Supplementary Information

I. **Transcript Analysis for TEF-pBLUESCRIPT SK Series**

To determine whether decreased efficiency across the multicloning site was due to translation or transcription limitations, yECitrine transcript levels resulting from the series developed above were measured relative to Alg9, a known reference gene in yeast (1). The transcript levels of yECitrine were not found to significantly vary and thus do not correlate with measured fluorescence (**Supplementary Figure 1**). As a result, it was concluded that the observed restriction site-dependent performance was predominantly a translation-level effect. Therefore, transcriptional effects such as the presence/absence of transcription factor binding sites do not lead to the phenomenon seen in **Figure 1**.

II. **Initial Multicloning Site Design**

Our initial hypothesis was that expression inhibition was due to the folding energy and structure of the entire 5’UTR. Therefore, promoter-specific MCSs were redesigned with the aim of increasing the ensemble folding energy of the 5’UTR (See Materials and Methods). Both reordering of enzyme sites and insertion of base-pairs to remove secondary structure were allowed. The resulting MCSs, dubbed TEF_1 and $CYC1_1$ for use after the TEF and CYC promoters, respectively, are shown in **Supplementary Table 2**. yECitrine was inserted at each restriction site as for the pBLUESCRIPT SK MCS, and the results are shown in **Supplementary Figure 2**. Despite the crudeness of this original model for MCS performance, both TEF_1 and $CYC1_1$ showed improved desirable performance.

The redesigned MCS TEF_1, using the TEF promoter, is remarkable in that it maintains a narrow range of reporter expression between the 2nd and 9th restriction sites. In this region, the expression from $pTEF_1xYFP$ ranges between 0.69 and 0.42, whereas the expression from pBLUESCRIPT SK ranges between 1.03 and 0.26 in the same region. This property makes TEF_1 more appropriate for applications in which consistency in expression across varying sites within the MCS is desired.

The redesigned MCS $CYC1_1$, using the CYC1 promoter, yields yECitrine expression equal to or greater than the pBLUESCRIPT SK for all but one of the available restriction sites, making this multicloning site desirable. Furthermore, the 2nd, 5th, and 6th sites attain the same level of expression as 1st, allowing more cloning possibilities without decreasing effective promoter strength. It is interesting to note that increases in expression can be attained by adding nucleotides to the 5’UTR (exemplified by $pCYC1_15YFP$ and $pCYC1_16YFP$), illustrating that MCS inhibition is not simply due to length. This observation also required a different model for inhibition, as free energy of folding of the 5’UTR always decreases as more base pairs are added to the MCS. The assumption that the entire 5’UTR produces translation-inhibiting secondary structure was therefore incorrect, and so the model was reevaluated to create better multiple cloning sites for each of the three promoters.

III. **Supplemental Materials and Methods**

Plasmid Construction: *yECitrine Insert Series* **(Supplementary Table 1)**

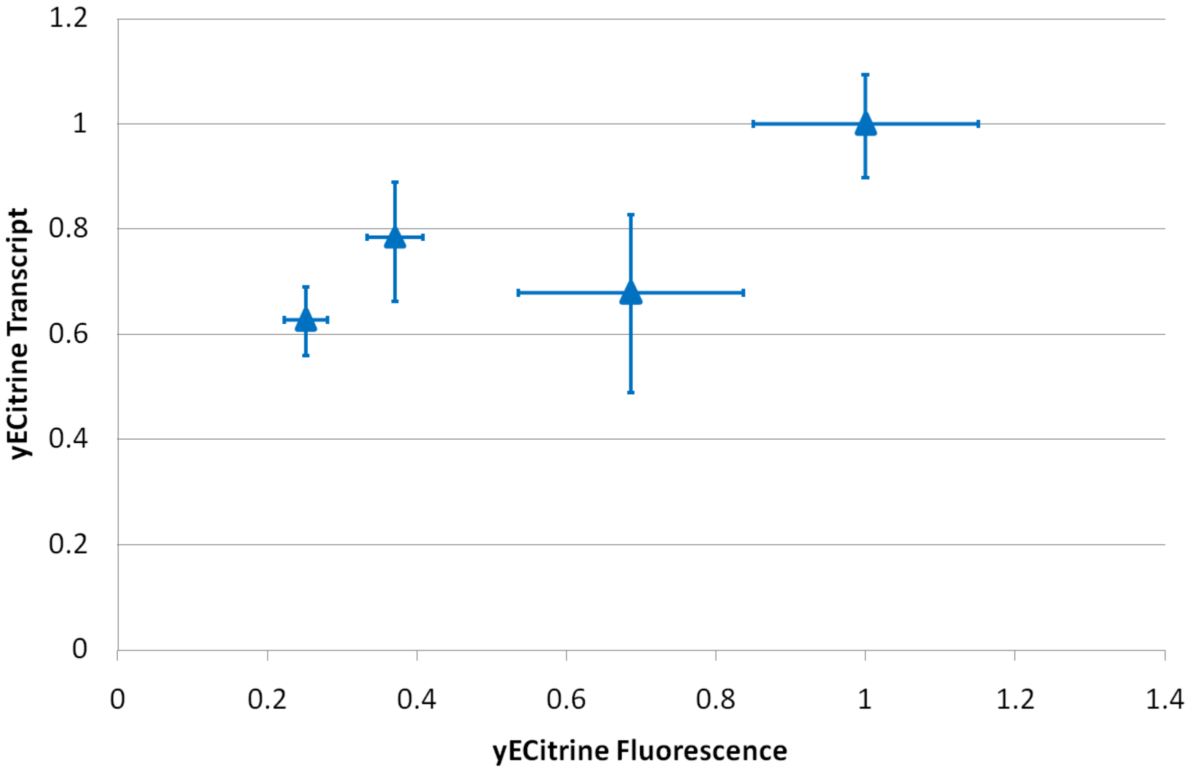
Oligos 5-15 (**Supplementary Table 3**) were annealed by combining 750 pmol of each complementary oligo in 1X T4 DNA Ligase Buffer (NEB) and incubating at 95 °C for 150 sec. The mixture was then steadily cooled from 75 °C to 25 °C over 24 min. The annealed product was cleaned with a MERmaid Spin Kit (Qbiogene), digested with XbaI, and ligated to the phosphatased XbaI fragment of a p416 (2) vector expressing yECitrine with either a mutant TEF promoter (TEFpmut5 (3)), GPD, or CYC1. Vector and insert digestions were performed for 3 hours at 37 °C and cleaned with a QIAquick PCR Purification Kit (Qiagen) and MERmaid Spin Kit, respectively. Ligations were performed at room temperature for 30 min, followed by heat inactivation. Plasmids from distinct *E. coli* colonies were isolated, sequenced, and transformed into yeast.

RT-PCR Assay

For each tested variant, the replicate yielding the most typical fluorescence measurement was grown to an optical density of 0.5 and its RNA was extracted (Ribopure Yeast Kit, Ambion).

