Supplementary Information

Design and synthesis of synthetic UP elements for modulation of gene expression in Escherichia coli

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Figure S1

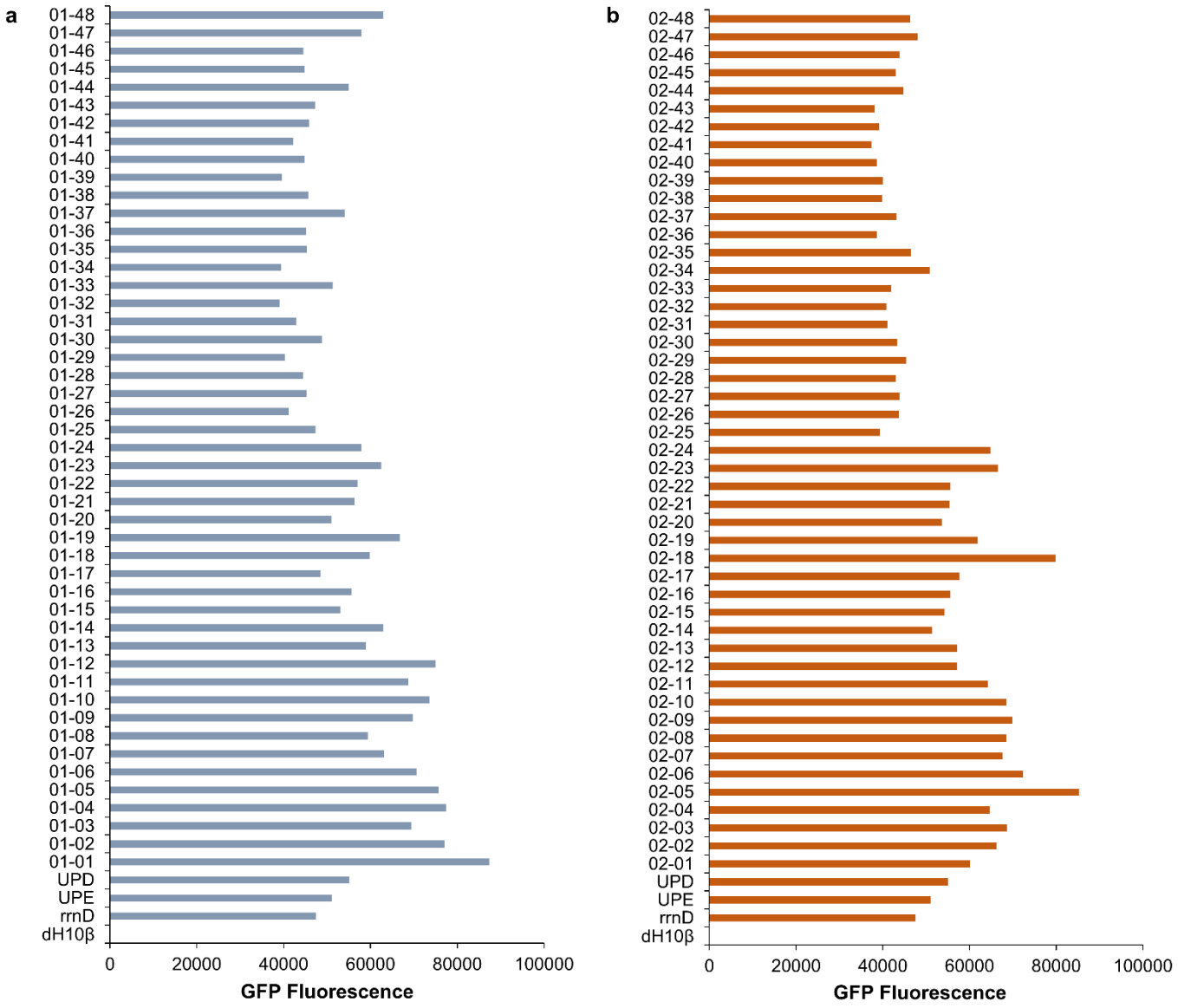


Figure S1: Final candidates from hexameric randomization libraries. Final 48 colonies picked from Library UPD-01 (**a**) and Library UPE-02 (**b**), after screening for top 10% of fluorescence with FACS, then **stationary phase** fluorescence measurement with flow cytometry. Library UPD-01 based on UPD sequence and Library UPE-02 based on UPE sequence. Positive controls UPD and UPE, and negative controls *rrnD* and untransformed dH10β in black.

