

Title: Quantitative tracking of combinatorially engineered populations with multiplexed binary assemblies

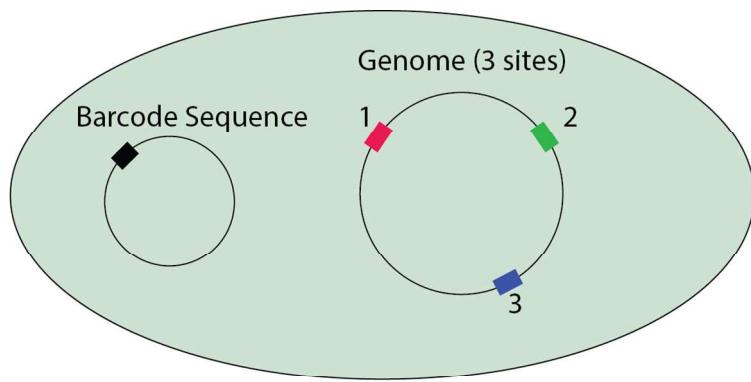
Author Information

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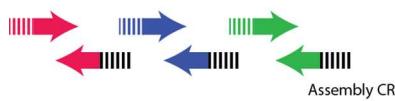
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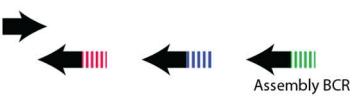
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Site Specific Primers with
Linkers and Universal Ends