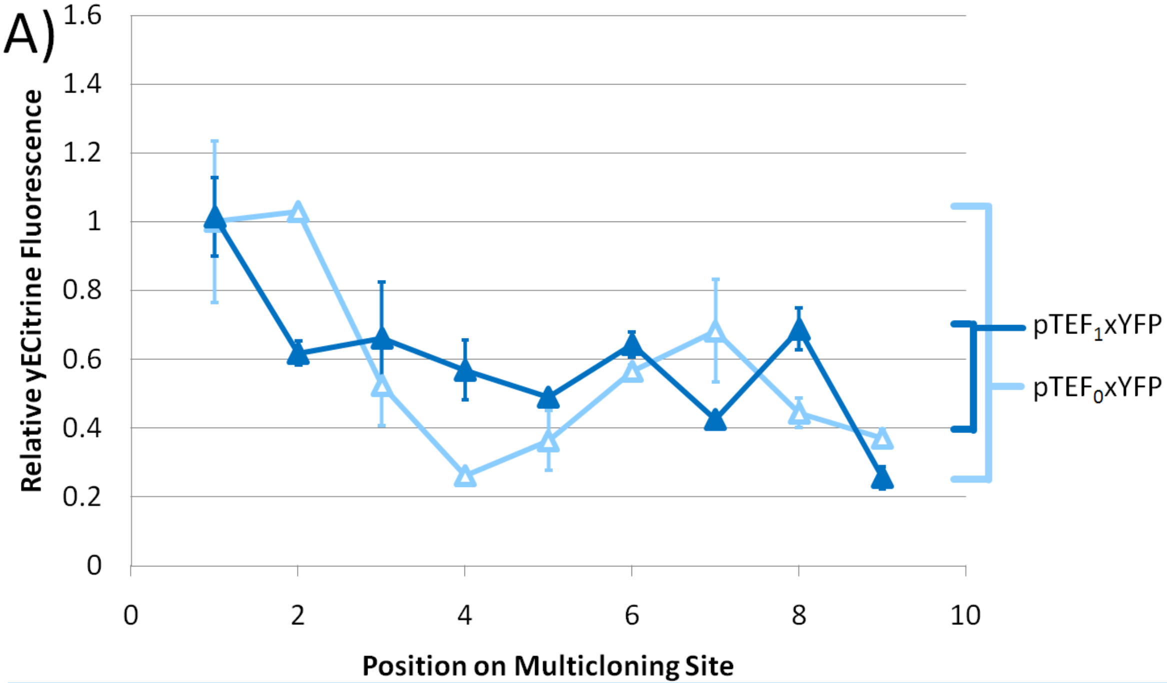
100 ng RNA was reverse-transcribed and quantified in triplicate using an iScript One-Step RT-PCR Kit with SYBR Green (Biorad) immediately after RNA extraction. yECitrine transcript levels were measured relative to that of ALG9 (Primers 1-4) on a 7900HT Real Time PCR Instrument (Applied Biosystems).

IV. **Supplementary Figure 1**



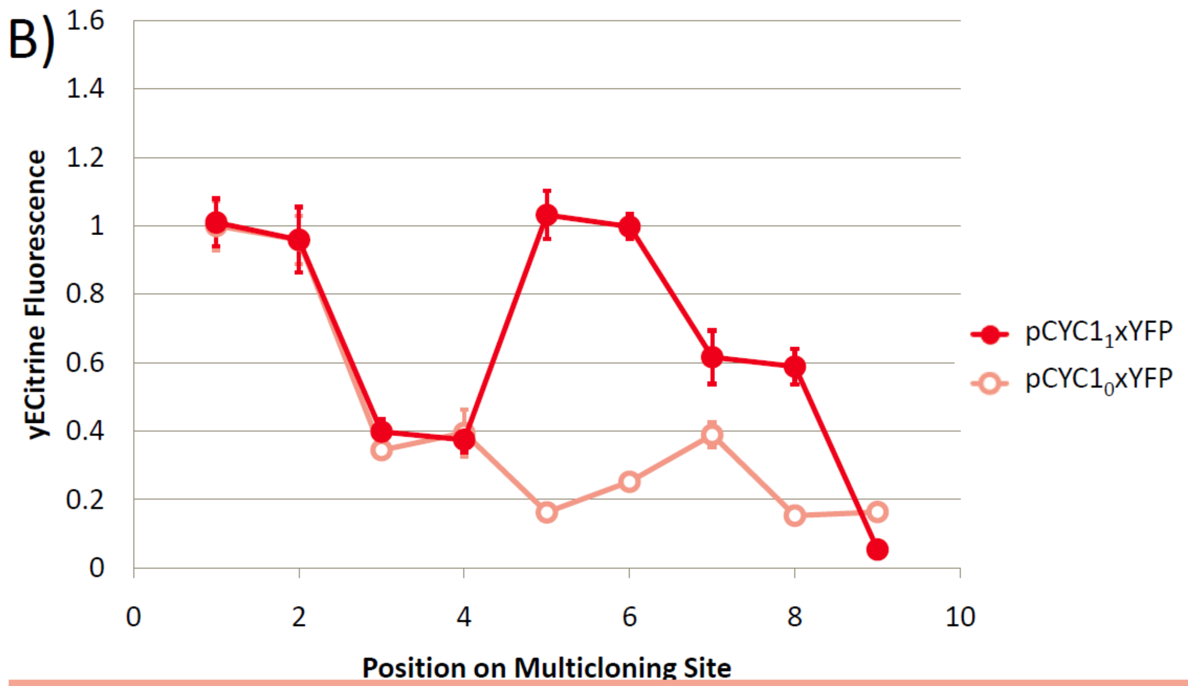
Supplementary Figure 1: *yECitrine Transcript Levels vs yECitrine Fluorescence in the p416-TEF multicloning site series*. yECitrine transcript levels were quantified in pTEF$_0$1YFP, pTEF$_0$4YFP, pTEF$_0$7YFP, and pTEF$_0$9YFP (**Supplementary Table 4**) and compared with fluorescence values obtained for that construct. Error bars in transcript level correspond to the standard deviation resulting from three measurements of the same RNA extract, and error bars in fluorescence level correspond to those shown in **Figure 1**. There is a non-correlation between transcript level and fluorescence level, thus suggesting translational inhibition.