Figure S2

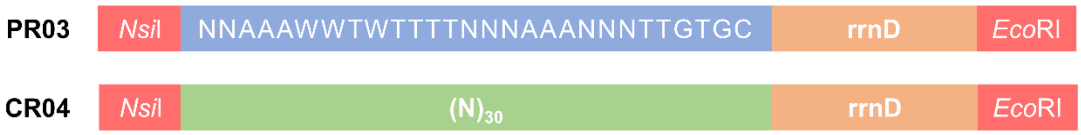
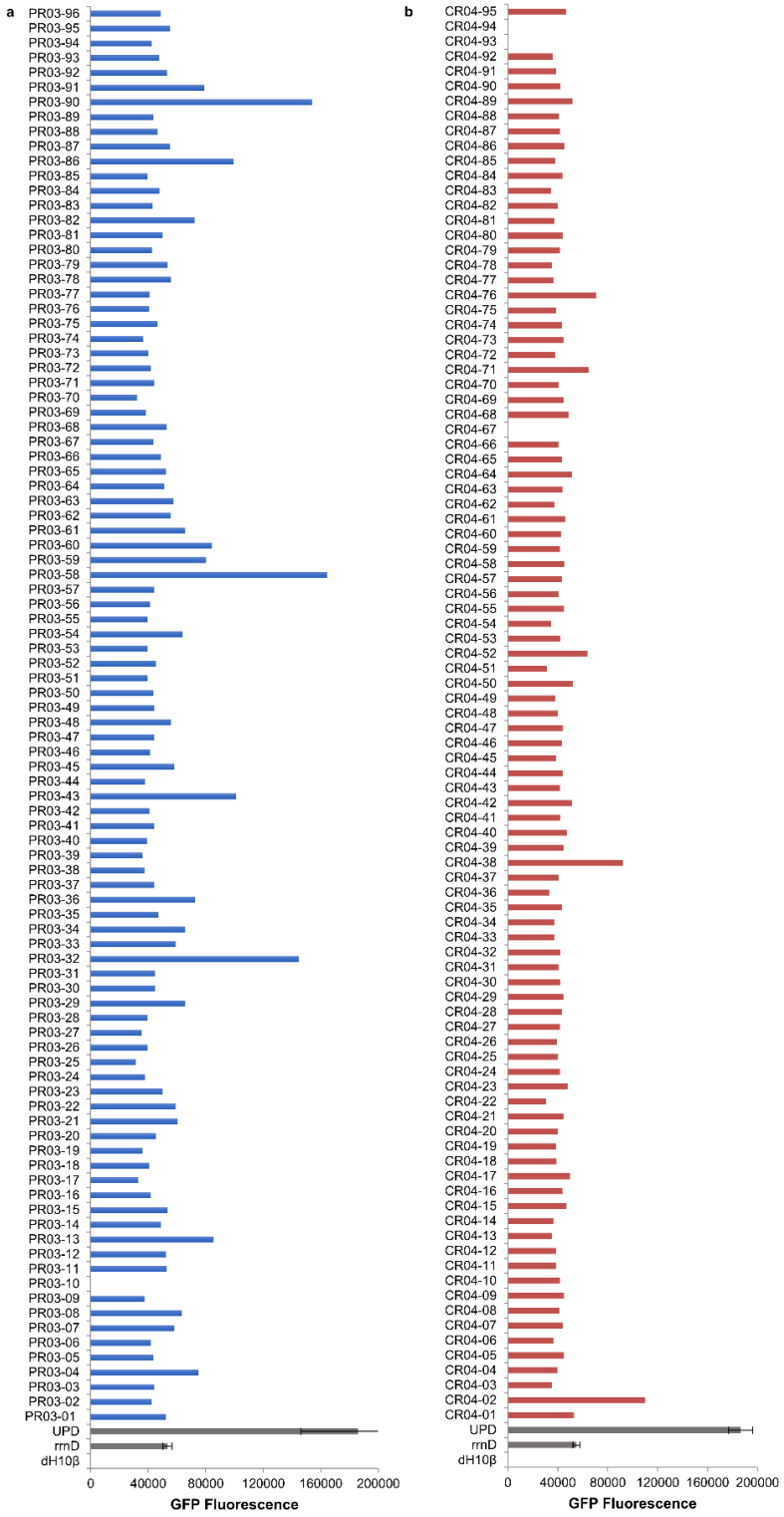


Figure S2: Design of partial and complete randomization libraries. To explore the potential sequence space of UP elements beyond that of UPD, two libraries were created based on partial randomization of an UP element consensus sequence previously identified (Estrem et al. 1999), and complete randomization of an extended UP element sequence space. These libraries were termed PR03 and CR04, respectively. W's signify A/T's

