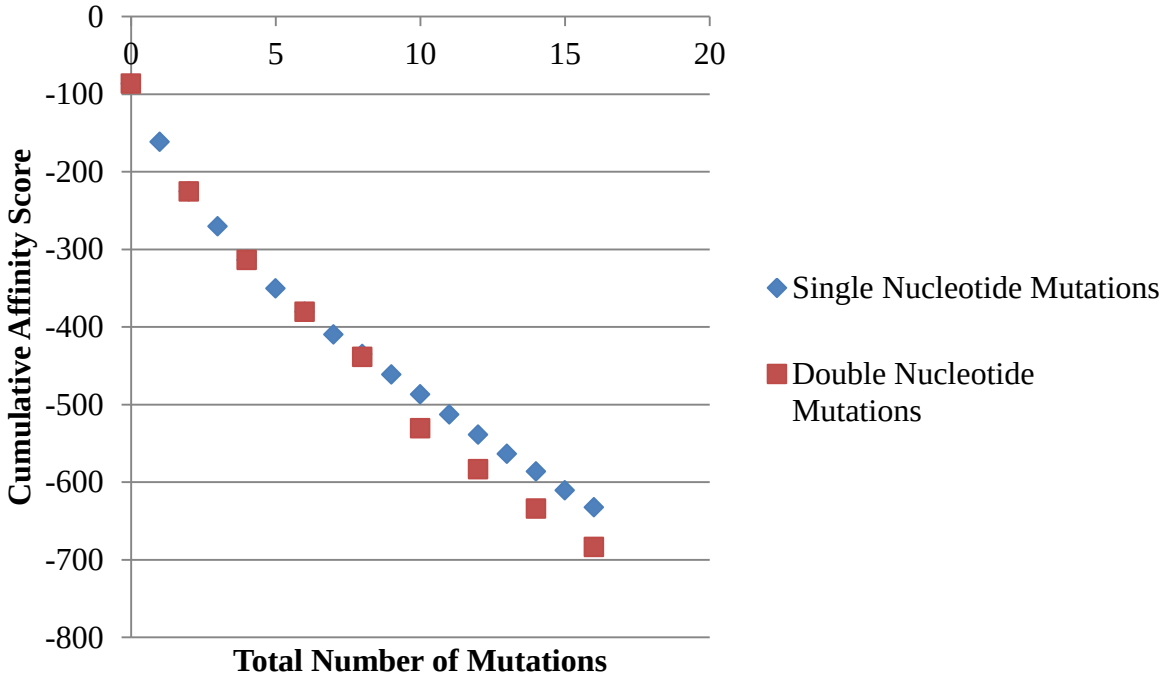
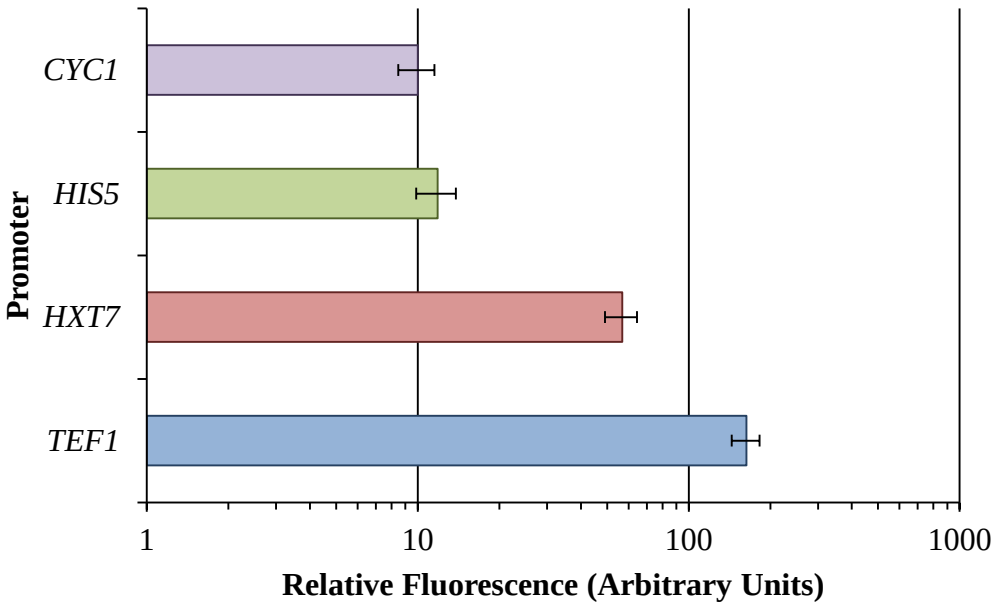
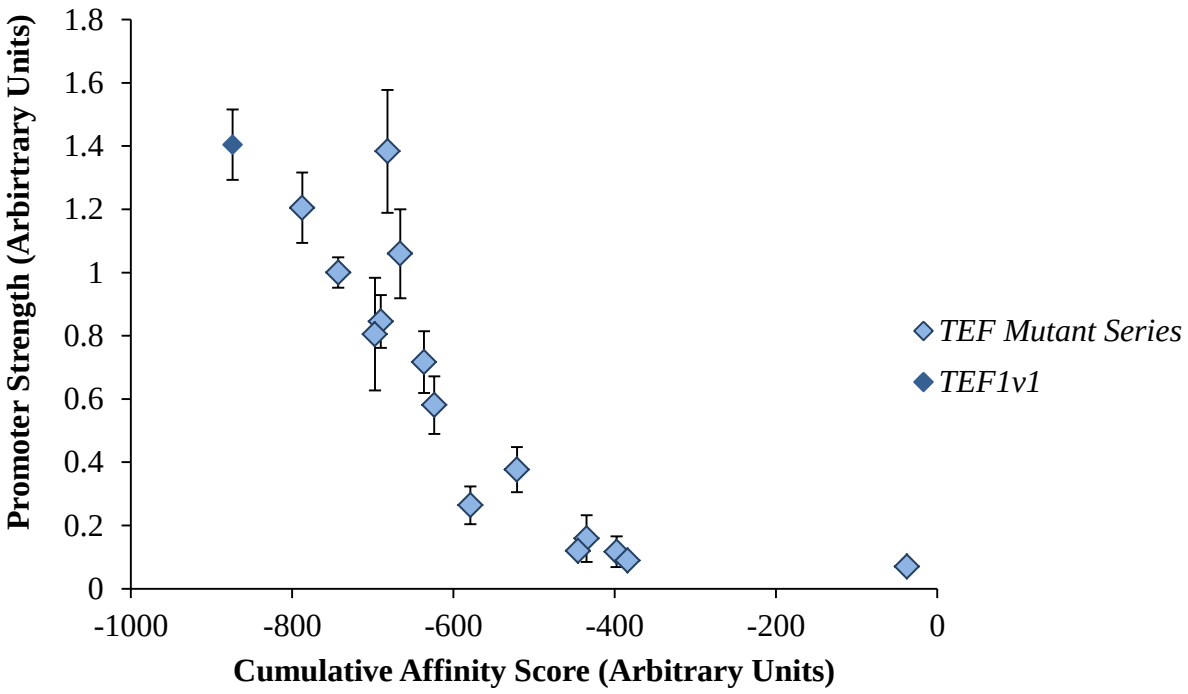
Supplementary Figures