V. Supplementary Figure 2



|TCTAGA|ACTAGT|GGATCC|CCCGGG|CTGCAG|GAATTC|GATATC|AAGCTT|ATCGAT|ACC|GTCGAC|CTCGAG|
XbaI **1** SpeI **2** BamHI **3** XmaI **4** PstI **5** EcoRI **6** EcoRV **7** HindIII **8** ClaI **9** SalI **10** XhoI **11**

|ATCGAT|AATAC|GGATCC|CC|ACTAGT|ACAAA|GAATTC|CCCCC|CTGCAG|CCC|CTCGAG|CCC|TCTAGA|AAAAA|
ClaI **1** BamHI **2** SpeI **3** EcoRI **4** PstI **5** XhoI **6** XbaI **7**
AAGCTT|ACAAC|GTCGAC|ACAAA|GATATC|AAAAA|CCCGGG|
HindIII **8** SalI **9** EcoRV **10** XmaI **11**

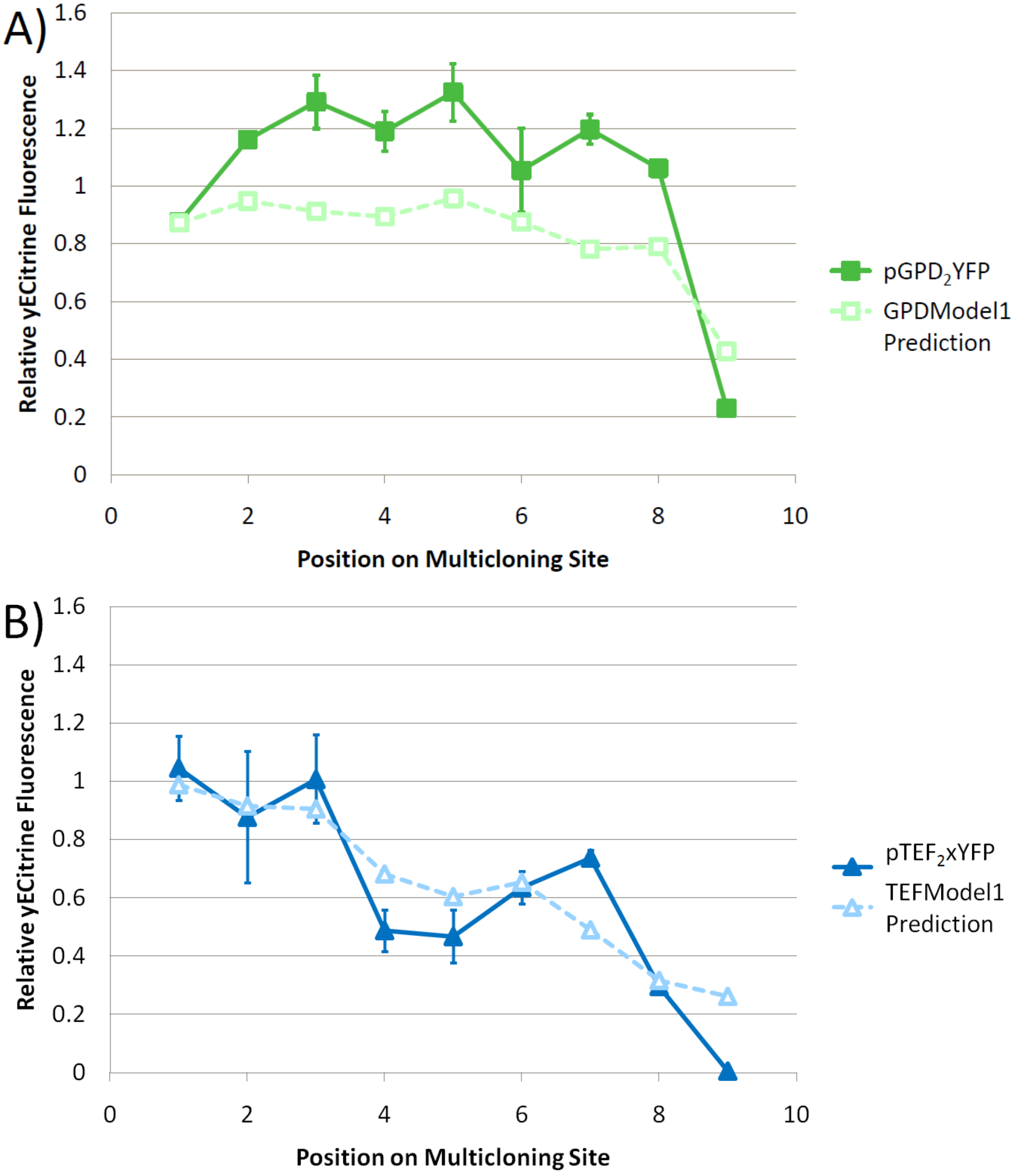


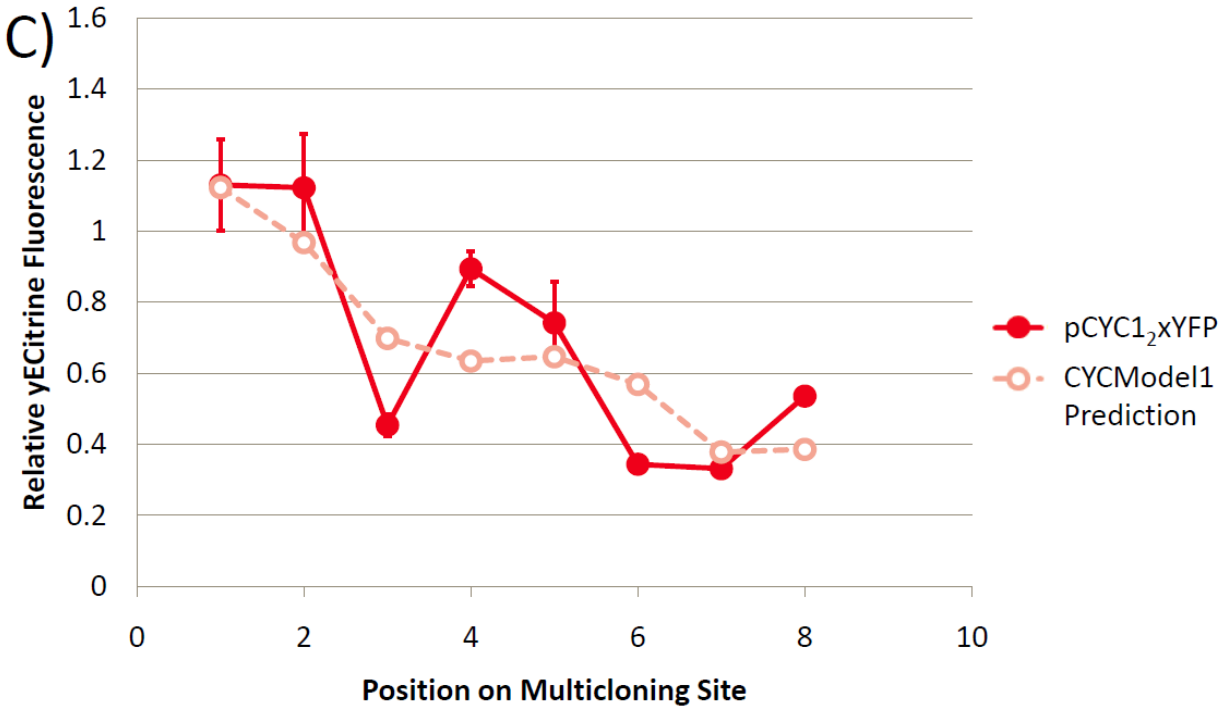
|TCTAGA|ACTAGT|GGATCC|CCCGGG|CTGCAG|GAATTC|GATATC|AAGCTT|ATCGAT|ACC|GTCGAC|CTCGAG|
Xbal **1** Spel **2** BamHI **3** Xmal **4** PstI **5** EcoRI **6** EcoRV **7** HindIII **8** ClaI **9** SalI **10** XhoI **11**

|CTCGAG|AAAAA|GTCGAC|AAC|GGATCC|AAAAA|AAGCTT|AAAAA|ACTAGT|ACAC|ATCGAT|AAAAA|GAATTC|
XhoI **1** SalI **2** BamHI **3** HindIII **4** SpeI **5** ClaI **6** EcoRI **7**
AA|CTGCAG|CC|TCTAGA|ACAAT|GATATC|AAAAA|CCCGGG|
PstI **8** XbaI **9** EcoRV **10** XmaI **11**