Figure S3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GFP Fluorescence
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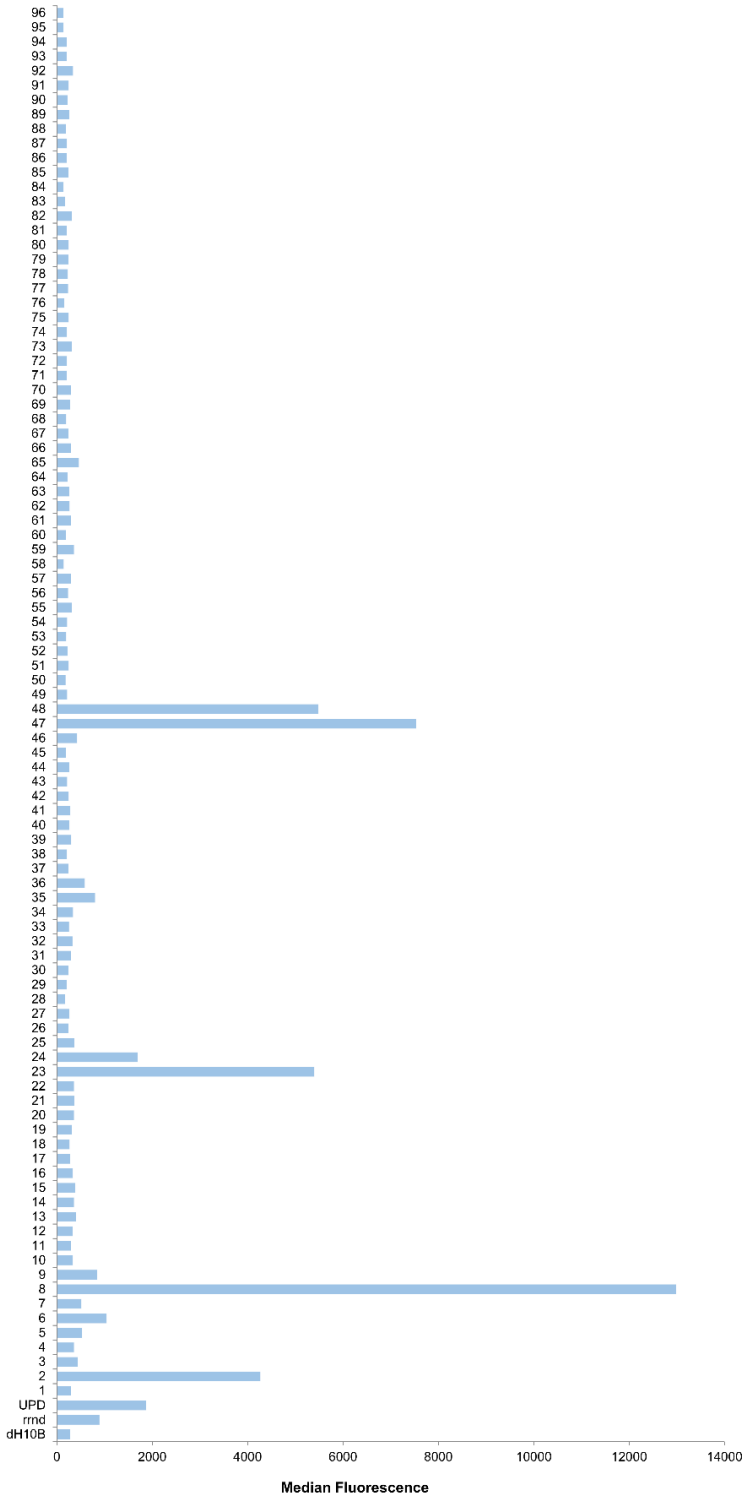
GFP Fluorescence

Figure S3: Performance of libraries PR03 and CR04. GFP fluorescence measurements of Library PR03 (**a**) and CR04 (**b**). Measurements made by flow cytometry in exponential phase. Positive control UPD and negative control untransformed dH10β shown in grey. Error bars represent standard deviation from technical triplicate.

Figure S4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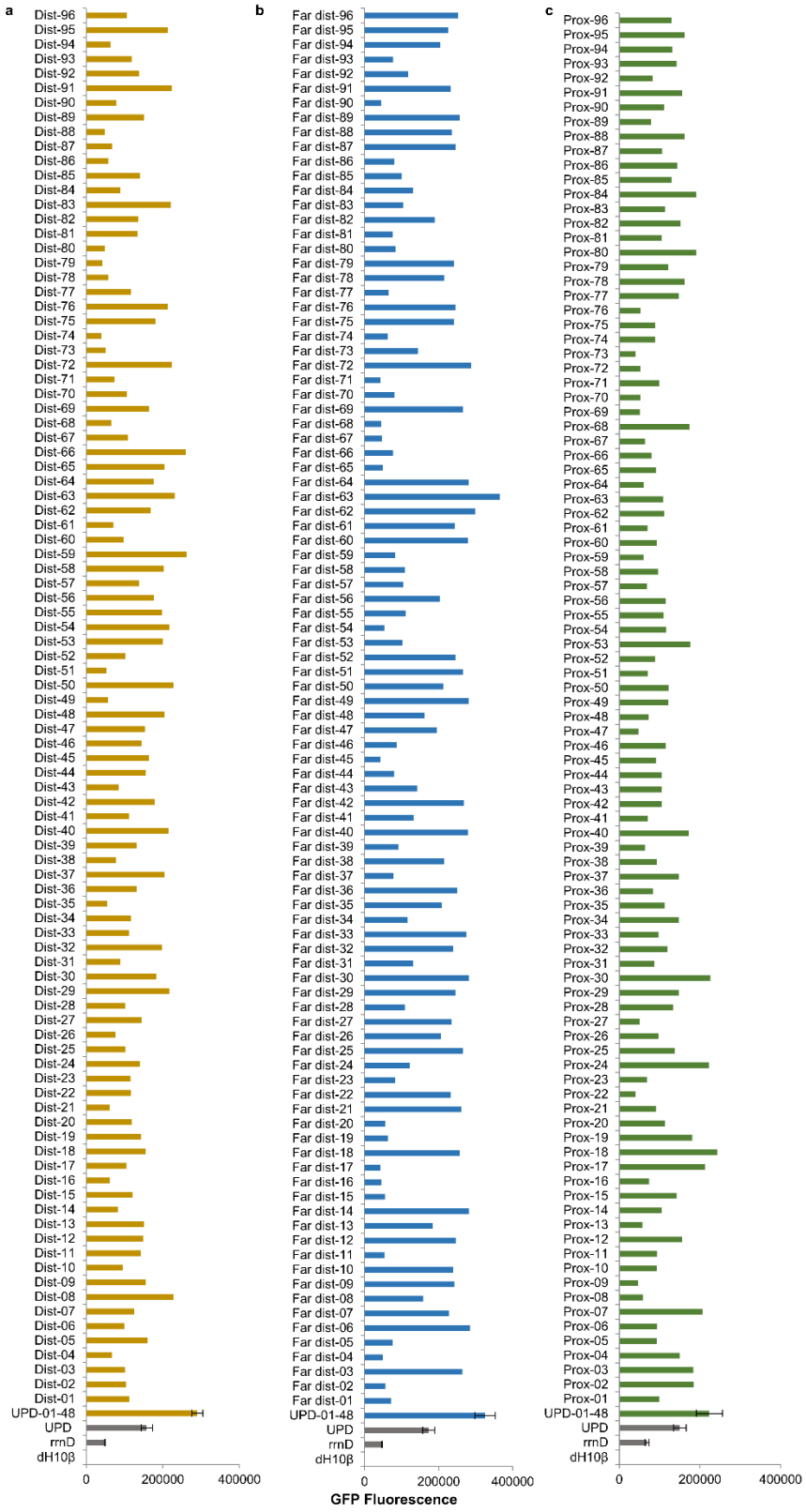
Median Fluorescence