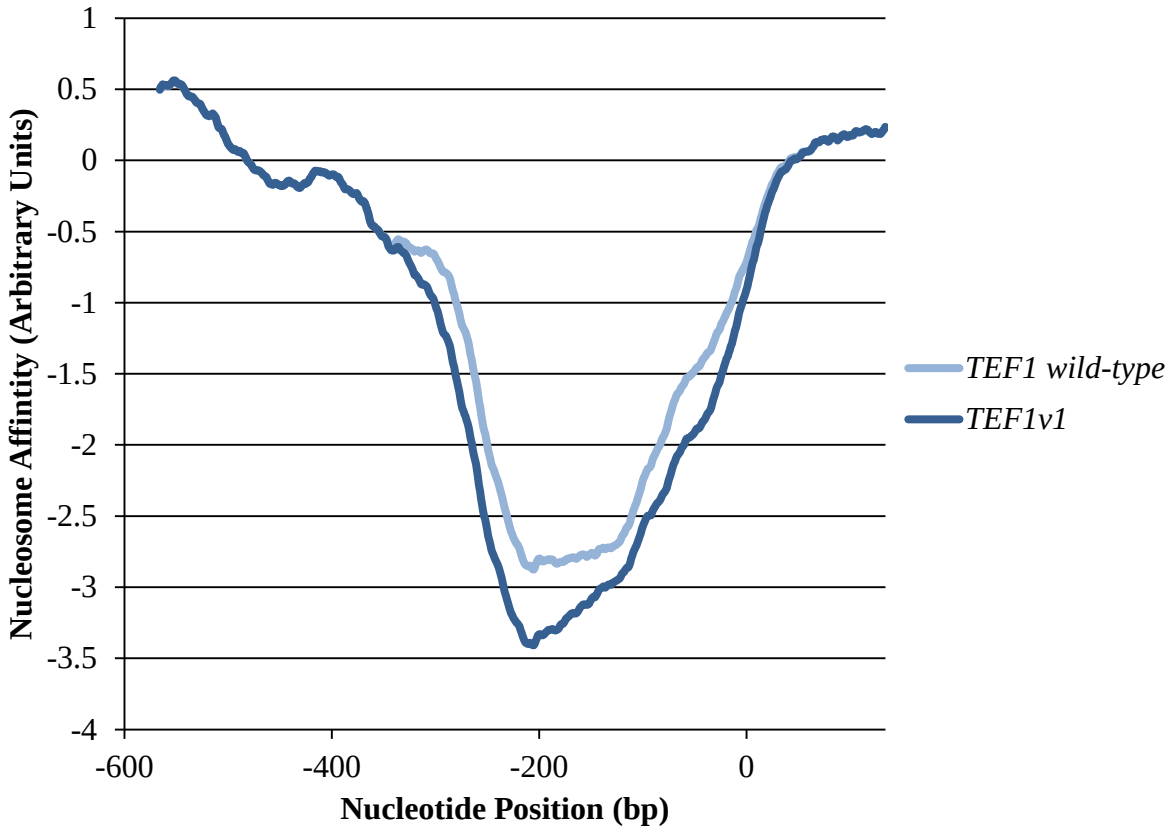
Supplementary Figure 1.
Comparison of single- and double-nucleotide greedy algorithms. Nucleosome affinity was minimized for the *CYC1* promoter using a greedy algortithm searching over all possible single nucleotide substitutions per round (used in this study) and a modified algorithm which takes into consideration of all double nucleotide substitutions. It can be seen that the algorithm considering double nucleotide substitutions performs slightly better than the single nucleotide algorithm.



