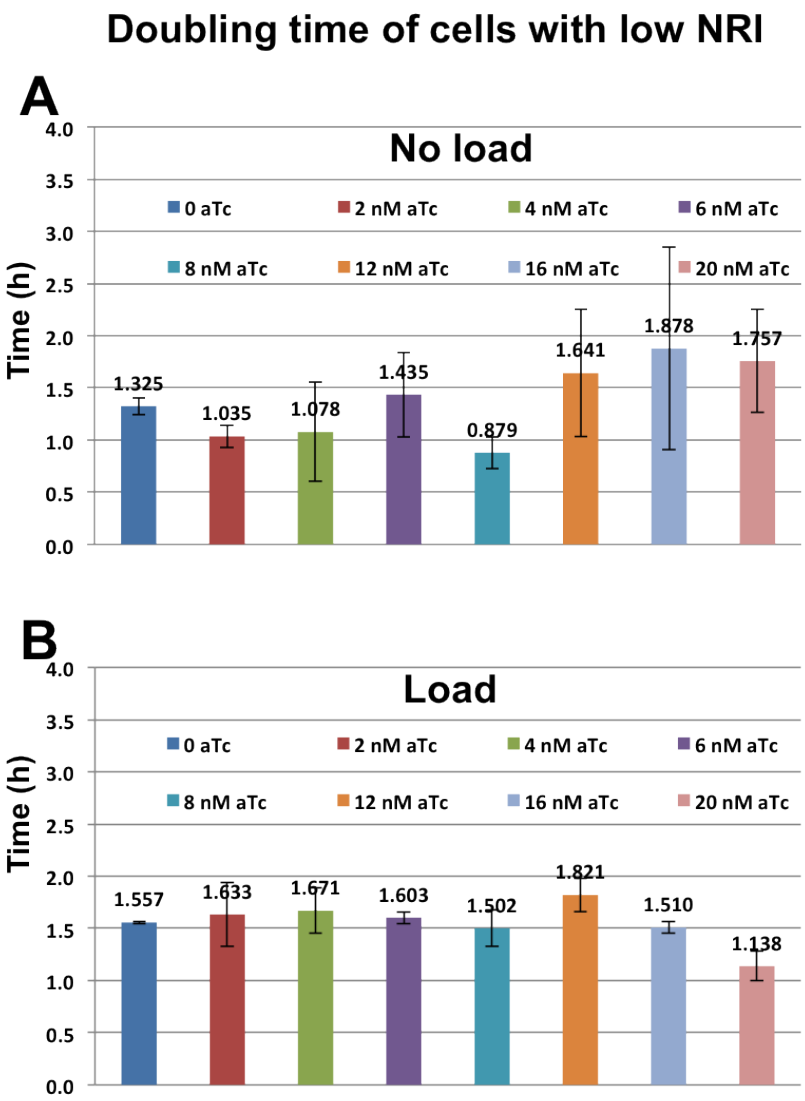


Fig. S13 Doubling time of cells with low NRI (L). The doubling time of the cells containing the gene circuit constitutively expressing low NRI and induced for varying kinase levels (2-20 nM aTc) during the assay. The bars indicate mean doubling time of the cells from three replicates and the error bars indicate standard deviation.