Figure S4: Comparison of stationary and exponential phase evaluation. Median fluorescence values of Library CR04 in stationary phase. Fluorescence values in stationary phase suffer from higher standard deviations and smaller percentages of cells exhibiting fluorescence within individual colonies when compared to exponential sort (**Fig. S3**).

Figure S5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Prox-02
Prox-01
UPD-01-48
UPD
rrnD
dH10β
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400000

Figure S5: Performance of libraries based on partial randomization of UPD-01-48. GFP fluorescence measurements of Libraries UPD48-Dist (**a**), UPD48-Far dist (**b**), and UPD48-Prox (**c**). Measurements made by flow cytometry in exponential phase. Individual libraries evaluated on different days. Positive control UPD and negative control untransformed dH10β shown in grey. Error bars represent standard deviation from technical triplicate.

Figure S6

Figure S6: Sequence alignment between proximal regions previously reported by Estrem *et al*. WebLogo y-axis represents relative nucleotide frequencies instead of bits.

Figure S7

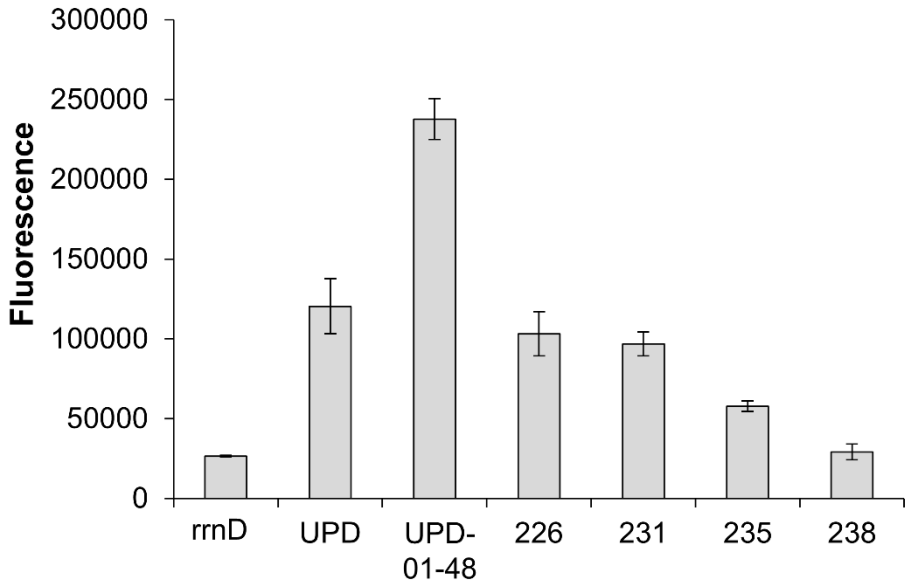

Figure S7: Performance of rationally designed tandem UP elements. GFP fluorescence measurements of rationally designed tandemly placed UP elements. Positive control UPD and negative controls rrnD and untransformed dH10β shown in grey. Error bars represent standard deviation of technical triplicates. Sequences shown in Table SV.