Supplementary Figure 2: *Performance of designed multicloning sites* (A) TEF$_1$ and (B) CYC1$_1$. Two MCSs were designed to minimize the ensemble free energy of the 5'UTR when placed after TEF or CYC1, respectively. Data in (A) has been scaled to the fluorescence of pTEF$_0$1YFP and in (B) to pCYC1$_1$1YFP. Position on the MCS has been measured according to the unique restriction sites in the p416 vector. Error bars represent the standard deviation in fluorescence observed across biological triplicates.

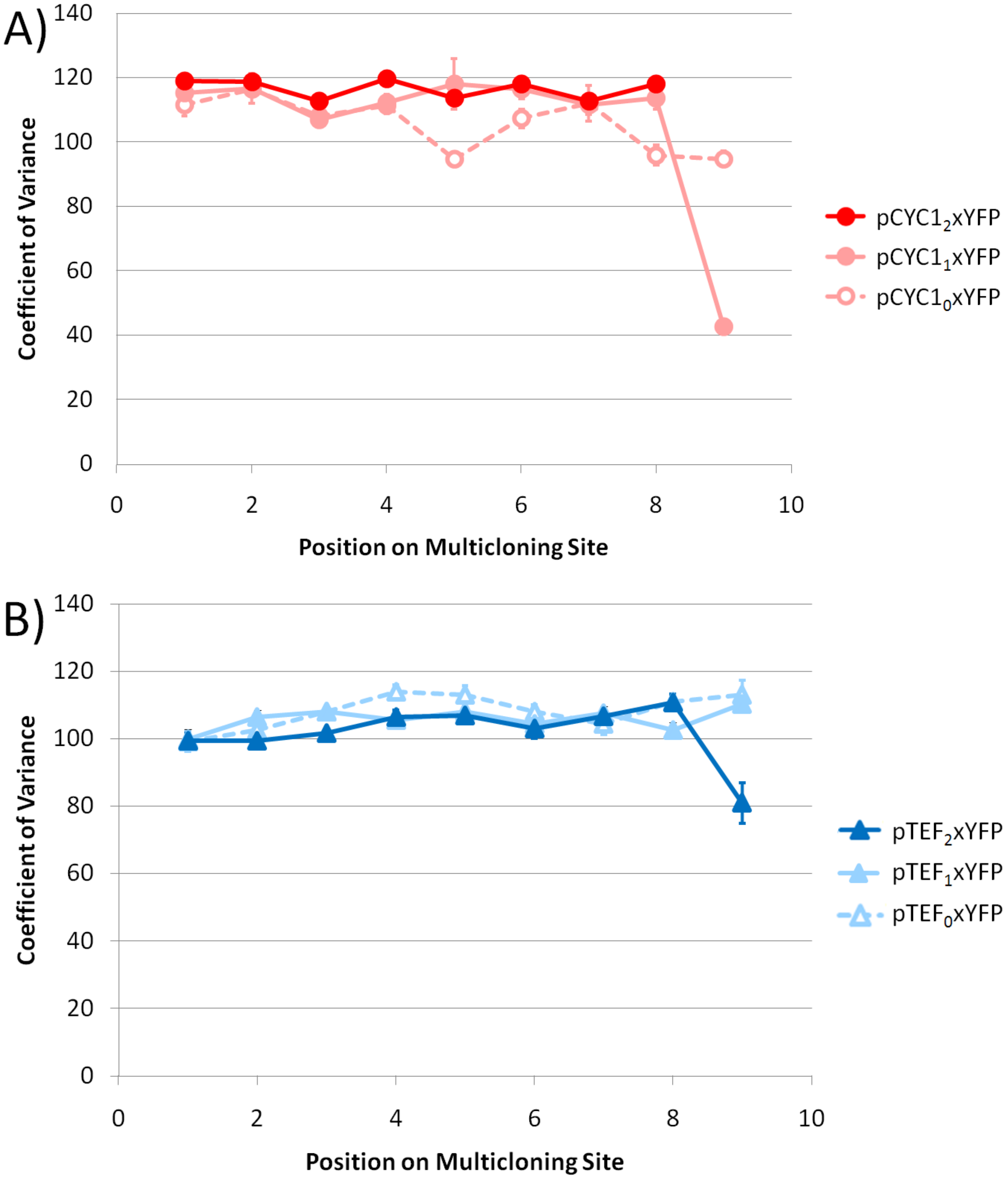
VI. **Supplementary Figure 3**

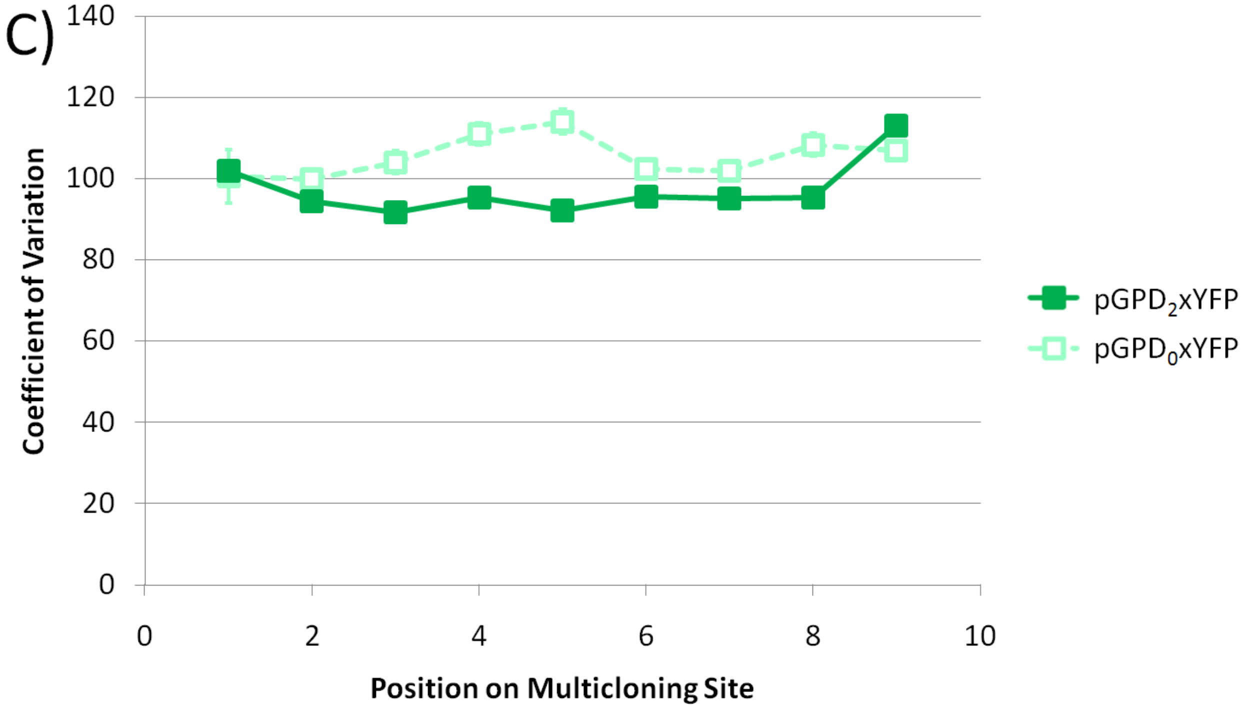




Supplementary Figure 3: *Predicted Performance of Designed Multicloning Sites* (A) GPD$_2$, (B) TEF$_2$, and (C) CYC1$_2$. Prospective MCSs were designed according to the procedures described in Materials and Methods. Observed values for the expression levels allowed by each designed multicloning site are plotted as in **Figure 5**. Position on the MCS has been measured according to the unique restriction sites in the p416 vector. Error bars represent the standard deviation in fluorescence observed across three biological replicates. Designed multicloning sites show good agreement with model predictions.

VII. **Supplementary Figure 4**





Supplementary Figure 4: *Effects of Designed MCSs on Expression Noise in* (A) *CYC1 MCSs,* (B) *TEF MCSs,* and (C) *GPD MCSs*. Expression noise is seen to be largely invariant with respect to restriction site and MCS. pCYC$1_1$9YFP and pTEF$_2$9YFP had fluorescence near the detection limits of our flow cytometer, leading to the decreased coefficients of variants seen in these constructs.

VIII. **Supplementary Table 1:** yECitrine Insert Series

pT5Y	TEFpmut5-TCTAGA-AAA-YFP
pT21(1) F	TEFpmut5-TCTAGA-GAATTC-TCTAGA-AAA-YFP