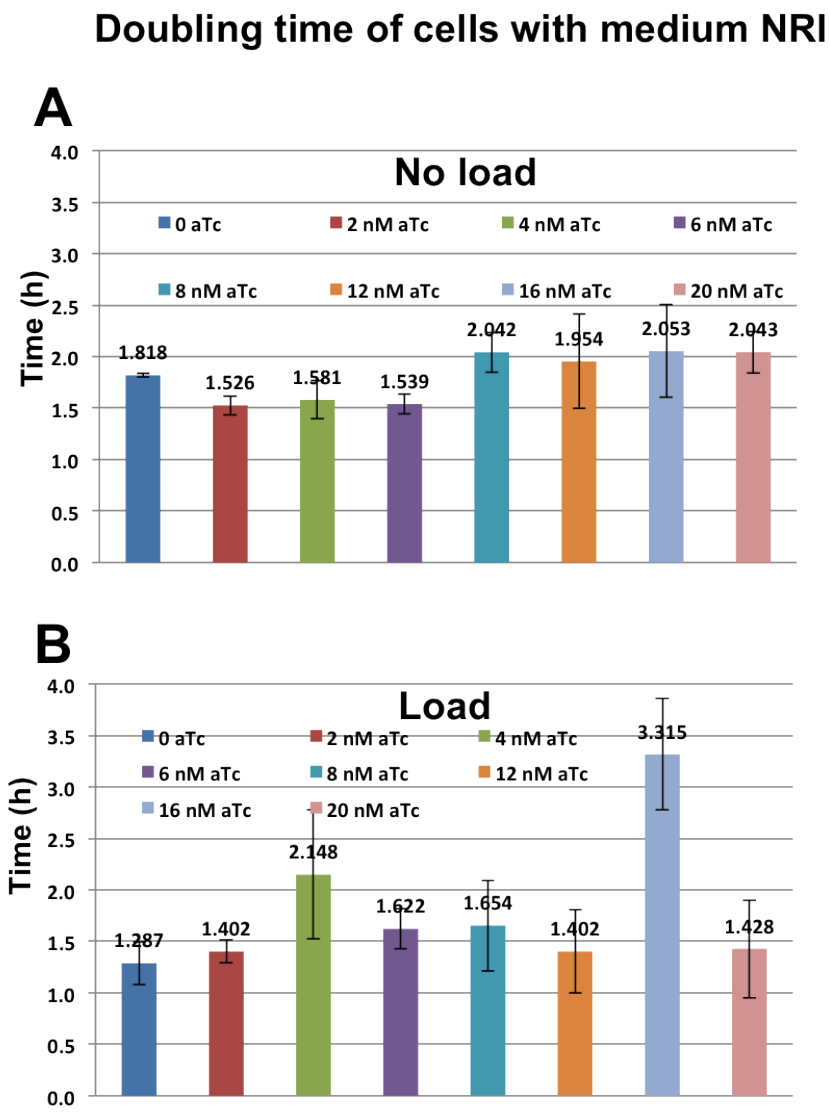


Fig. S14 Doubling time of cells with medium NRI (M). The doubling time of the cells containing the gene circuit constitutively expressing medium NRI and induced for varying kinase levels (2-20 nM aTc) during the assay. The bars indicate mean doubling time of the cells from three replicates and the error bars indicate standard deviation