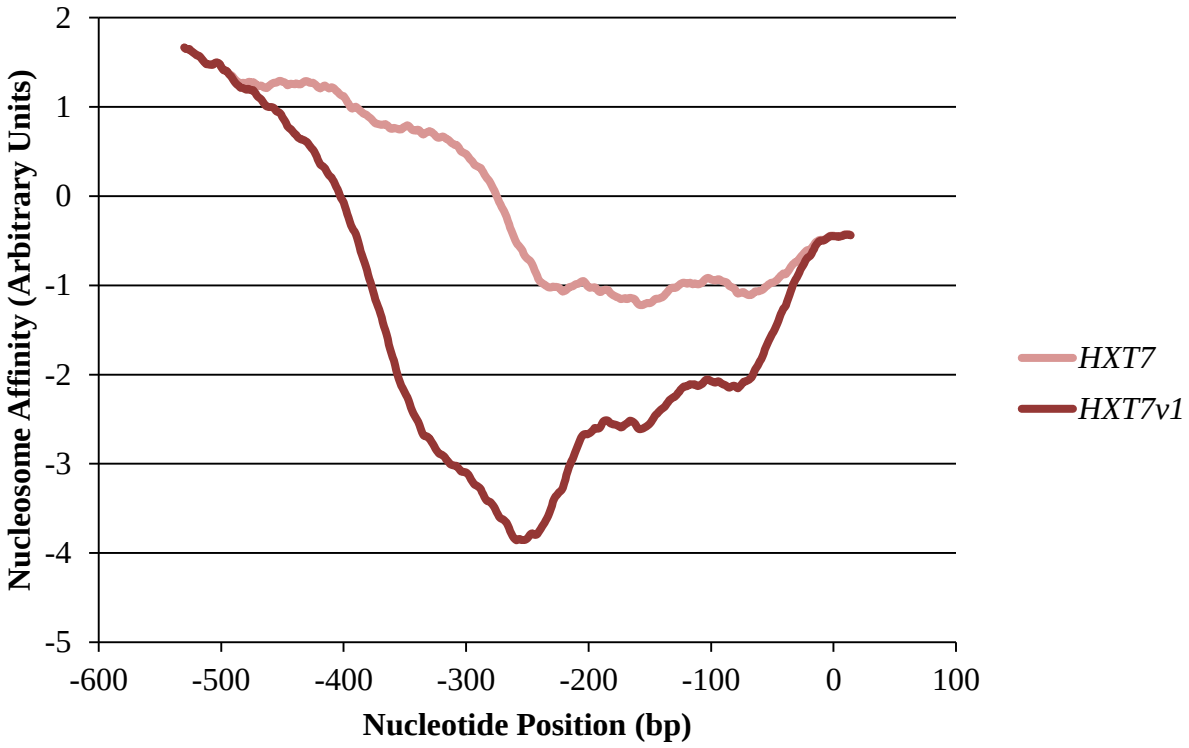
Supplementary Figure 2.
Expression levels of wild-type promoter scaffolds used in this study. Error bars represent standard deviation from three biological replicates.



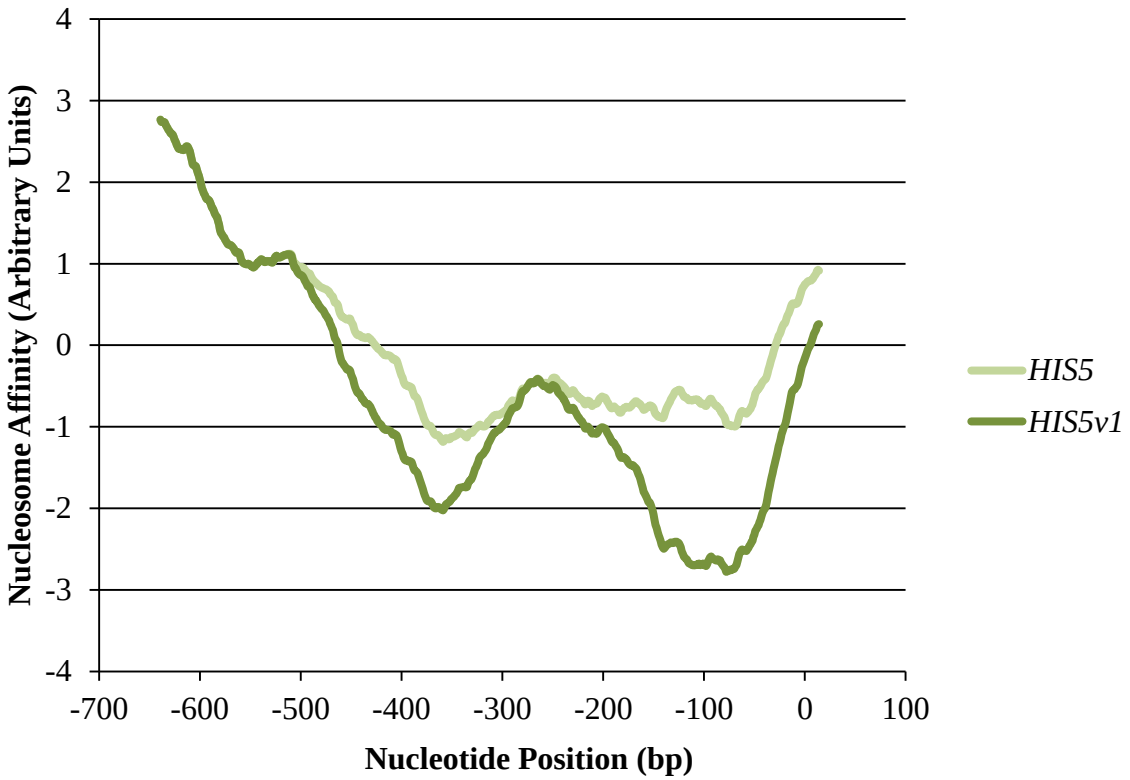
Supplementary Figure 3.
Experimental promoter strength as a function of the cumulative predicted nucleosome affinity scores for the *TEF1* mutant promoter series and for the redesigned variant, *TEF1v1*. Promoter strength was measured via flow cytometry with strains expressing a yellow fluorescent protein gene, *yECitrine*. Error bars represent standard deviation of biological triplicates.