pT21(1) FF	TEFpmut5-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-AAA-YFP
pT21(1) FFFF	TEFpmut5-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-AAA-YFP
pT21(1) FFFFFF	TEFpmut5-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-GAATTC-TCTAGA-AAA-YFP
pT21(2) F	TEFpmut5-TCTAGA-GGTTGG-TCTAGA-AAA-YFP
pT21(2) RR	TEFpmut5-TCTAGA-CCAACC-TCTAGA-CCAACC-TCTAGA-AAA-YFP
pT21(2) RRR	TEFpmut5-TCTAGA-CCAACC-TCTAGA-CCAACC-TCTAGA-CCAACC-TCTAGA-AAA-YFP
pT21(3) F	TEFpmut5-TCTAGA-GGGCCC-TCTAGA-AAA-YFP
pT21(3) FF	TEFpmut5-TCTAGA-GGGCCC-TCTAGA-GGGCCC-TCTAGA-AAA-YFP
pT21(4) F	TEFpmut5-TCTAGA-AAATTT-TCTAGA-AAA-YFP
pT21(4) FF	TEFpmut5-TCTAGA-AAATTT-TCTAGA-AAATTT-TCTAGA-AAA-YFP
pT26 F	TEFpmut5-TCTAGA-GA-GAATTC-AGG-TCTAGA-AAA-YFP
pT26 RR	TEFpmut5-TCTAGA-CCT-GAATTC-TC-TCTAGA-CCT-GAATTC-TC-TCTAGA-AAA-YFP
pT36 F	TEFpmut5-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pT46 F	TEFpmut5-TCTAGA-AGGGACAAACTT-GAATTC-ATTTAGGCGTAGT-TCTAGA-AAA-YFP
pGY	GPD-TCTAGA-AAA-YFP
pG21(1) F	GPD-TCTAGA-GAATTC-TCTAGA-AAA-YFP
pG21(2) RR	GPD-TCTAGA-CCAACC-TCTAGA-CCAACC-TCTAGA-AAA-YFP
pG21(2) R	GPD-TCTAGA-CCAACC-TCTAGA-AAA-YFP
pG21(2) F	GPD-TCTAGA-GGTTGG-TCTAGA-AAA-YFP
pG21(3)	GPD-TCTAGA-GGGCCC-TCTAGA-AAA-YFP
pG21(4)	GPD-TCTAGA-AAATTT-TCTAGA-AAA-YFP
pG26 FRRR	GPD-TCTAGA-GA-GAATTC-AGG-TCTAGA-CCT-GAATTC-TC-TCTAGA-CCT-GAATTC-TC-TCTAGA-CCT-GAATTC-TC-TCTAGA-AAA-YFP
pG26 FRFF	GPD-TCTAGA-GA-GAATTC-AGG-TCTAGA-CCT-GAATTC-TC-TCTAGA-GA-GAATTC-AGG-TCTAGA-GA-GAATTC-AGG-TCTAGA-AAA-YFP
pG26 FFFR	GPD-TCTAGA-GA-GAATTC-AGG-TCTAGA-GA-GAATTC-AGG-TCTAGA-GA-GAATTC-AGG-TCTAGA-CCT-GAATTC-TC-TCTAGA-AAA-YFP
pG26 R	GPD-TCTAGA-CCT-GAATTC-TC-TCTAGA-AAA-YFP
pG36 FF	GPD-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pG36 RRF -1	GPD-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AGTACC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pG36 RFF	GPD-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP