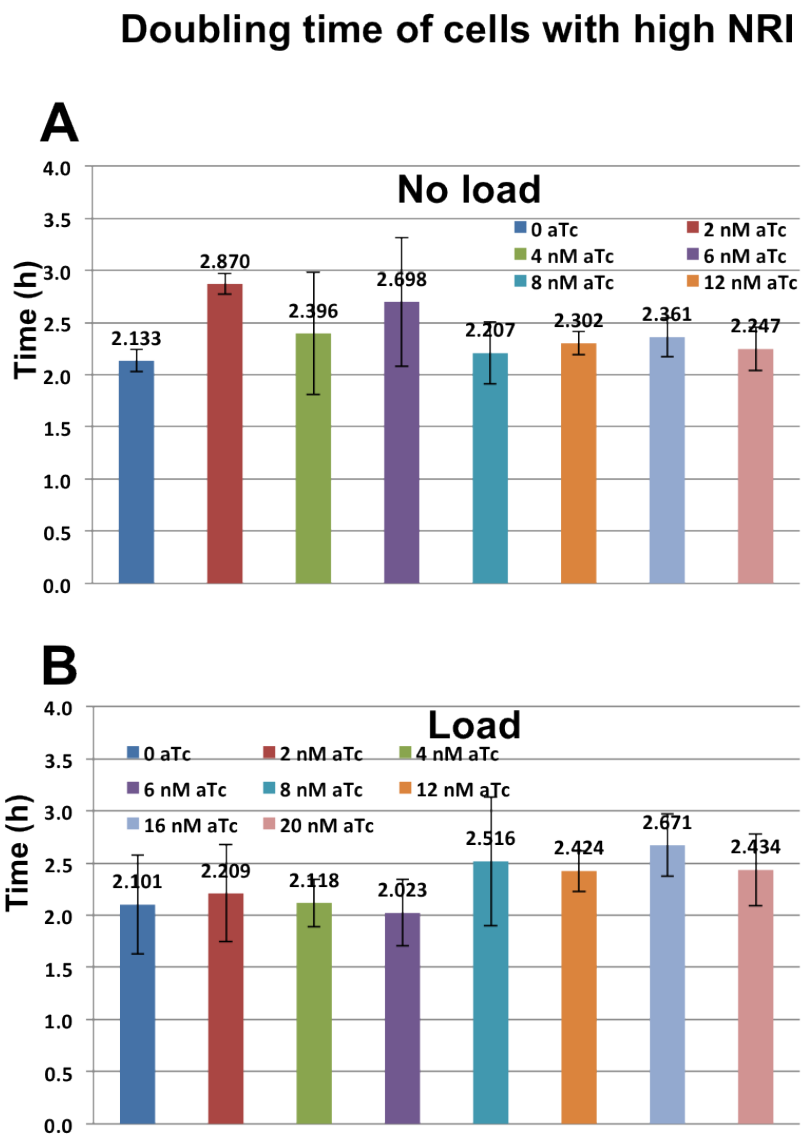


Fig. S15 Doubling time of cells with high NRI (H). The doubling time of the cells containing the gene circuit constitutively expressing high NRI and induced for varying kinase levels (2-20 nM aTc) during the assay. The bars indicate mean doubling time of the cells from three replicates and the error bars indicate standard deviation.

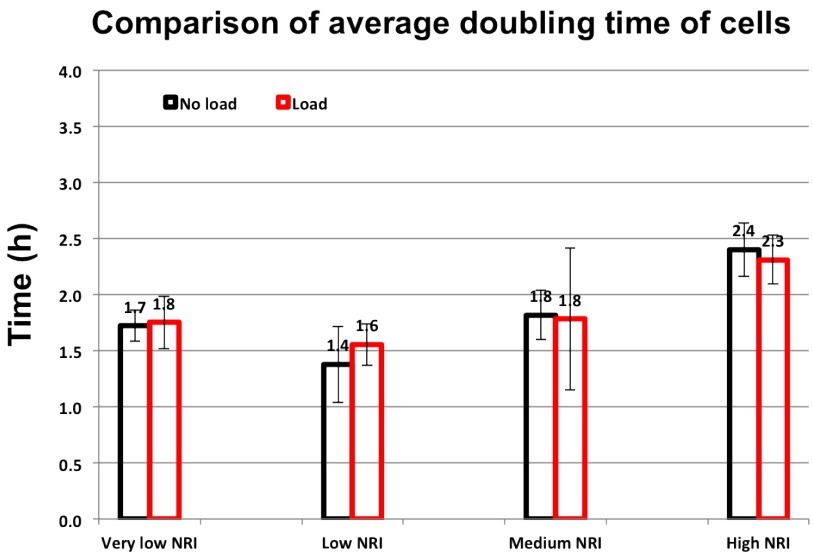


Fig. S16 Comparison of average doubling time of cells with varying NRI. The average doubling time of the cells containing the gene circuits with different amounts of NRI after induction for varying kinase levels (2-20 nM aTc) during the assay. The cells showed a marginal increase in doubling time with high NRI although the unloaded and DNA loaded cells of the respective gene circuits did not show much variation. The bars indicate mean doubling time of the cells induced with varying aTc levels for a constant amount of NRI and the error bars indicate standard deviation.

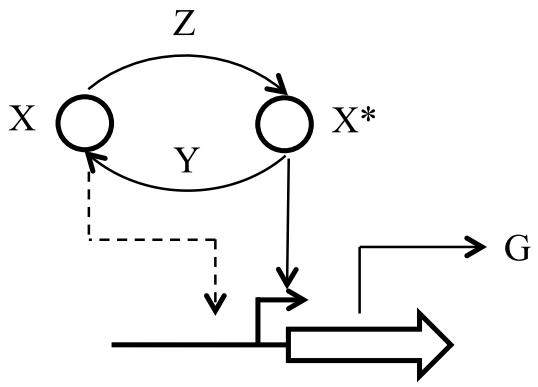


Fig. S17 Phosphorylation cycle diagram. Diagram of a phosphorylation cycle where the cycle protein regulates expression of protein G.