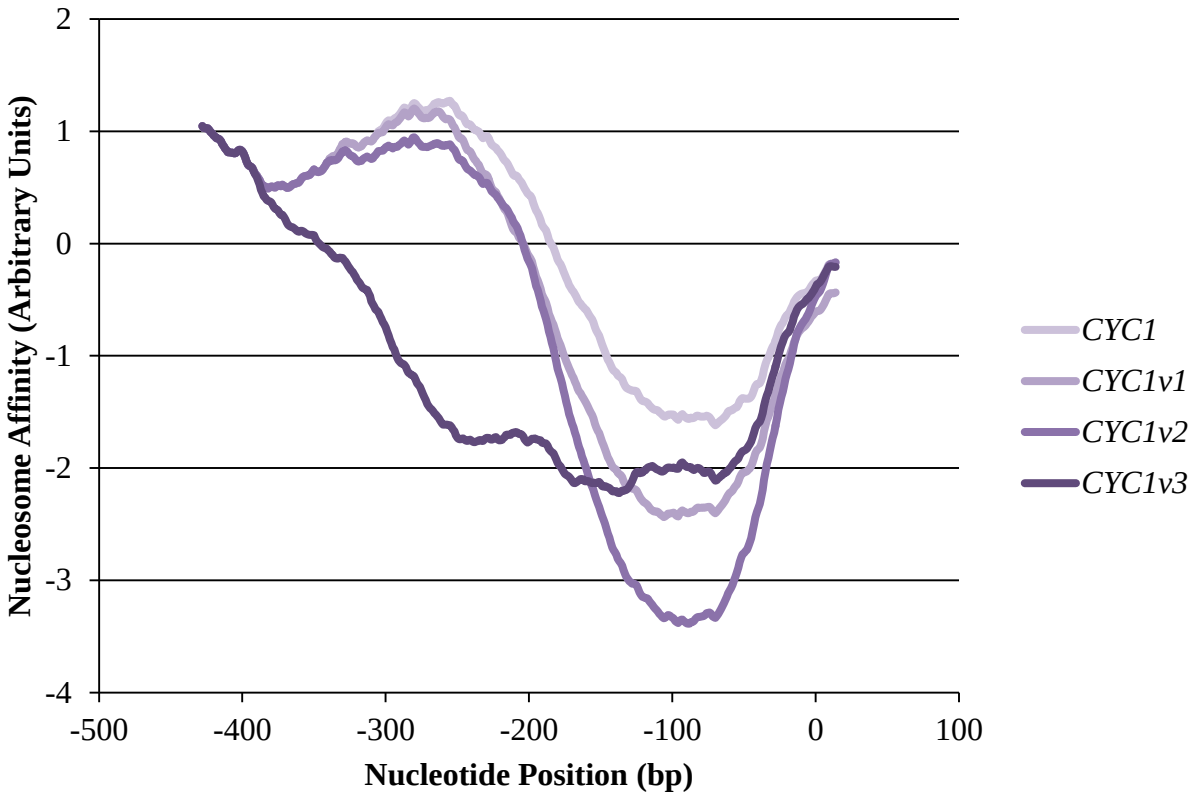
Supplementary Figure 4.
Computational nucleosome affinity profiles generated using a hidden Markov model[1] for *TEF1v1* and *TEF1* wild-type.