Figure S8

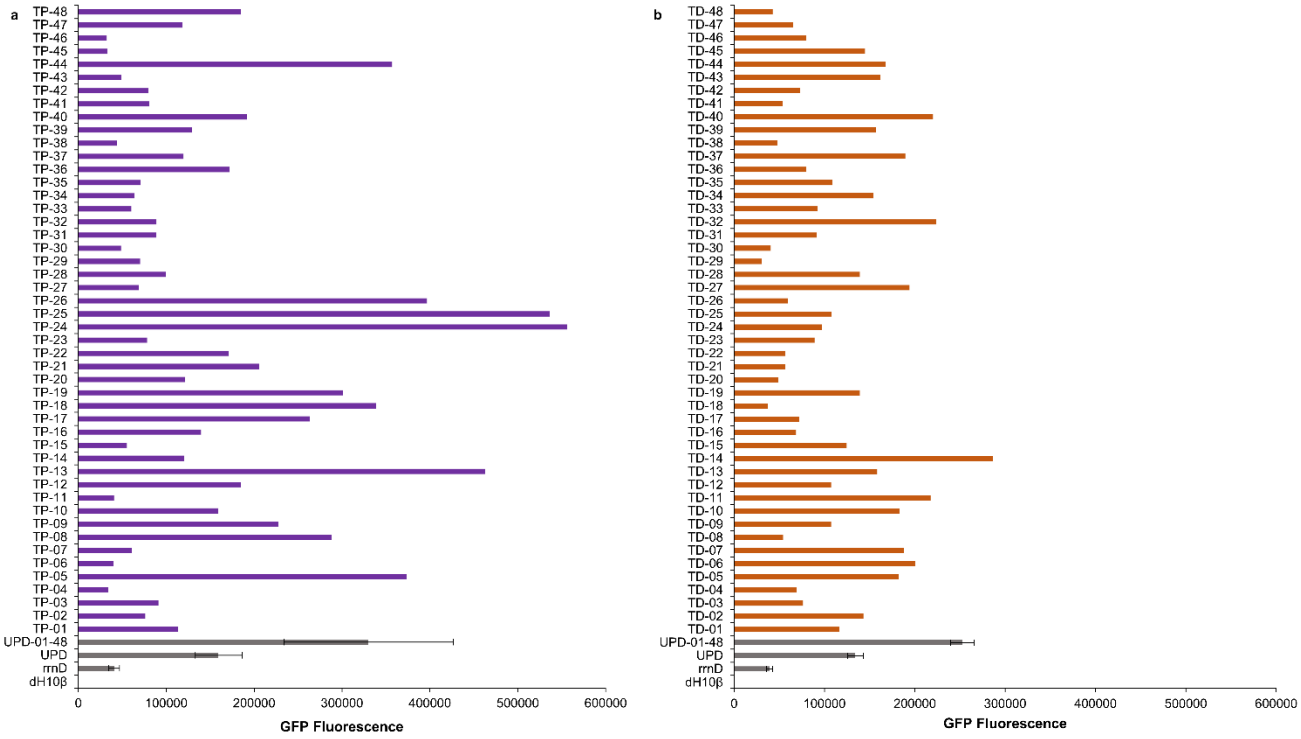

Figure S8: Tandem UP element libraries. GFP fluorescence of Tandem Proximal Library (**a**) and Tandem Distal Library (**b**), measured by flow cytometry. Positive control UPD and negative controls rrnD and untransformed dH10β shown in grey. Error bars represent standard deviation from technical triplicate.

Figure S9

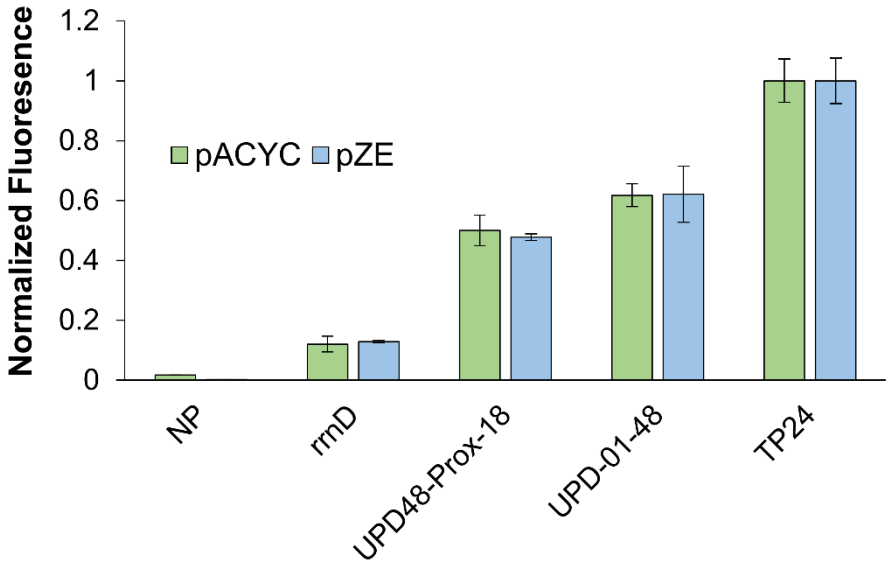

Figure S9: Normalized GFP fluorescence levels across variable plasmid backgrounds.