Barcode Specific Primers



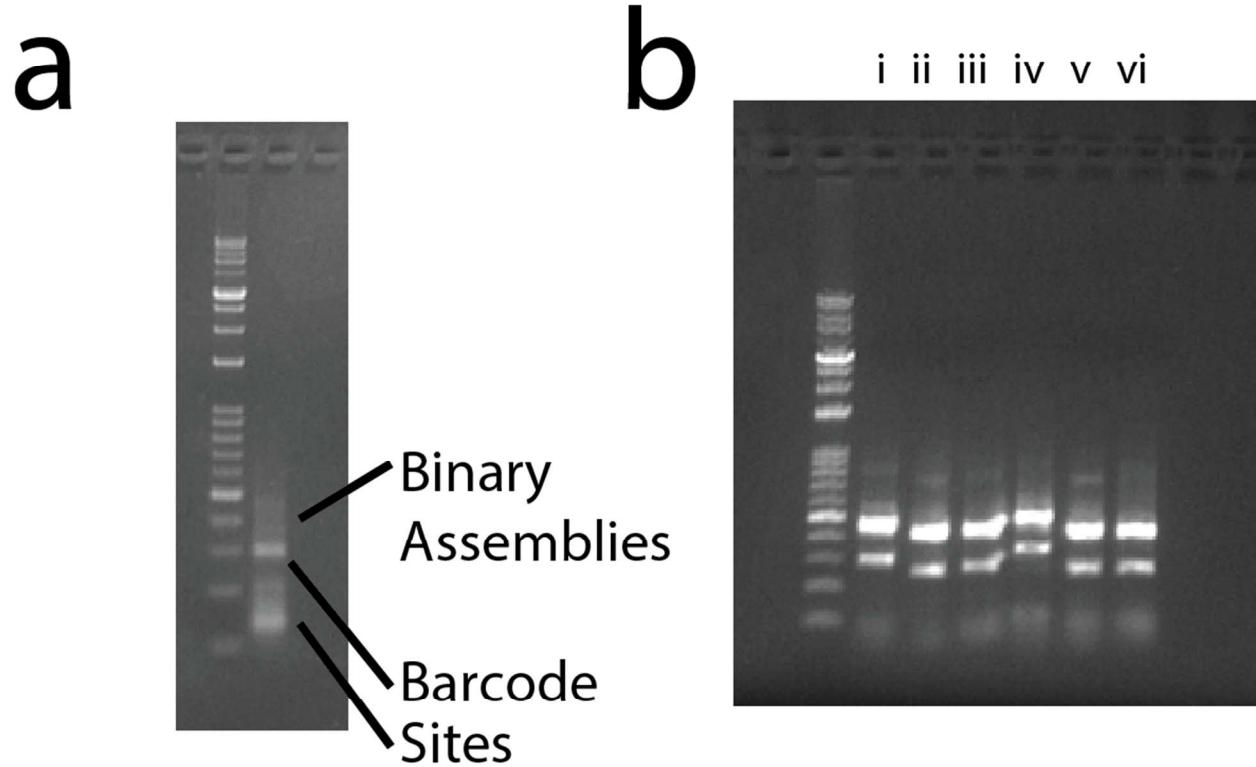
Barcode - Site Assemblies



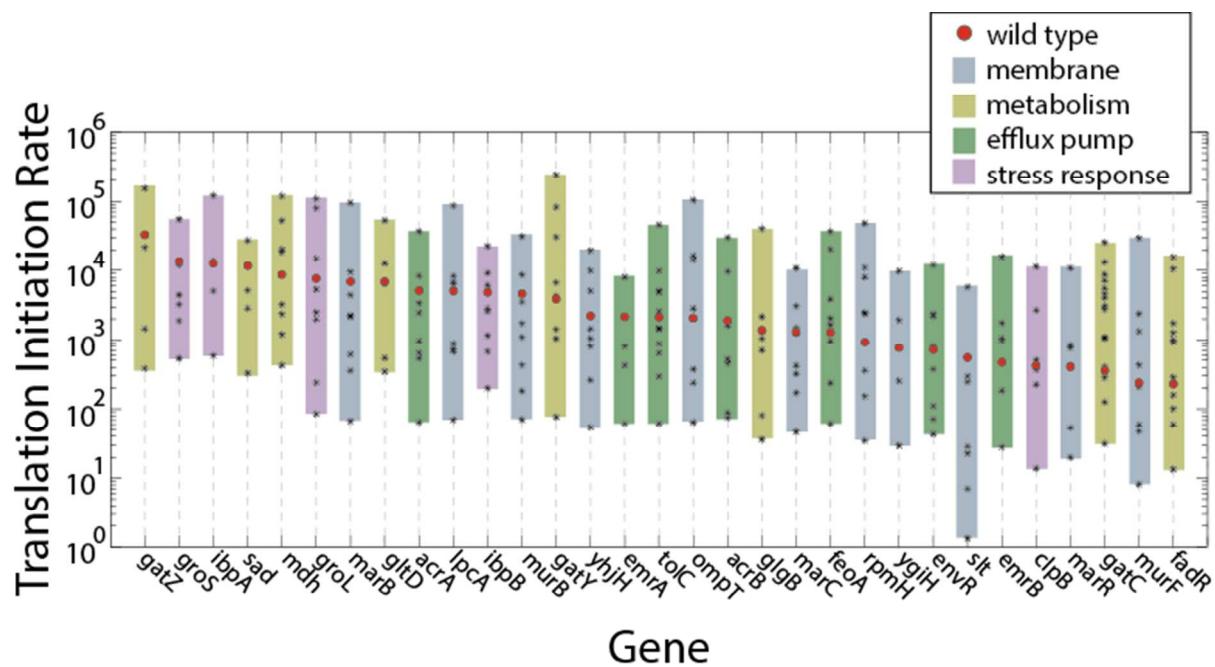
MiSeq Adapter Primers



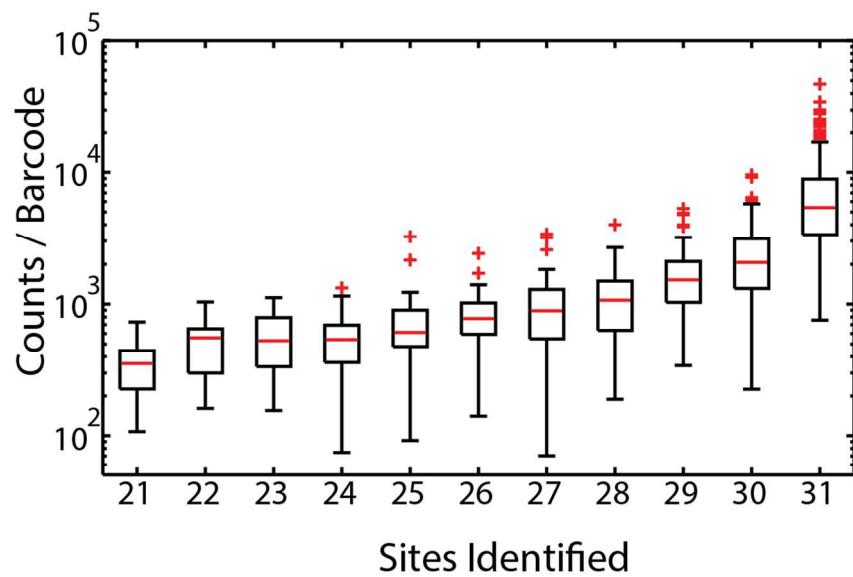
Supplemental Figure 1: Primer design for multiplexed binary assembly. Assembly BCR and Assembly CR primers are labelled.