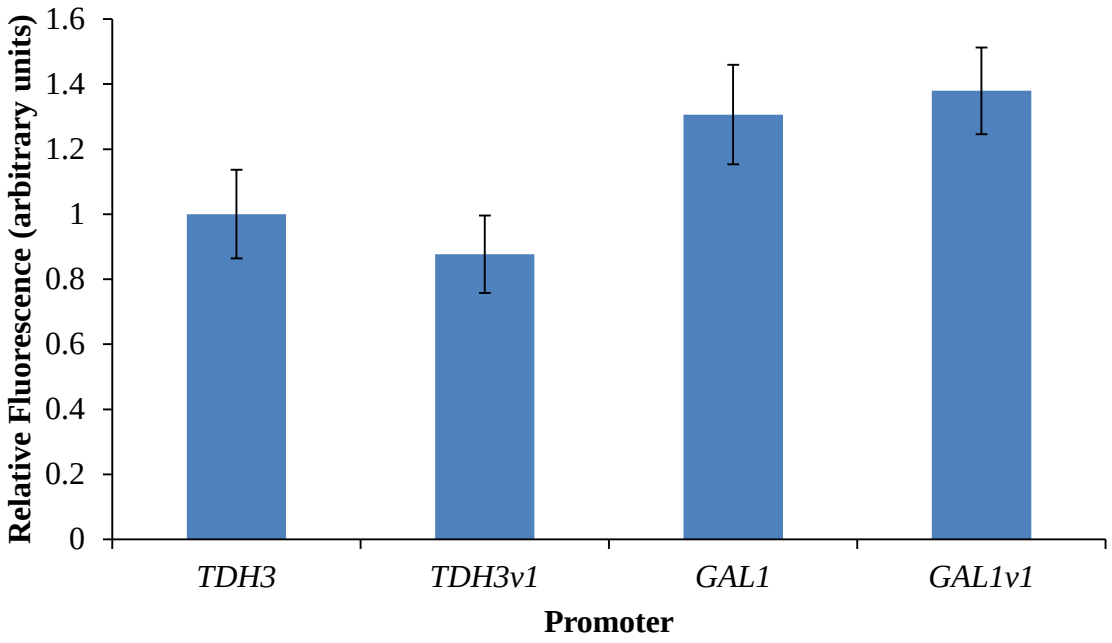
Supplementary Figure 5.
Computational nucleosome affinity profiles generated using a hidden Markov model[1] for *HXT7v1* and *HXT7* wild-type.



Supplementary Figure 6.
Computational nucleosome affinity profiles generated using a hidden Markov model[1] for *HIS5v1* and *HIS5* wild-type.



Supplementary Figure 7.
Computational nucleosome affinity profiles generated using a hidden Markov model[1] for *CYC1v1-3* and *CYC1* wild-type.



Supplementary Figure 8.
Relative fluorescence of TDH3, GAL1, and redesigned promoter constructs. Both redesigned promoters had the same fluorescence relative to wild-type, demonstrating that nucleosome architecture is not limiting in these very strong promoters. Error bars represent standard deviation from biological triplicate.

Supplementary Tables

Supplementary Table 1.
Sequences of redesigned and synthetic promoters. Underlined sequences in the synthetic promoters *Psynth1* and *Psynth2* are designated transcription factor binding sites and 5' UTR sequences.

Promoter Name	Sequence
TEF1v1	ATAGCTTCAAAATGTTTCTACTCCTTTTTTACTCTTCCAGATTTTCTCGGA CTCCGCGCATCGCCGTACCACTTCAAAACACCCAAGCACAGCATACTAA ATTTCCCCTCTTTCTTCCTCTAGGGTGTCGTTAATTACCCGTACTAAAGG TTTGGAAAAGAAAAAAAAGACCGCCTCGTTTCTTTTTTTTCGTCGAAAA AGGCAATAAAAATTTTTTTCACGTTTCTTTTTCTTGAAAATATTTTTTTTG ATTTTTTTCTCTTTCGATGACCTCCCATTGATATTTAAGTTAATAAACGG TCTTCAATTTCTCAAGTTTCAGTTTCATTTTTTTTGTTCTATTACAACTTT TTTTACTTCTTGCTCATTAAAAAGAAAGCATAGCAATCTAATCTAAGTTT
HXT7v1	CTCGTAGGAAAAATTTCGGGCCCCTGCGTTTTTTTCTGAGGTTCATTTTT TACATTTGCTTCTGCTGGATAATTTTCAGAGAAAAAAAGGAAAAATTAT ATGAAAAAAAGTTTTTTTTCAAGGAAAAAACCCTATTTTTTTTCGAGATC CCCTGTAACTTATTGGCAACTGAAAGAATGAAAAGGAAAAAAATAAAA AATATACTAGAACTGAAAAAAAATTAGTATAAATAGAGACGATATATG CCAATACTTCACAATGTTCGAATCTTTTTTTTTATTTTCAGCTATTGAAAA AAAATAAAACATCAAGAACAAACAAGCTCAACTTGTCTTTTCTAAGAAC AAAGAATAAACACAAAAACAAAAAGTTTTTTTAATTTTAATCAAAAA
HIS5v1	AAATGGTTAAAAATTGTTATCATAAATAAGGTGACCGGTTATATTGAGA CCTTTCCTGGACAGTAACTAATACAGAAGCCATTGGTAATGCAATAATT TTTTTGATCATGTGACTACGATCCGGGTGAGACTATTAAAAAAAGGAGT CAAGCATTGAAATAATTAATGACTAATCCGAAGTTAATTGTTAGGAGTC AATTGTTTTTTCCAATGAATGGAATCTGAGATGACTAAACTACCAATTTT CAATAGTTCATGGTATAGTGACGTAGTTAGTGCTTTTTTTTCTTGGATCT GTTGACTCACTTCAATTGATGTTTCTTACCCTGACATGACATACTTGATT TTTTATCTCTCACGTTATATAACTTGAAAAGGATGCACACAGTTCTGTTC AATATACCCTCCAATATGTAAAAAAAGTTTTTTCATTGATTACTCTTAAT TTTTTTCCTGCTAAACCAGCAGTACGTGTGTGCCGTATATATTAAAATTA CACT
CYC1v1	ATTTGGCGAGCGTTGGTTGGTGGATCAAGCCCACGCGTAGGCAATCCTC GAGCAGATCCGCCAGGCGTGTATATATAGCGTGGATGGCCAGGCAATTT TAGTGCTGACACATACAGGCATATATATATGTGTGCGACGAAAAATGAT CATATGGCATGCATGTGCTCTGTATGTATATAAAACTCTTGTTTTCTTTT TTTCTCTAAATATTCTTTCCTTATACATTAGGACCTTTGCAGCATAAATT ACTATACTTCTATAGACACGCAAAAACAAATACACACACTAA