Figure S10

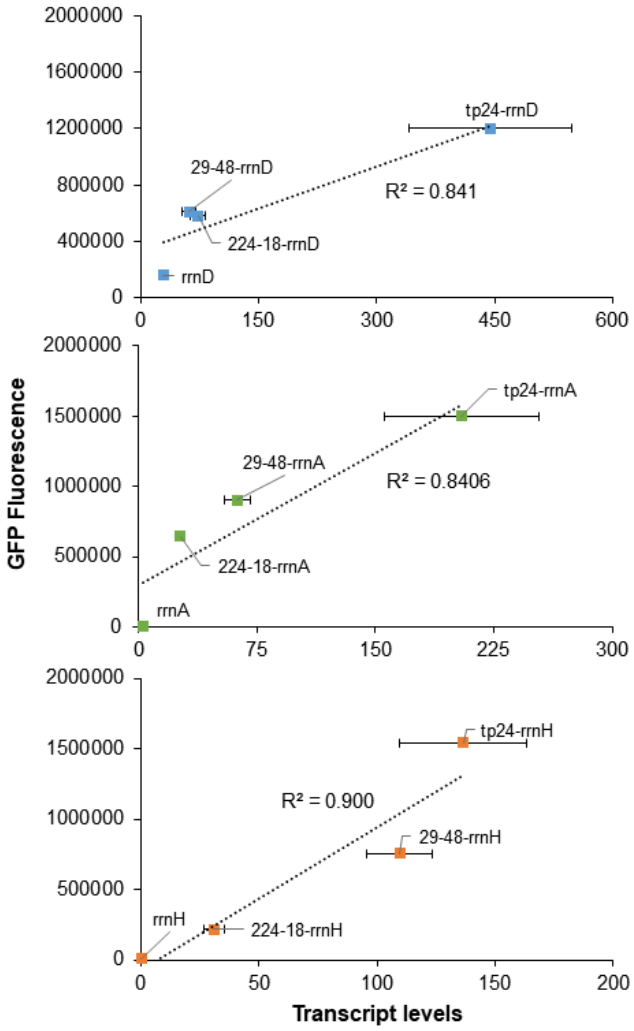

Figure S10: GFP fluorescence levels vs transcript levels for select UP elements upstream of variable rRNA cores. Top (blue): *rrnD* core promoters. Middle (green): *rrnA* core promoters. Bottom (orange): *rrnH* core promoters. Error bars represent standard deviation of technical triplicates. All transcript levels normalized to levels of no-promoter no-UP element pZE control. GFP fluorescence levels representative of the single biological sample from which the RNA was isolated.

Table SI

Vector	Sequence
pZE	tttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgactagtgcttggattctcaccaata aaaaacgcccggcggcaaccgagcgttctgaacaaatccagatggagttctgaggtcattactggatctatcaacaggag tccaagcgagctctcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatgcgctgcgaa tcgggagcggcgataccgtaaagcacgaggaagcggtcagcccattcgccgccaagctcttcagcaatatcacgggta gccaacgctatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcggccattttcca ccatgatattcggcaagcaggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggc gaacagttcgggctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtac gtgctcgctcgatgcgatgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgcattgcat cagccatgatggatactttctcggcaggagcaaggtgagatgacaggagatcctgccccggcacttcgcccaatagcag ccagtcccttcccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggccagccacgatagccg cgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtcttgacaaaaagaaccgggcgcccctgcgctgac agccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctccacccaagcgg ccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttgatcccctgc gccatcagatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagct ggcaattccatcgatggcatgcatggaaaatttttttaaaaaaatcgtgcttgtgcaaaaaattgggatccctataatgcgcc tcgggaattcattaaagaggagaaaggtaccgcatgcgtaaaggagaagaacttttcactggagttgtcccaattcttgttg aattagatggtgatgttaatgggcacaaattttctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttacccttt aaatttatttgcactactggaaaactacctgttccatggccaacacttgtcactactttcggttatggtgttcaatgctttgcgag atacccagatcatatgaaacagcatgacttttttcaagagtgccatgcccgaaggttatgtacaggaaagaactatatttttcaa agatgacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttgttaatagaatcgagttaaaaggtatt gattttaaagaagatggaaacattcttggacacaaattggaatacaactataactcacacaatgtatacatcatggcagacaa acaaaagaatggaatcaaagttaacttcaaaattagacacaacattgaagatggaagcgttcaactagcagaccattatcaa caaaatactccaattggcgatggccctgtccttttaccagacaaccattacctgtccacacaatctgccctttcgaaagatcc caacgaaaagagagaccacatggtccttcttgagtttgtaacagctgctgggattacacatggcatggatgactatacaaaa ggcctgcagcaaacgacgaaaactacgctgcatcagtttaataagctgatcccatggtacgcgtgctagaggcatcaaat aaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgctctcctgagtaggacaaatc cgccgccctagacctagggcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacaga atcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttg ctggcgtttttccataggctccgccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgac aggactataaagataccaggcgtttcccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatac ctgtccgcctttctcccttcgggaagcgtggcgctttctatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgc tccaagctgggctgtgtgcacgaacccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaac ccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgcta cagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagccagttac cttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagcag attacgcgcagaaaaaaggatctcaagaagatcctttgatct
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Table SI: Vectors used in this work. Location of antibiotic resistance marker shown in red, UP element in blue (UPD-01-48 as shown), *rrn* core (*rrnD* as shown) in purple, reporter gene (GFP as shown) shown in green, and origin of replication shown in gold.