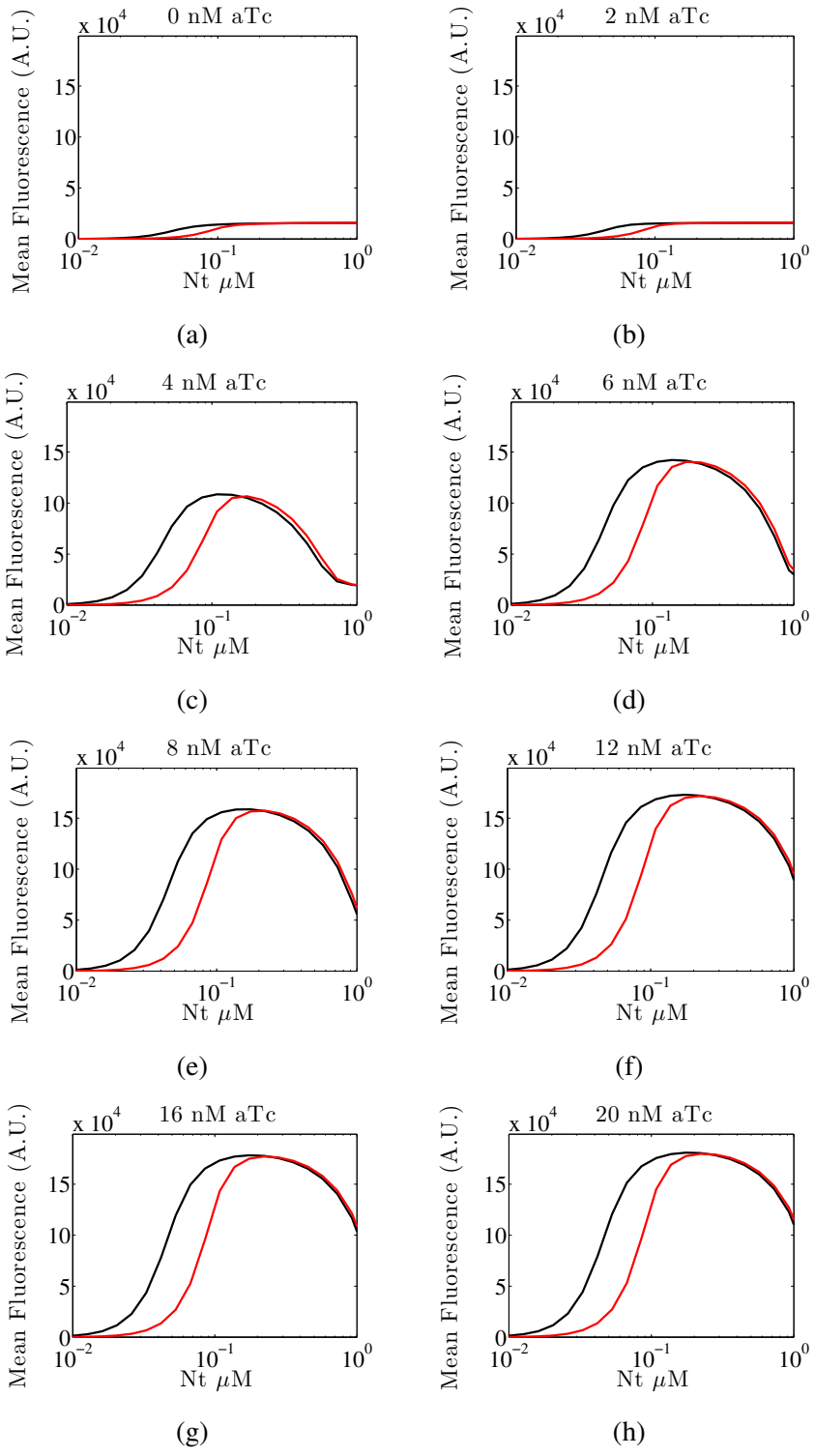
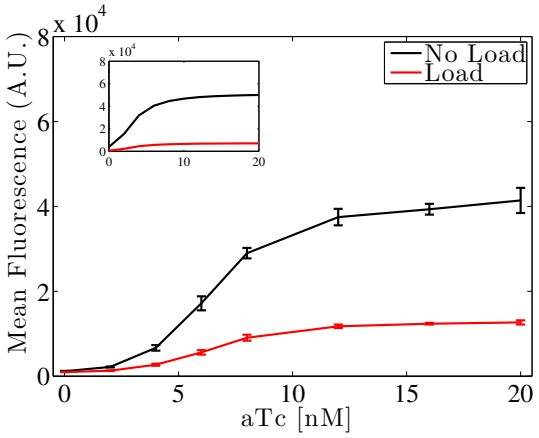
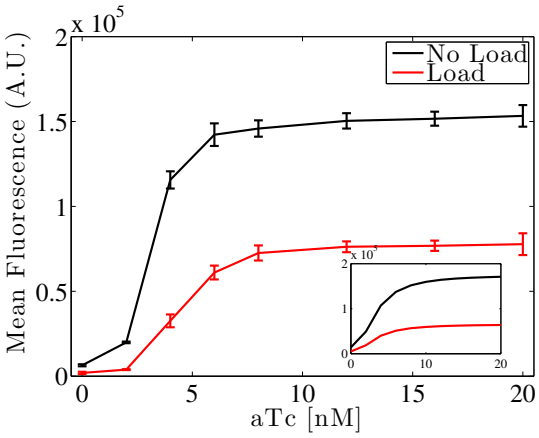