Promoter Name	**Sequence**
CYC1v2	ATTTGGCGAGCGTTGGTTGGTGGATCAAGCCCACGCGTAGGCAATCCTC GAGCAGATCCGCGAGGCGTGTATATATAGCGTGGATGGCCAGGCAACT TTAGTGCTGACACATACAGGCATATATATATGTGTGCGACGACACATGA TCATATGGCATGTATGTGCTCTGTATGTATATAAAACTCTTTTTTTCTTTT TTTCTCTAAATTTTTTTTCCTTATACATTAGGACCTTTGCAGCATAAATTA CTATACTTCTATAGACACGCAAACACAAATACACACACTAA
CYC1v3	ATTTCGCGCGCGTTGGTTAGTAAAAAAAGCCCACGCGTAGGGAATCCTC GAGCATATACGCGAGGCGCGTATATATAGCGCGTATGTTCAGGTAAATT TAGTGCTGACACATACAGGCATATATATATGTGCGCGTATATACATGAT TATATGGCATGTATGTGCTCTGTATGTATATAAAACTCTTTTTTTCTTTTT TTCTCTAAATTTTTTTTCCTTATACATTAGGACCTTTGCAGCATAAATTA CTATACTTCTATAGACACGCAAATACAAATACACACACTAA
TDH3v1	AGTTTATCATTATCAATACTCGCCATTTCAAAGAATACGTAAATAATTA ATAGTAGTGATTTTCCTAACTTTTTTTAGTCAAAAAATTAGCCTTTTAAT TCTGCTGTAACCCGTACATGCCCAAAATAGGGGGCGGGTTACACAGAAT ATATAACATCGTAGGTGTCTGGGTGAACAGTTTATTCCTGGCATCCACT AAATATAATGGAGCCCGCTTTTTAAGCTGGCATCCAGAAAAAAAATGA ATCCCAGCACCAAAATATTTTTTTCTTCACCAACCATCAGTTCATAGGTC CATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCAAAAA ACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGAT GACACAAGGCAATTTACCCGCGCATGTATCTATCTCATTTTTTTACACCT TCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGT TGAAACCAGTTCCCTGAAATTATTCCCCTACTTGACTAATAAGTATATA AAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCT TAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTAAAAAACCAGAA CTTAGTTTCGACGGAT
GAL1v1	ACGGATTAGAAGCCGCCGAGCGGGCGACAGCCCTCCGACGGAAGACTC TCCTCCGCGCGTCCGCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATG TGCCTCGCGCCGCACTGCTCCGAAAAATAAAGATTCTACAATACTAGCT TTTTTGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACC TTCAAATTAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAG TTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTG ATCTATTAACAGATATATAAATGAAAAAGCTGCATAACCACTTTAACTA ATACTTTCAACATTTTCAGTTTTTATTACTTCTTATTCAAATGTCATAAA AGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGA GAAAAAAC