Table SII

Primer Name	Sequence	Description
MFH-24-UPA-F	cccacacatgcataaaaaaacagaaaaatattttgaaaaaaagacgtcttcgctc	Mix and match UP Element Design
MFH-23-UPA-R	gagcgaagacgtctttttttcaaaatatttttctgttttttatgcatgtgtggg	
MFH-22-UPB-F	cccacacatgcatggaaaattttttttaaaaaaagagacgtcttcgctc	
MFH-21-UPB-R	gagcgaagacgtctctttttttaaaaaaaattttccatgcatgtgtggg	
MFH-20-UPC-F	cccacacatgcatagaaaaatattttgaaaaaaagagacgtcttcgctc	
MFH-19-UPC-R	gagcgaagacgtctctttttttcaaaatatttttctatgcatgtgtggg	
MFH-18-UPD-F	cccacacatgcatggaaaattttttttaaaaaaagacgtcttcgctc	
MFH-17-UPD-R	gagcgaagacgtctttttttaaaaaaaattttccatgcatgtgtggg	
MFH-14- rrnd EcoRi-end3'	TTGTGCAAAAAATTGGGATCCCTATAATGCGCCTCCGGACGTCTTCGCTC	
MFH-13-5'end-NsiI-UPD-primer (rrnd)	CCCACACATGCATGGAAAATTTTTTTTAAAAAAATTGTGCAAAAAATTGGGATCCCTA	
MFH-12-5'end-NsiI-UPE-primer (rrnd)	CCCACACATGCATAGAAAAATATTTTGAAAAAAATTGTGCAAAAAATTGGGATCCCTA	

MFH027-GFPprimerUpstream	ttgcatcaccttcaccctctccactga	Sequencing primer
MFH028-rrnd EcoRi correct	gagcgaagaattcccgaggcgcattatagggatcccaatttttgcacaa	Library UPD-01-48
MFH0029-NsiI-UPDAatIIrrnd	cccacacatgcatggaaaatttttttttaaaaaaannnnnnttgtgcaaaaaattgggat	
MFH115-5'NsiIendfullconseqrrnd	cccacaatgcatnnaaawwtwttttnnnaaannnttgtgcaaaaaattgggatcc	Libraries PR03 and CR04
MFH116-3'endrrnd-EcoRI	gagcgaagaattccggaggcgcattatagggatcccaatttttgcacaa	
MFH117-5'NsiIend30Nrrnd	cacaatgcatnnnnnnnnnnnnnnnnnnnnnnnnnnnnnttgtgcaaaaaattgggatc	
MFH215-29-48 distal 10N library F	cccacacatgcatggaaaatnnnnnnnnnnaaaatcgtgcttgtgcaaaaaattgggatc	Library UPD48-Dist and UPD48-Far Dist
MFH216-29-48 library R	ttcgctcgaattcccgaggcgcattatagggatcccaatttttgcacaagc	
MFH217-Far distal 7N	cccacacatgcatnnnnnnnttttttttaaaaaaatcgtgcttgtgcaaaaaattgggatc	
MFH224-PROX_29-48	cccacacatgcatggaaaatttttttttaaannnnnnnnnnttgtgcaaaaaattgggatc	Library UPD48-Prox
MFH-225-REV_LIBPROX_29-48	ttcgctcgaattcccgaggcgcattatagggatcccaatttttgcacaa	
MFH226-p1_2x-29-48-rrnd	acacatgcatggaaaatttttttttaaaaaaatcgtgcggaaaatttttttttaaaaaaatc	Rational tandem UP element design

MFH227-p2_2x-29-48-rrnd	gggatcccaatttttgcacaagcacgatttttttaaaaaaaattttccgcacg	
MFH228-p3_29-48rrnd	tgtgcaaaaaattgggatccctataatgcgcctccggaattcttcg	
MFH229-Amplify 3' end	cgaagaattccggaggcg	
MFH230-Amplify 5'end	acacatgcatggaaaattttttttaaa	
MFH231-p1_29-1_29-48-rrnd	acacatgcatggaaaattttttttaaaaaaatagcggggaaaatttttttaaaaaaatc	
MFH232-p2_29-1_29-48-rrnd	gggatcccaatttttgcacaagcacgatttttttaaaaaaaattttccccgct	
MFH233-p1-UPD2948	acacatgcatggaaaattttttttaaaaaaaatgcatggaaaattttttttaaaaaaatc	
MFH234-p2-UPD2948	gggatcccaatttttgcacaagcacgatttttttaaaaaaaattttccatgcatttttt	
MFH235-p1_115-48_29-48	acacatgcattaaaatatttttttttaaaagagggaaaatttttttttaaaaaaatcg	
MFH236-p2_115-48_29-48	gggatcccaatttttgcacaagcacgatttttttaaaaaaaattttccctctttaaaa	
MFH237-5'amplify_115-48_29-48	acacatgcattaaaatatttttttttaaaagagg	
MFH238-p1_115-89_29-48	acacatgcattgaaaatttttttaggaaaatgggaaaatttttttttaaaaaaatc	