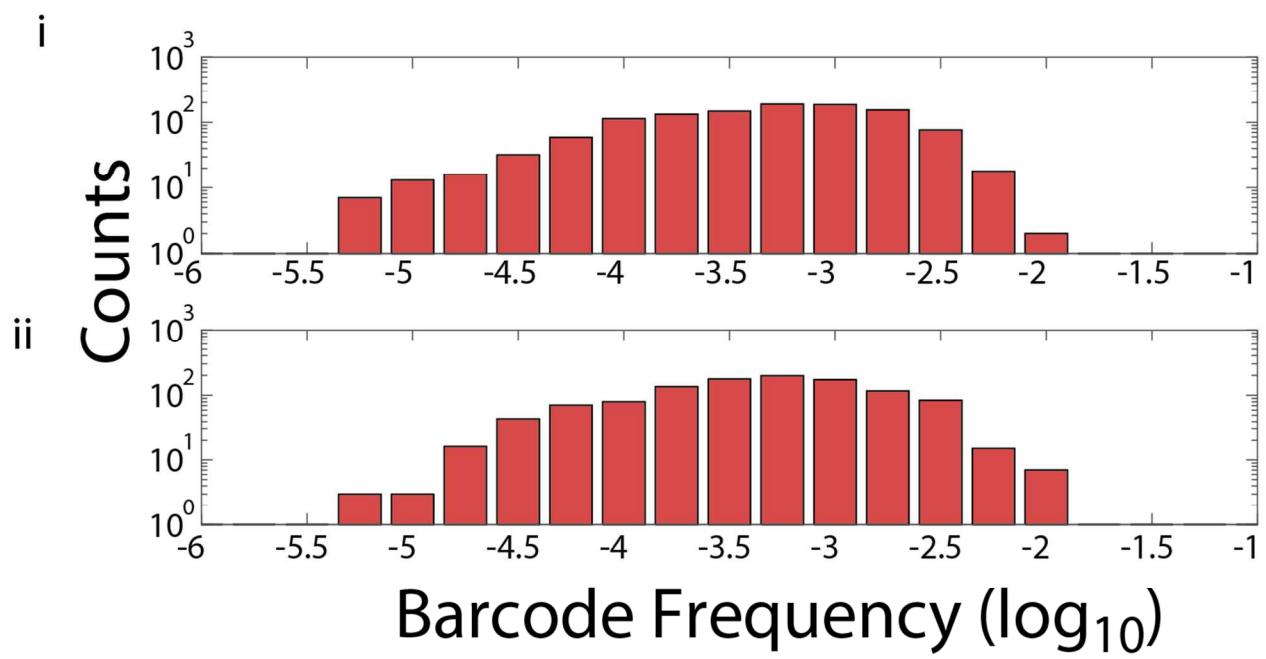
Supplemental Figure 2: Multiplexed binary assemblies. a) Gel electrophoresis image of the multiplex assembly of 6 sites with barcodes in a one-pot reaction. 100 bp ladder used. b) Amplification of each individual site from the gel extracted binary assembly. The indicies i-vi represent the 6 assembly genes (*murB*, *murF*, *lpcA*, *ompT*,*ygiH*, *slt*). A 100 bp ladder is used.



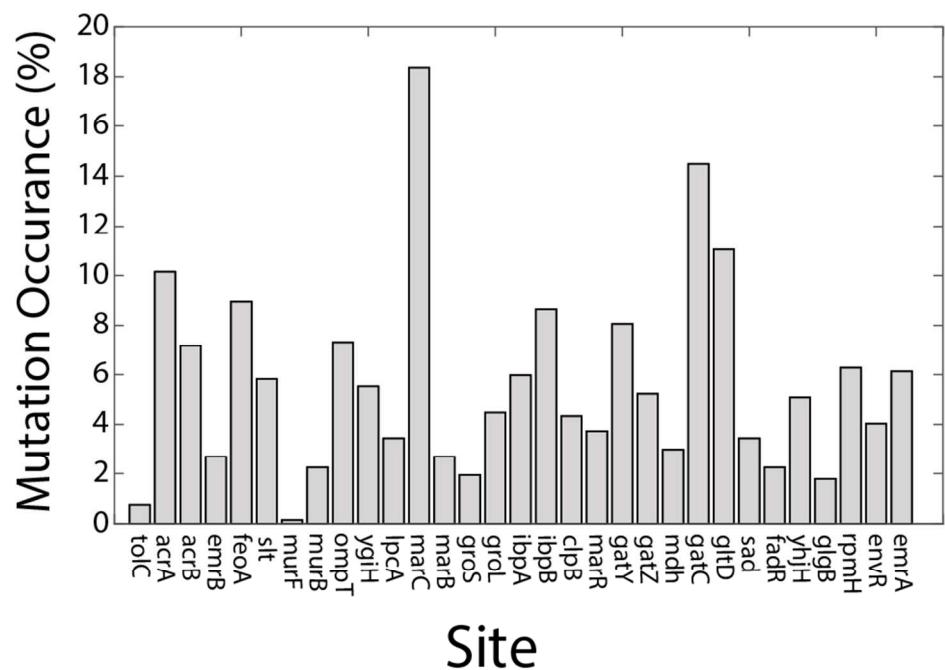
Supplemental Figure 3: Translation initiation rate design for RBS library.