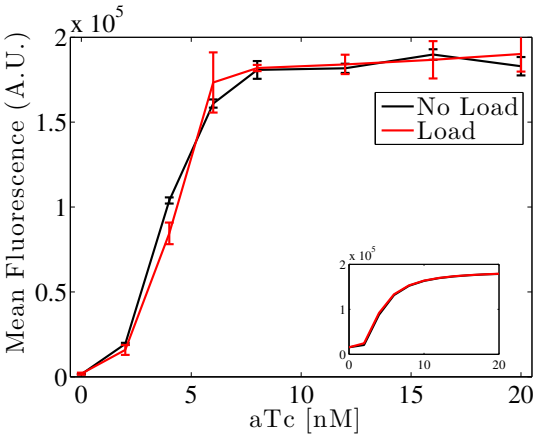
Fig. S18 Biphasic behavior of the system, simulation results. In all plots, black corresponds to the unloaded system (69)-(80) and red to the loaded system (53)-(68). For low amounts of aTc (aTc $\leq$ 2nM), the steady state results of both loaded and unloaded systems saturate to a low steady state, while for higher concentrations of aTc the system presents a biphasic behavior.



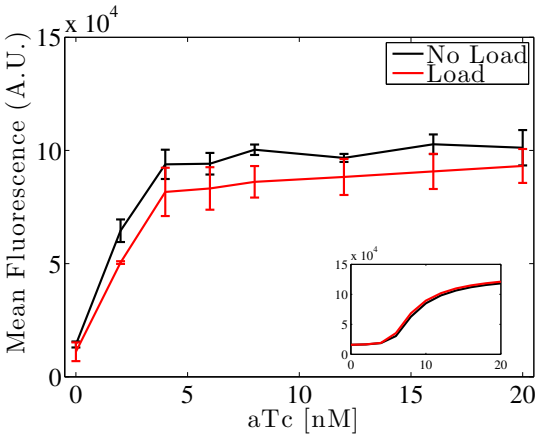
(a) Very low NRI concentration



(b) Low NRI conentration



(c) Medium NRI concentration



(d) High NRI concentration

Fig. S19 Steady state results for varying aTc. The simulation results of both loaded and unloaded systems for all concentrations of aTc are given by the insets in figures (a)-(d). In this and all simulations Very low NRI is given by $k_N/\delta = 0.0329\mu$M, Low NRI is given by $k_N/\delta = 0.0700\mu$M, Medium NRI is given by $k_N/\delta = 0.2807\mu$M, HIgh NRI is given by $k_N/\delta = 0.9237\mu$M. For medium and high values of NRI, the insulator is able to attenuate the effect of the load on the steady state of the system.