MFH239-p2_115-89_29-48	gggatcccaatttttgcacaagcacgatttttttaaaaaaaattttcccattttcctaa	
MFH240-amplify_115-89_29-48	acacatgcattgaaaatttttttagga	Libraries Tandem Proximal (TP) and Tandem Distal (TD)
MFH256-tandemproxlib	cacacatgcatggaaaatttttttttaaannnnnnnnnnnggaaaatttttttttaaaaaatcgtgcttgtgcaaaaaattgggatccc	
MFH256-AmplifytandemUP	gagcgaagaattcccgaggcgcattatagggatcccaatttttgcacaa	
MFH258-tandemDistalUP	cacacatgcatnnnnnnnnnnttttaaaaaaatcgtgcggaaaatttttttttaaaaaaatcgtgcttgtgcaaaaaattgggatccc	
KP103_F1_hcaT_qPCR	TGGAGCGTCTGGCTTAAAGG	qPCR primers for housekeeping gene, hcaT
KP104_R1_hcaT_qPCR	AGAGAAGTGTCAGCAGTGCC	
KP111_F1_GFP_qPCR	ACCTGTCCACACAATCTGCC	qPCR primers for GFP
KP112_F1_GFP_qPCR	GCAGCGTAGTTTTCGTCGTT	
KP134_GFP_UP_into_pACYC	aagcaaattcgacccggtcgGCGAAACGATCCTCATCC	Insertion into pACYC background
KP135_GFP_UP_into_pACYC	AGGGCATCGGTCGAGATCCCgccttttacggttcctgg	
KP136_pACYC_F	GGGATCTCGACCGATGCC	
KP137_pACYC_R	cgaccgggtcgaatttgc	

KP142_AmpR_into_UPpZE	ATGAGTATTCAACATTTCCGTG	Substitution of β-lactamase for GFP
KP133_AmpR_into_UPpZE	gtctgacagttaccaatgc	
KP079_inv_PCR_rev	GAATTCATTAAAGAGGAGAAAGGTACC	Variable rRNA core promoters
KP080_29-48-and-tp24-rrnA-invPCR	TAgggagttattccggccTGACAAGCACGATTTTTTTAAAAAAAATTTTCC	
KP081_29-48-and-tp24-rrnH-invPCR	tagggagtcggctcaggaagACAAGCACGATTTTTTTAAAAAAAATTTTCC	
KP082_rrnAcoreonly-invPCR	TAgggagttattccggccTGACAAatgCATgccATCGAT	
KP083_rrnHcoreonly-invPCR	tagggagtcggctcaggaagACAAatgCATgccATCGAT	
KP084_rrnA_core_insert_fwd	CAggccggaataactcccTATAAtgcgccaccagaattcattaaagaggagaaaggtacc	
KP085_rrnH_core_insert_fwd	cttcctgagccgactccctataatgcgcctccagaattcattaaagaggagaaaggtacc	
KP086_rrnA_core_insert_rev	ggtacctttctcctctttaatgaattctggtggcgcaTTATAgggagttattccggccTG	
KP087_rrnH_core_insert_rev	ggtacctttctcctctttaatgaattctggaggcgcattatagggagtcggctcaggaag	

Table SII: Primers used in this work

Table SIII

Region	Sequence	Relative Activity	UP element present in
Proximal1	aaaaaaaga	170	UPA, UPB, UPC, UPD, UPE
Proximal2	aaaaaaaca	160	UPA
Distal1	agaaaaatattttg	16	UPA, UPC, UPE
Distal2	ggaaaatttttttt	16	UPB, UPD

Table SIII: Proximal and distal UP element regions used in rational design. Regions were identified through RNA polymerase binding assays. Relative activity determined elsewhere (Estrem et al. 1999) is a measure of β-galactosidase expression.

Table SIV

Construct	Sequence
rrnD	TTGTGCAAAAAATTGGGATCCCTATAATGCGCCTCCG
UPA	AAAAAAACAAGAAAAATATTTTGAAAAAAAGA
UPB	GGAAAATTTTTTTTAAAAAAAGAGACGTC
UPC	AGAAAAATATTTTGAAAAAAAGAGACGTC
UPD	GGAAAATTTTTTTTAAAAAAAGACGTC
UPE (previously UP1)	AGAAAAATATTTTGAAAAAAAGACTGC
UPD-01-48	GGAAAATTTTTTTTAAAAAAATCGTGC
UPD48-Prox-18	GGAAAATTTTTTTTAAAGGGGGGACCC
UPD48-Dist-66	GGAAAATCGGGTTGCTAAAAATCGTGC
UPD48-Far dist-62	CATCATCTTTTTTTAAAAAAATCGTGC
TP-24	AGACTAAGTGTTTTAAAAAAATCGTGCGGAAAATTTTTTTTAAAAAAATCGTGC

Table SIV: Sequences of selected UP elements.

Table SV

Tandem Up Element Name	Sequence	Primer Source1	Primer Source 2
226	ggaaaatttttttaaaaaaatcgtgcggaaaatttttttt aaaaaaatcgtgc	MF226	Mf227
231	ggaaaatttttttaaaaaaatagcggggaaaatttttttt taaaaaaatcgtgc	MF231	MF232
235	taaaatatttttttaaaagagggaaaatttttttttaaaaa aatcgtgc	MF235	MF236
238	tgaaaatttttttaggaaaatgggaaaatttttttttaaaa aaatcgtgc	MF238	MF239

Table SV: Rational tandem library. Combinations of UP element sequences used in construction of mix-and-match rational tandem repeat library.