Promoter Name	Sequence
Psynth1v1	TCCGGGTAACGCCGACACAGTAAGTAACGAGATGTATGGGTGTCCTAAC TAAAAGGCTTCCAACTCAACATTGAATCAGGTAATCCTAGATCAAGGCT TCCATACACAGGTTTATATTAATACATATACGACAACTCTCCAATTCGCT CATAATTACAACAAAGATCGAACTGAGAGAGACTTAGACTCGTACAAC TACATTTTTCGTTAACTTTTTAACATACGCGAGGGTATTAAACTTAGCTG ACGCAACTCTAGTTGTATCTCGCGATAATTTCTTTTTACTTGTCTATTTAT AAAAACCAAGCTAATAACTTCATACGTCTTATTGTATTTAGACTATTTCT TTTTAACCTAACTATAGCAGAACCCGCGGGTAATTACTTAAAACACCAA GAACTTAGTTTCGAATAAACACACATAAACAAACAAA
Psynth1v2	TCCGGGTAACGCCGAAAAAATAAGTAACGCGATGTATGGGTGTACTAA AAAAAAGGCTTCCAATAAAAAATTGAATCAGGTAATCCTATATCAAGG CTTCCATATATAGGTTTATATTAATACATATACGAAAAAACTCTTTTTCG CGCATAATTATAATAAAAATCGAACTGAGAGAGACTTAGACTCGTACA ACTATTTTTTTTGTTAATTTTTTTATATACGCGCGGGTATTAAACTTAGCT GACGCGATTCTATTTGTATCTCGCGATAATTTCTTTTTTCTTCTCTATTTA TAAAAACCAAGCTAATAACTTCATACGTCTTTTTGTATATAGACTTTTTC TTTTTTTCCTAACTATAGGAGAACCCGCGGGTAATTTTTTAAAACACCAA GAACTTAGTTTCGAATAAACACACATAAACAAACAAA
Psynth1v3	TCCGGGTAACGCCGAAAAAATTATATACGCGATGTATGGGTGTATTAAA AAAAAGGCTTCCAATAAAAAAAAGAATCAGGTAATCCTTTTTCGCGGCT TCCATATATATTTTTTTATTAATACATATACGAAAAAAGTCTTTTTCGCG GGTAATTATAATAAAAATCGAACTGAGAGAGACTTTCACACGTACTACT ATTTTTTTTATTATTTTTTTTATATACGCGCGGGTAAAAAAATTAACTAA CGCGATTTTTTTTCTTTCGCGCGAAAATTTCTTTTTTCTTCTCTATTTATA AAAAGGAAGGAAAAAAGTTCTTACCTCTTTTTGTATATACACTTTTTCTT TTTTTCCTTAGTAAAGGAGAACGCGCGGGTATTTTTTTAAAACACCAAG AACTTAGTTTCGAATAAACACACATAAACAAACAAA
Psynth2v1	TCCGGGTAACTGCGGGTGACCGCAATCTTAGATGTATGGGTGTTAACTG AGCTAGGCTTCCATGCATTTAGAGAACTTATTAACTGAATAGTTAGGCT TCCAAACGAGCTAGTTCTCGCGTGTCATCTAAAAAAATTCTAGACTGGT GATACTTATAACTATAAAAAAACTGACACTTCTCCCTAATCGTAGTATT GTATATATTTTTTTAAAAAAAACTTGCAACCATTTAAAACACCAAGAAC TTAGTTTCGAATAAACACACATAAACAAACAAA
Psynth2v2	TCCGGGTAACCGCGGGTAACCTCAATCTTAGATGTATGGGTGTTAACTG AGCTCGGCTTCCATGTATTAAAAAAAATTATTAACTGAAAAAAAAGGCT TCCAAACTAGTTTTTTTTCGCGTGTGATCAAAAAAAATTCTAGACGGGT AATACATATAAGTATAAAAAAACTGACACTTCTCCCTAATCGTAGTATT GTATATATTTTTTTAAAAAAAACTTGCAACCATTTAAAACACCAAGAAC TTAGTTTCGAATAAACACACATAAACAAACAAA

Promoter Name	Sequence
Psynth2v3	TCCGGGTAACCGCGGGTAACATATATATTAGATGTATGGGTGTAAAAAA AGCGCGGCTTCCATGTATTAAAAAAAATTTTTTTCTGAAAAAAAAGGCT TCCATACTAATTTTTTTTCGCGCGGGTAGAAAAAAAATACTAGTCGGGT AATACATATAAGTATAAAAAAAGAGACACTTCTCCCTAATCGTACTATT GTATATATTTTTTTAAAAAAAAGTTCGAAGCTTTTAAAACACCAAGAAC TTAGTTTCGAATAAACACACATAAACAAACAAA

Supplementary Table 2.

Glycolytic promoter architecture and design of Psynth1 and Psynth2. The positions and lengths between various transcription factors in a collection of yeast glycolytic promoters were catalogued in order to design *Psynth1* and *Psynth2*. All lengths refer to the distance in basepairs between the start of each binding site. Column Descriptions: *1.* Number of Gcr1p binding sites. *2.* Number of Rap1p binding sites. *3.* Number of Reb1p binding sites. *4.* 5' UTR length. *5.* Length between TATA box and transcription start site. *6.* Length between Gcr1p binding site and TATA box. *7.* Length between two Gcr1p binding sites when they occur in close proximity to a Rap1p or Reb1p binding site. Values of N/A mean that the sites did not occur in a pair. A value of 40 bp was chosen for the synthetic promoters because *PGK1* and *GPM1* were identified as outliers in this category. *8.* Length between Rap1p binding site and Gcr1p binding site when they occur in close proximity to each other. Values of N/A mean that the sites did not occur close together or there was no Rap1p binding site. *9.* Length between Rap1p and Reb1p binding sites. Values of N/A mean that the promoter did not have both a Rap1p site and a Reb1p site. The minimum distance was chosen in this category because the three values had a large distribution and some of the promoters that lack a Reb1p binding site have an Abf1 binding site in a similar position.

Promoter	**1 Gcr1**	**2 Rap1**	**3 Reb1**	**4 5' UTR**	**5 TATA -> TSS**	**6 Gcr1 -> TATA**	**7 Gcr1 -> Gcr1**	**8 Rap1 -> Gcr1**	**9 Rap1 -> Reb1**
TDH3	2	1	1	41	93	309	38	27	31
FBA1	3	1	0	11	105	255	42	N/A	N/A
TPI1	2	0	1	31	140	167	40	N/A	N/A
ADH1	2	1	0	39	82	207	N/A	N/A	N/A
PGK1	2	1	1	42	104	279	17	24	88
CDC19	5	1	1	29	163	64	47	12	170
TDH2	4	0	1	33	93	170	45	N/A	N/A
GPM1	2	1	0	11	128	196	16	37	N/A
Average	**2.75**	**0.75**	**0.625**	**29.6**	**113.5**	**205.9**	**35**	**25**	**96.3**
Minimum	**2**	**0**	**0**	**11**	**82**	**64**	**16**	**12**	**31**
Psynth1	2	1	1	41	100	200	40	24	30
Psynth2	2	1	1	41	80	65	40	24	30

Supplementary Table 3.