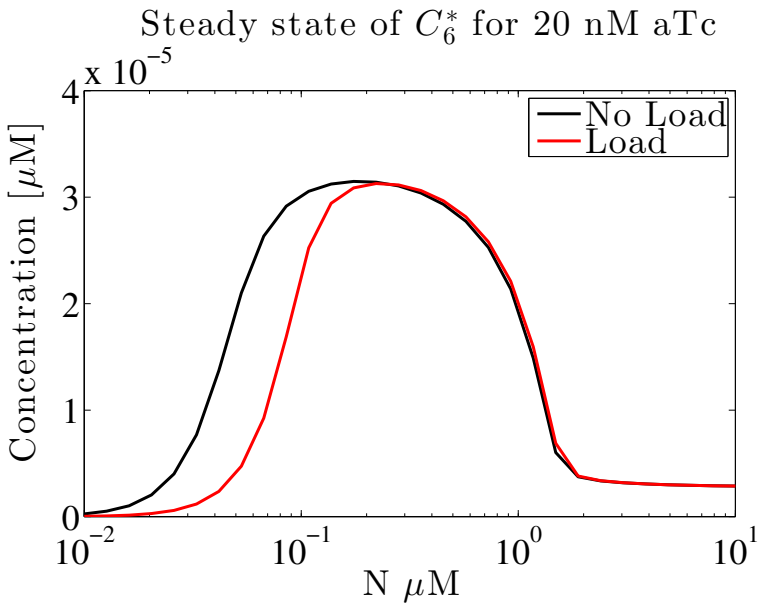
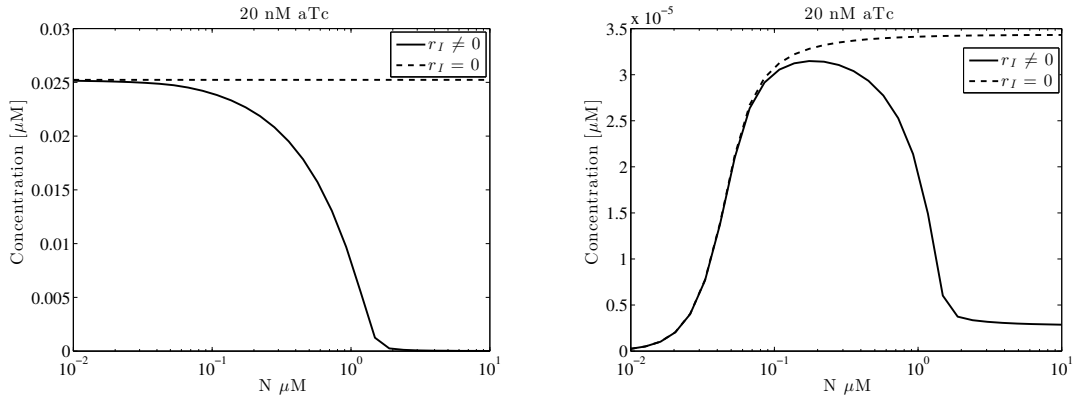


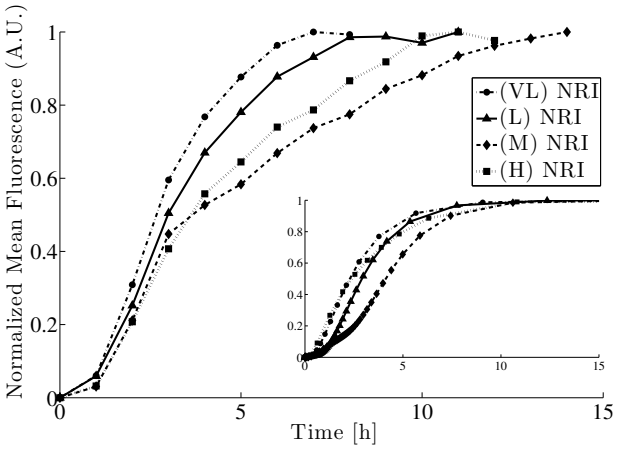
Fig. S20 Steady state of C_6^* for varying $N = k_N/\delta$ concentrations. The No Load plot corresponds to the steady state of C_6^* in system (69) - (80) where $p_{TL} = 0\mu$M and the Load plot corresponds to the steady state of C_6^* in system (53) - (68) where $p_{TL} = 0.1495\mu$M



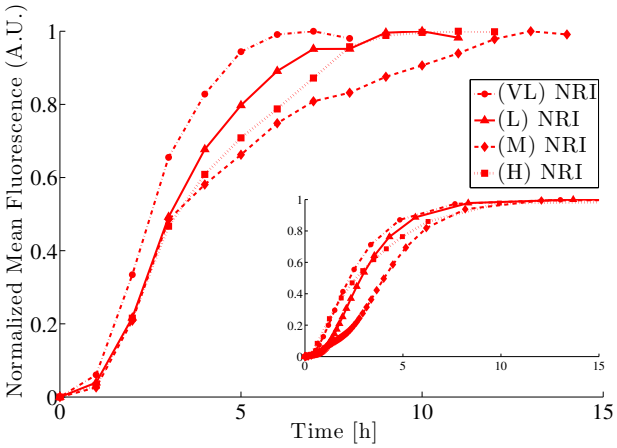
(a) K Steady State

(b) C_6^* Steady State

Fig. S21 (a) Steady state value of K for varying $N = k_N/\delta$ in system (69)-(80). (b) Steady state value of C_6^* for varying $N = k_N/\delta$ in system (69)-(80). (a)-(b) Solid lines correspond to the unloaded system (69)-(80), the dash lines correspond to the unloaded system (69)-(80) when additionally the terms over brace r_I in (69) are equated to zero.