pG46 RR	GPD-TCTAGA-ACTACGCCTAAAT-GAATTC-AAGTTTGTCCCT-TCTAGA-ACTACGCCTAAAT-GAATTC-AAGTTTGTCCCT-TCTAGA-AAA-YFP
pG46 R	GPD-TCTAGA-ACTACGCCTAAAT-GAATTC-AAGTTTGTCCCT-TCTAGA-AAA-YFP
pG46 FF -1	GPD-TCTAGA-AGGACAAACTT-GAATTC-ATTTAGGCGTAGT-TCTAGA-AGGGACAAACTT-GAATTC-ATTTAGGCGTAGT-TCTAGA-AAA-YFP
pG46 F	GPD-TCTAGA-AGGGACAAACTT-GAATTC-ATTTAGGCGTAGT-TCTAGA-AAA-YFP
pCY	CYC1-TCTAGA-AAA-YFP
pC21(1) F	CYC1-TCTAGA-GAATTC-TCTAGA-AAA-YFP
pC21(2) R	CYC1-TCTAGA-CCAACC-TCTAGA-AAA-YFP
pC21(2) RF	CYC1-TCTAGA-CCAACC-TCTAGA-GGTTGG-TCTAGA-AAA-YFP
pC21(2) RFF	CYC1-TCTAGA-CCAACC-TCTAGA-GGTTGG-TCTAGA-GGTTGG-TCTAGA-AAA-YFP
pC21(2) FFF	CYC1-TCTAGA-GGTTGG-TCTAGA-GGTTGG-TCTAGA-GGTTGG-TCTAGA-AAA-YFP
pC21(3) F	CYC1-TCTAGA-GGGCCC-TCTAGA-AAA-YFP
pC21(3) FF	CYC1-TCTAGA-GGGCCC-TCTAGA-GGGCCC-TCTAGA-GGGCCC-TCTAGA-AAA-YFP
pC21(4) F	CYC1-TCTAGA-AAATTT-TCTAGA-AAA-YFP
pC26 R	CYC1-TCTAGA-CCT-GAATTC-TC-TCTAGA-AAA-YFP
pC26 RFRF	CYC1-TCTAGA-CCT-GAATTC-TC-TCTAGA-GA-GAATTC-AGG-TCTAGA-CCT-GAATTC-TC-TCTAGA-GA-GAATTC-AGG-TCTAGA-AAA-YFP
pC26 RFRR	CYC1-TCTAGA-CCT-GAATTC-TC-TCTAGA-GA-GAATTC-AGG-TCTAGA-CCT-GAATTC-TC-TCTAGA-CCT-GAATTC-TC-TCTAGA-AAA-YFP
pC36 F	CYC1-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pC36 RF	CYC1-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pC36 RRF	CYC1-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pC36 RRRF	CYC1-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-AAA-YFP
pC46 F	CYC1-TCTAGA-AGGGACAAACTT-GAATTC-ATTTAGGCGTAGT-TCTAGA-AAA-YFP
pC46 RR	CYC1-TCTAGA-ACTACGCCTAAAT-GAATTC-AAGTTTGTCCCT-TCTAGA-ACTACGCCTAAAT-GAATTC-AAGTTTGTCCCT-TCTAGA-AAA-YFP

IX. **Supplementary Table 2:** pTEF$_1$xYFP, pTEF$_2$xYFP, pGPD$_2$xYFP, pCYC1$_1$xYFP, and pCYC1$_2$xYFP.

pTEF$_1$1YFP	TEF-ATCGAT-YFP
pTEF$_1$2YFP	TEF-ATCGAT-AATAC-GGATCC-YFP
pTEF$_1$3YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-YFP
pTEF$_1$4YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-ACAAA-GAATTC-YFP
pTEF$_1$5YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-ACAAA-GAATTC-CCCCC-CTGCAG-CCC-CTCGAG-YFP
pTEF$_1$6YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-ACAAA-GAATTC-CCCCC-CTGCAG-CCC-CTCGAG-CCC-TCTAGA-YFP
pTEF$_1$7YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-ACAAA-GAATTC-CCCCC-CTGCAG-CCC-CTCGAG-CCC-TCTAGA-AAAAA-AAGCTT-YFP
pTEF$_1$8YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-ACAAA-GAATTC-CCCCC-CTGCAG-CCC-CTCGAG-CCC-TCTAGA-AAAAA-AAGCTT-ACAAC-GTCGAC-YFP
pTEF$_1$9YFP	TEF-ATCGAT-AATAC-GGATCC-CC-ACTAGT-ACAAA-GAATTC-CCCCC-CTGCAG-CCC-CTCGAG-CCC-TCTAGA-AAAAA-AAGCTT-ACAAC-GTCGAC-ACAAA-GATATC-AAAAA-CCCGGG-YFP
pTEF$_2$1YFP	TEF-ACCCC-ATCGAT-YFP
pTEF$_2$2YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-YFP
pTEF$_2$3YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-YFP
pTEF$_2$4YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-CCCCC-GAATTC-YFP
pTEF$_2$5YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-CCCCC-GAATTC-CCCCC-AAGCTT-YFP
pTEF$_2$6YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-CCCCC-GAATTC-CCCCC-AAGCTT-TAAA-GTCGAC-YFP
pTEF$_2$7YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-CCCCC-GAATTC-CCCCC-AAGCTT-TAAA-GTCGAC-CCCCC-CTCGAG-YFP
pTEF$_2$8YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-CCCCC-GAATTC-CCCCC-AAGCTT-TAAA-GTCGAC-CCCCC-CTCGAG-ACCCC-GGATCC-YFP
pTEF$_2$9YFP	TEF-ACCCC-ATCGAT-CACCC-ACTAGT-TCTAGA-CCCCC-GAATTC-CCCCC-AAGCTT-TAAA-GTCGAC-CCCCC-CTCGAG-ACCCC-GGATCC-CCCGGG-YFP
pGPD$_2$1YFP	GPD-A-AAGCTT-YFP
pGPD$_2$2YFP	GPD-A-AAGCTT-ACTAGT-YFP
pGPD$_2$3YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-YFP
pGPD$_2$4YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-YFP
pGPD$_2$5YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-TCTAGA-YFP
pGPD$_2$6YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-TCTAGA-GGATCC-YFP
pGPD$_2$7YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-TCTAGA-GGATCC-CTGCAG-CTCGAG-YFP
pGPD$_2$8YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-TCTAGA-GGATCC-CTGCAG-CTCGAG-GTCGAC-YFP
pGPD$_2$9YFP	GPD-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-TCTAGA-GGATCC-CTGCAG-CTCGAG-GTCGAC-CCCGGG-YFP