Primer sequences for cloning of promoters, yECitrine and LacZ genes, knockout and integration cassettes, and primers for qPCR of yECitrine. All redesigned and synthetic promoters were cloned using the "general promoter primers" with the exception of *HIS5v1*, which was cloned using the *HIS5* promoter primer set.

Primer set name	Forward Primer	Reverse Primer
yECitrine	GGCGCTACTAGTATGTCTAAAGGTGAAGAATTATTCACTGG	ACGCGTCGACTTATTTGTACAATTCATCCATACCATG
LacZ	GGCGCTTCTAGAACTAGTATGACCATGATTACGGATTCACT	ACGCGTCGACTTATTTTTGACACCAGACCAACTG
General promoter primers	TAAAGGGAACAAAAGCTGGAGCTC	CAGTGAATAATTCTTCACCTTTAGACATACTAGTTCTAGA
HXT7 promoter	TGACTGAGCTCCTCGTAGGAACAATTTCGGG	GGCGCTACTAGTTCTAGATTTTTGATTAAAATTAAAAAAACTTTTTGTTTT
HIS5 promoter	TGACTGAGCTCAAATGGTTAAAAATTGTTATCATA	GGCGCTACTAGTTCTAGAAGTGTAATTTTAATATATACGGCA
P_{CYC1} knockout cassette	TGAATCTAAAATTCCCGGGAGCAAGATCAAGATGTTTTCACAGCTGAAGCTTCGTACGC	TAGCACCTTTCTTAGCAGAACCGGCCTTGAATTCAGTCATGCATAGGCCACTAGTGGATCTG
K. lactis URA3	TGACTGAGCTCCAGCTGAAGCTTCGTACGC	GCATAGGCCACTAGTGGATCTG
TRP1 integration cassette	TGGAGATATTCCTTATGGCATGTCTGGCGATGATAATAAAGGGAACAAAAGCTGGAGCTC	ACACCAATAACGCCATTTAATCTAAGCGCATCACCAACGGTACCCAATTCGCCCTATAGT
yECitrine qPCR primers	TTCTGTCTCCGGTGAAGGTGAA	TAAGGTTGGCCATGGAACTGGCAA
ALG9 qPCR primers	ATCGTGAAATTGCAGGCAGCTTGG	CATGGCAACGGCAGAAGGCAATAA

Supplementary Table 4.

Primers for nucleosome mapping tiling array. Primers sets marked with a * were used for both *CYC1* and *CYC1v3*. All other sets were used for a specific promoter as noted.

Primer set	Mid-amplicon location relative to start codon	Forward primer	Reverse primer
CYC1_1	-313.5	CGCGCAATTAACCCTCACTAA	AACCAACGCTCGCCAAAT
CYC1_2	-271.5	ATTTGGCGAGCGTTGGT	CGGATCTGCTCGAGGATTG
CYC1_3	-222.5	GCAATCCTCGAGCAGATCC	GCCTGTATGTGTCAGCACTAA
CYC1_4	-189	ATGGCCAGGCAACTTTAG	GTGTCGTCGCACACATA
CYC1_5	-170.5	TAGTGCTGACACATACAGG	CACATGCATGCCATATGAT
CYC1_6	-120.5	GTGCGACGACACATGAT	GGTCCTAATGTATAAGGAAAGAATATTTAG
CYC1v3_1	-315.5	CGCGCAATTAACCCTCACTAAA	AACGCGCGCGAAATGAG
CYC1v3_2	-270.5	GCGCGCGTTGGTTAGTAAA	ATATGCTCGAGGATTCCCTACG
CYC1v3_3	-233.5	CCCACGCGTAGGGAATC	TCAGCACTAAATTTACCTGAACATAC
CYC1v3_4	-208.5	GTATATATAGCGCGTATGTTCAGGTA	GCCTGTATGTGTCAGCACTAA
CYC1v3_5	-179.5	TAGCGCGTATGTTCAGGTAAA	CAGAGCACATACATGCCATATAATC
CYC1v3_6	-167	GTGCTGACACATACAGGCATA	CAGAGCACATACATGCCATATAATC
CYC_7*	-52	TTTCCTTATACATTAGGACCTTTGCAG	AGTGTGTGTATTTGTATTTGCGTGT
CYC_8*	-11	GACACGCAAATACAAATACACACA	TTGGGACAACACCAGTGAATAA
CYC_9*	129.5	TTCTGTCTCCGGTGAAGGTGAA	TAAGGTTGGCCATGGAACTGGCAA
Ampicillin control*	N/A	TGTAACTCGCCTTGATCGTTGGGA	TTGTTGCCATTGCTACAGGCATCG

Supplementary Reference

1. Xi, L. et al. Predicting nucleosome positioning using a duration Hidden Markov Model. *BMC Bioinformatics* **11**, 346 (2010).