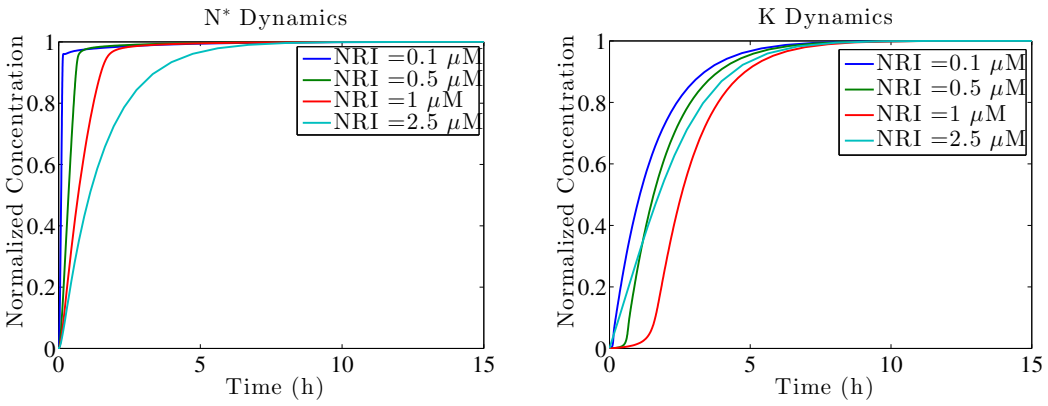


(a) Unloaded system



(b) Loaded System

Fig. S22 GFP dynamics at all values of NRI. (a)-(b): Data for the 20 nM aTc induction dynamic experiments and simulation insets. Data in black is for the unloaded system and data in red is for the loaded system. In both plots, there is a slow down in the dynamics for increasing amounts of NRI until the highest value. Insets are the simulation results for 20 nM aTc induction of the unloaded system (a) (69)-(80) and the loaded system (b) (53)-(68).



(a) (b)

Fig. S23 Simplified model simulations for NRI and NRII. (a)-(b) Normalized dynamics of N^* in (a) and K in(b) in the unloaded system (69)-(80) for a 20 nM aTc induction at various NRI levels.

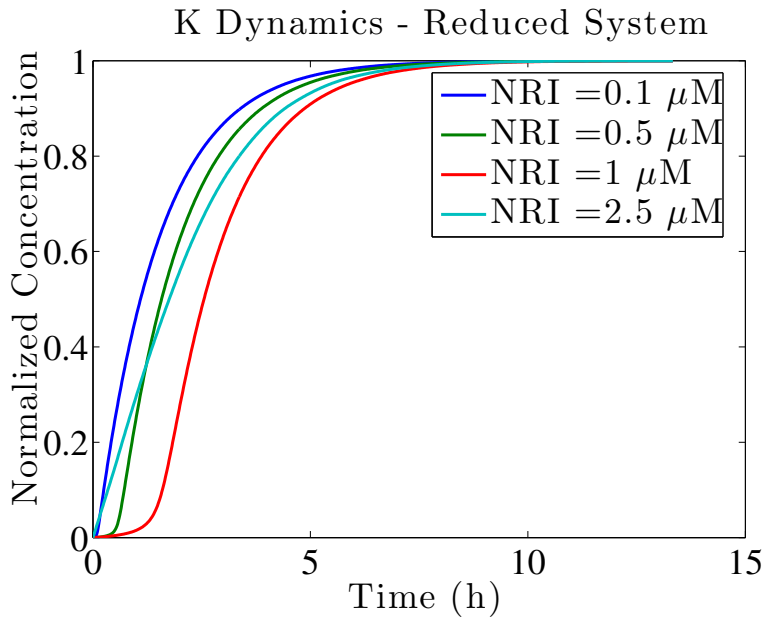


Fig. S24 Reduced order NRII dynamics. Dynamics of system (83) for various concentrations of N_T. We see that, as in the model before applying singular perturbation, the dynamics start slowing down with increasing amounts of N_T, until the final value in which it speeds up.

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