pCYC1$_{1}$1YFP	CYC1-CTCGAG-YFP
pCYC1$_{1}$2YFP	CYC1-CTCGAG-AAAAA-GTCGAC-YFP
pCYC1$_{1}$3YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-YFP
pCYC1$_{1}$4YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-AAAAA-AAGCTT-YFP
pCYC1$_{1}$5YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-AAAAA-AAGCTT-AAAAA-ACTAGT-YFP
pCYC1$_{1}$6YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-AAAAA-AAGCTT-AAAAA-ACTAGT-ACAC-ATCGAT-YFP
pCYC1$_{1}$7YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-AAAAA-AAGCTT-AAAAA-ACTAGT-ACAC-ATCGAT-AAAAA-GAATTC-YFP
pCYC1$_{1}$8YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-AAAAA-AAGCTT-AAAAA-ACTAGT-ACAC-ATCGAT-AAAAA-GAATTC-CCAA-CTGCAG-CC-TCTAGA-YFP
pCYC1$_{1}$9YFP	CYC1-CTCGAG-AAAAA-GTCGAC-AAC-GGATCC-AAAAA-AAGCTT-AAAAA-ACTAGT-ACAC-ATCGAT-AAAAA-GAATTC-CCAA-CTGCAG-CC-TCTAGA-ACAAT-GATATC-AAAAA-CCCGGG-YFP
pCYC1$_{2}$1YFP	CYC1-AACCC-GGATCC-YFP
pCYC1$_{2}$2YFP	CYC1-AACCC-GGATCC-AAGCTT-YFP
pCYC1$_{2}$3YFP	CYC1-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-YFP
pCYC1$_{2}$4YFP	CYC1-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-AACCA-ACTAGT-YFP
pCYC1$_{2}$5YFP	CYC1-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-AACCA-ACTAGT-ACCCA-ATCGAT-YFP
pCYC1$_{2}$6YFP	CYC1-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-AACCA-ACTAGT-ACCCA-ATCGAT-CCTAA-GAATTC-YFP
pCYC1$_{2}$7YFP	CYC1-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-AACCA-ACTAGT-ACCCA-ATCGAT-CCTAA-GAATTC-AAAA-CCCGGG-YFP
pCYC1$_{2}$8YFP	CYC1-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-AACCA-ACTAGT-ACCCA-ATCGAT-CCTAA-GAATTC-AAAA-CCCGGG-AGAAG-CTGCAG-TAAAA-GTCGAC-YFP

X. **Supplementary Table 3:** Oligos (IDT)

1	RT YFP Fwd	TTCTGTCTCCGGTGAAGGTGAA
2	RT YFP Rev	TAAGGTTGGCCATGGAACTGGCAA
3	RT ALG9 Fwd	ATCGTGAAATTGCAGGCAGCTTGG
4	RT ALG9 Rev	CATGGCAACGGCAGAAGGCAATAA
5	21(1)	GC-TCTAGA-GAATTC-TCTAGA-GC
6	21(2)F	GC-TCTAGA-GGTTGG-TCTAGA-GC
7	21(2)R	GC-TCTAGA-CCAACC-TCTAGA-GC
8	21(3)	GC-TCTAGA-GGGCCC-TCTAGA-GC
9	21(4)	GC-TCTAGA-AAATTT-TCTAGA-GC
10	26F	GC-TCTAGA-GA-GAATTC-AGG-TCTAGA-GC
11	26R	GC-TCTAGA-CCT-GAATTC-TC-TCTAGA-GC
12	36F	GC-TCTAGA-AGTAGCC-GAATTC-TGTCAGTT-TCTAGA-GC
13	36R	GC-TCTAGA-AACTGACA-GAATTC-GGCTACT-TCTAGA-GC
14	46F	GC-TCTAGA-AGGGACAAACTT-GAATTC-ATTTAGGCGTAGT-TCTAGA-GC
15	46R	GC-TCTAGA-ACTACGCCTAAAT-GAATTC-AAGTTTGTCCCT-TCTAGA-GC
16	YFPXbaIF	GC-TCTAGA-ATGTCTAAAGGTGAAGAATTATTCACTGG
17	YFPSpeIF	G-ACTAGT-ATGTCTAAAGGTGAAGAATTATTCACTGG
18	YFPBamHIF	CG-GGATCC-ATGTCTAAAGGTGAAGAATTATTCACTGG
19	YFPXmaIF	TCC-CCCGGG-ATGTCTAAAGGTGAAGAATTATTCACTGG
20	YFPEcoRIF	G-GAATTC-ATGTCTAAAGGTGAAGAATTATTCACTGG
21	YFPClaIF	CC-ATCGAT-ATGTCTAAAGGTGAAGAATTATTCACTGG
22	YFPSalIF	TAACGC-GTCGAC-ATGTCTAAAGGTGAAGAATTATTCACTGG
23	YFPXhoIF	CCCCG-CTCGAG-ATGTCTAAAGGTGAAGAATTATTCACTGG
24	YFPHindIIIF	CCCCCC-AAGCTT-ATGTCTAAAGGTGAAGAATTATTCACTGG
25	YFPXhoIR	CCCCG-CTCGAG-TTATTTGTACAATTCATCCATACCATGGG
26	YFPSalIR	TAACGC-GTCGAC-TTATTTGTACAATTCATCCATACCATGGG
27	YFP fwd	ATGTCTAAAGGTGAAGAATTATTCACTGGTG
28	6&8constfwd	TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT
29	6constrev	AGTGAATAATTCTTCACCTTTAGACAT-AAGCTT-GATATC-GAATTC-CTGC
30	8constrev	GAATAATTCTTCACCTTTAGACAT-GTCGAC-GGT-ATCGAT-AAGCTT-GATATC-GAATTC-CTG
31	TEFpF	CCCCCC-GAGCTC-ATAGCTTCAAAATGTTTCTAC
32	TEFpR	AAACTTAGATTAGATTCGTATGCTTTCTTTC
33	GPDpF	CCCCCC-GAGCTC-AGTTTATCATTATCAATACTCGCCA
34	GPDpR	ATCCGTCGAAACTAAGTTCTGG
35	CYC1pF	CCCCCC-GAGCTC-ATTTGGCGAGCG
36	CYC1pR	TTAGTGTGTGTATTTGTGTTTGCG
37	CYCtermF	ATTAGTTATGTCACGCTTACATTCACG
38	CYCtermR	GG-GGTACC-GGCCGCAAAT
39	TEFdesMCS1-1	TTGT-ACTAGT-GG-GGATCC-GTATT-ATCGAT-

		AAACTTAGATTAGATTCGTATGCTTTCTTTC
40	TEFdesMCS1-2	C-GGATCC-CC-ACTAGT-ACAAA-GAATTC-CCCCC-CTGCAG-CCC-CTCGAG-CCC-TCTAGA-AAAAA
41	TEFdesMCS1-3	CCCGGG-TTTTT-GATATC-TTTGT-GTCGAC-GTTGT-AAGCTT-TTTTT-TCTAGA-GGG-CTCGAG-G
42	TEFdesMCS1-4	ACAAA-GATATC-AAAAA-CCCGGG-TCATGTAATTAGTTATGTCACGCTTACATTCA
43	TEFdesMCS2-1	GAAAGCATAGCAATCTAATCTAAGTTT-ATCGAT-GTCGAC-GGATCC-ACTAGT-GAATTC-TCT
44	TEFdesMCS2-2	CTGCAG-GATATC-CTCGAG-CCCGGG-AAGCTT-TCTAGA-GAATTC-ACTAGT-GGATCC-G
45	TEFdesMCS2-3	TGAATGTAAGCGTGACATAACTAAT-CTGCAG-GATATC-CTCGAG-C
46	GPDdesMCS2-1	CCAGAACTTAGTTTCGACGGAT-A-AAGCTT-ACTAGT-ATCGAT-AAA-GAATTC-TCTAGA-GGAT
47	GPDdesMCS2-2	GATATC-CCCGGG-GTCGAC-CTCGAG-CTGCAG-GGATCC-TCTAGA-GAATTC-TTT-ATCGAT-ACT
48	GPDdesMCS2-3	CGAC-CCCGGG-GATATC-ATTAGTTATGTCACGCTTACATTCA
49	CYCdesMCS1-1	AAGCTT-TTTTT-GGATCC-GTT-GTCGAC-TTTTT-CTCGAG-TTAGTGTGTGTATTTGTGTTTGC
50	CYCdesMCS1-2	CAAC-GGATCC-AAAAA-AAGCTT-AAAAA-ACTAGT-ACAC-ATCGAT-AAAAA-GAATTC-CCAA-CTG
51	CYCdesMCS1-3	CCCGGG-TTTTT-GATATC-ATTGT-TCTAGA-GG-CTGCAG-TTGG-GAATTC-TTTTT-ATCGAT-G
52	CYCdesMCS1-4	TGAATGTAAGCGTGACATAACTAAT-CCCGGG-TTTTT-GATATC-ATTGT
53	CYCdesMCS2-1	GCAAACACAAATACACACACTAA-AACCC-GGATCC-AAGCTT-AAAAC-TCTAGA-AACCA-ACTA
54	CYCdesMCS2-2	T-CCCGGG-TTTT-GAATTC-TTAGG-ATCGAT-TGGGT-ACTAGT-TGGTT-TCTAGA-GTTTT-AAGCT
55	CYCdesMCS2-3	CTAA-GAATTC-AAAA-CCCGGG-AGAAG-CTGCAG-TAAAA-GTCGAC-TAAAC-CTCGAG-GATATC
56	CYCdesMCS2-4	TGAATGTAAGCGTGACATAACTAAT-GATATC-CTCGAG-GTTTA-GTCGA
57	YFPXmaIR	TCC-CCCGGG-TTATTTGTACAATTCATCCATACCATGGG
58	CD1-1R	AGTGAATAATTCTTCACCTTTAGACAT-CTCGAG-TTAGTGTGTGTATTTGTGTTTGC
59	LacZExtF	GC-TCTAGAAAA-ATGACCATGATTACGGATTCACTGG
60	LacZExtR	ACGCGTCGACGGTATCGAT-TTATTTTTGACACCAGACCAACTGG
61	LacZXbaIF	GC-TCTAGA-ATGACCATGATTACGGATTCACTGG
62	LacZXhoIR	CCCCG-CTCGAG-TTATTTTTGACACCAGACCAACTGGT
63	LacZF	ATGACCATGATTACGGATTCACTG
64	Mummcsfwd	GC-TCTAGA-ACTAGT-GGATCC-CCC
65	TL3F	TCTAGA-ACTAGT-GGATCC-ATGACCATGATTACGGATTCAC
66	TL57F	TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC
67	TL5R	GTGAATCCGTAATCATGGTCAT-GAATTC-CTGCAG-CCCG
68	TL7R	GTGAATCCGTAATCATGGTCAT-ATCGAT-AAGCTT-GATATC-GAATTC-CTGCAG-CCCG
69	TL9R	CTCGAG-GTCGAC-GGT-ATCGAT-AAGCTT-GATATC-GAATTC-CTGCAG-CCCG

70	TL9F	GATACC-GTCGAC-CTCGAG-ATGACCATGATTACGGATTCAC
71	GFPXbaIF	GC-TCTAGA-ATGCGTAAAGGAGAAGAACTTTT
72	GFPBamHIF	CG-GGATCC-ATGCGTAAAGGAGAAGAACTTTT
73	GFPEcoRIF	G-GAATTC-ATGCGTAAAGGAGAAGAACTTTT
74	GFPClaIF	CC-ATCGAT-ATGCGTAAAGGAGAAGAACTTTT
75	GFPXhoIF	CCCCG-CTCGAG-ATGCGTAAAGGAGAAGAACTTTT
76	GFPXhoIR	CCCCG-CTCGAG-TTAAACTGCTGCAGCGTAG

XI. **Supplementary Table 4:** pTEF$_0$xYFP, pGPD$_0$xYFP and pCYC1$_0$xYFP.

pTEF$_0$1YFP	TEF-TCTAGA-YFP
pTEF$_0$2YFP	TEF-TCTAGA-ACTAGT-YFP
pTEF$_0$3YFP	TEF-TCTAGA-ACTAGT-GGATCC-YFP
pTEF$_0$4YFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-YFP
pTEF$_0$5YFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-YFP
pTEF$_0$6YFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-YFP
pTEF$_0$7YFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-YFP
pTEF$_0$8YFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-YFP
pTEF$_0$9YFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-CTCGAG-YFP
pGPD$_0$1YFP	GPD-TCTAGA-YFP
pGPD$_0$2YFP	GPD-TCTAGA-ACTAGT-YFP
pGPD$_0$3YFP	GPD-TCTAGA-ACTAGT-GGATCC-YFP
pGPD$_0$4YFP	GPD-TCTAGA-ACTAGT-GGATCC-CCCGGG-YFP
pGPD$_0$5YFP	GPD-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-YFP
pGPD$_0$6YFP	GPD-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-YFP
pGPD$_0$7YFP	GPD-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-YFP
pGPD$_0$8YFP	GPD-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-YFP
pGPD$_0$9YFP	GPD-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-CTCGAG-YFP
pCYC1$_0$1YFP	CYC1-TCTAGA-YFP
pCYC1$_0$2YFP	CYC1-TCTAGA-ACTAGT-YFP
pCYC1$_0$3YFP	CYC1-TCTAGA-ACTAGT-GGATCC-YFP
pCYC1$_0$4YFP	CYC1-TCTAGA-ACTAGT-GGATCC-CCCGGG-YFP
pCYC1$_0$5YFP	CYC1-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-YFP
pCYC1$_0$6YFP	CYC1-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-YFP
pCYC1$_0$7YFP	CYC1-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-YFP
pCYC1$_0$8YFP	CYC1-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-YFP
pCYC1$_0$9YFP	CYC1-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-CTCGAG-YFP

XII. **Supplementary Table 5:** pTEF$_0$xLacZ

pTEF$_0$1LacZ	TEF-TCTAGA-LacZ
pTEF$_0$3LacZ	TEF-TCTAGA-ACTAGT-GGATCC- LacZ
pTEF$_0$5LacZ	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC- LacZ
pTEF$_0$7LacZ	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT- LacZ
pTEF$_0$9LacZ	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-CTCGAG- LacZ

XIII. **Supplementary Table 6:** pTEF$_0$xGFP

pTEF$_0$1GFP	TEF-TCTAGA-GFP
pTEF$_0$3GFP	TEF-TCTAGA-ACTAGT-GGATCC- GFP
pTEF$_0$5GFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC- GFP
pTEF$_0$7GFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT- GFP
pTEF$_0$9GFP	TEF-TCTAGA-ACTAGT-GGATCC-CCCGGG-CTGCAG-GAATTC-GATATC-AAGCTT-ATCGAT-ACC-GTCGAC-CTCGAG- GFP

XIV. **References**

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