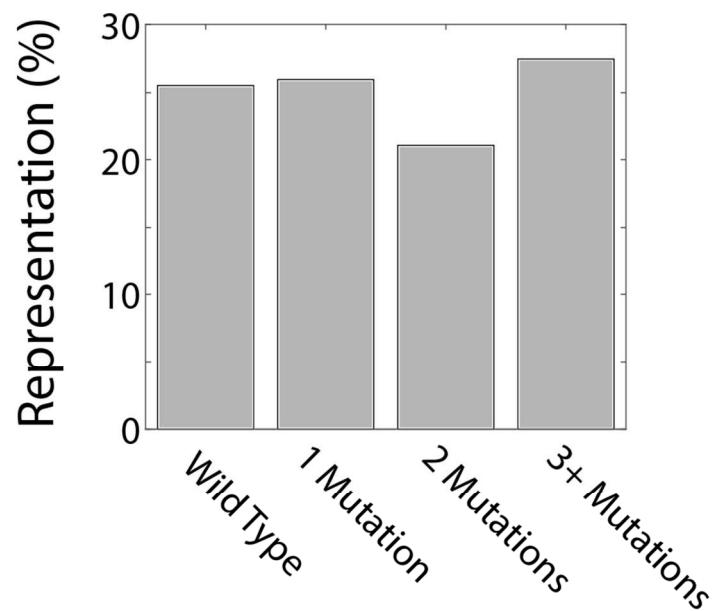
Supplemental Figure 4: Counts per barcode with respect to number of linkages identified per genotype.



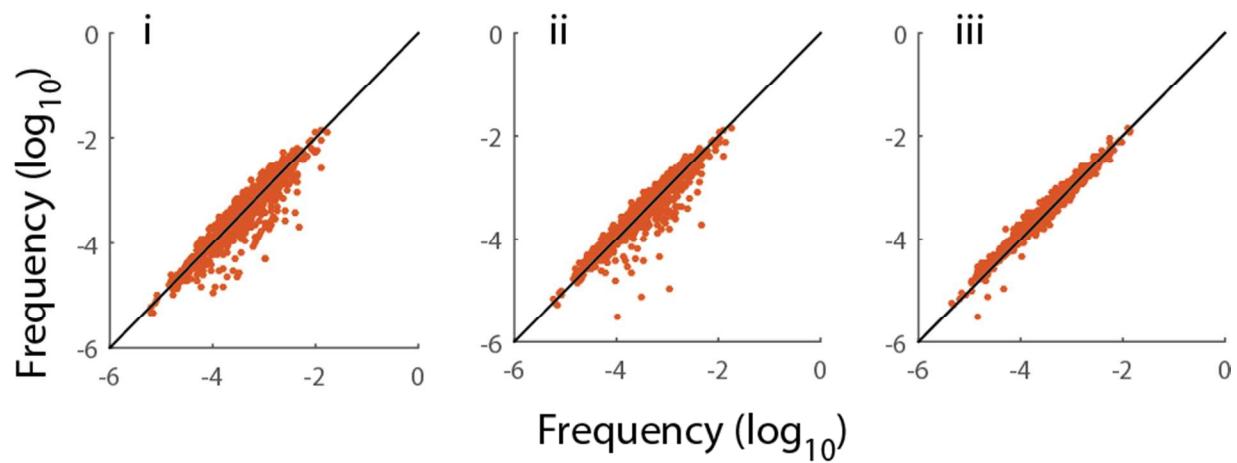
Supplemental Figure 5: Histograms of barcode frequency in the populations occurring during i. ePCR assembly; ii. quantitative barcode sequencing (of an initial population).



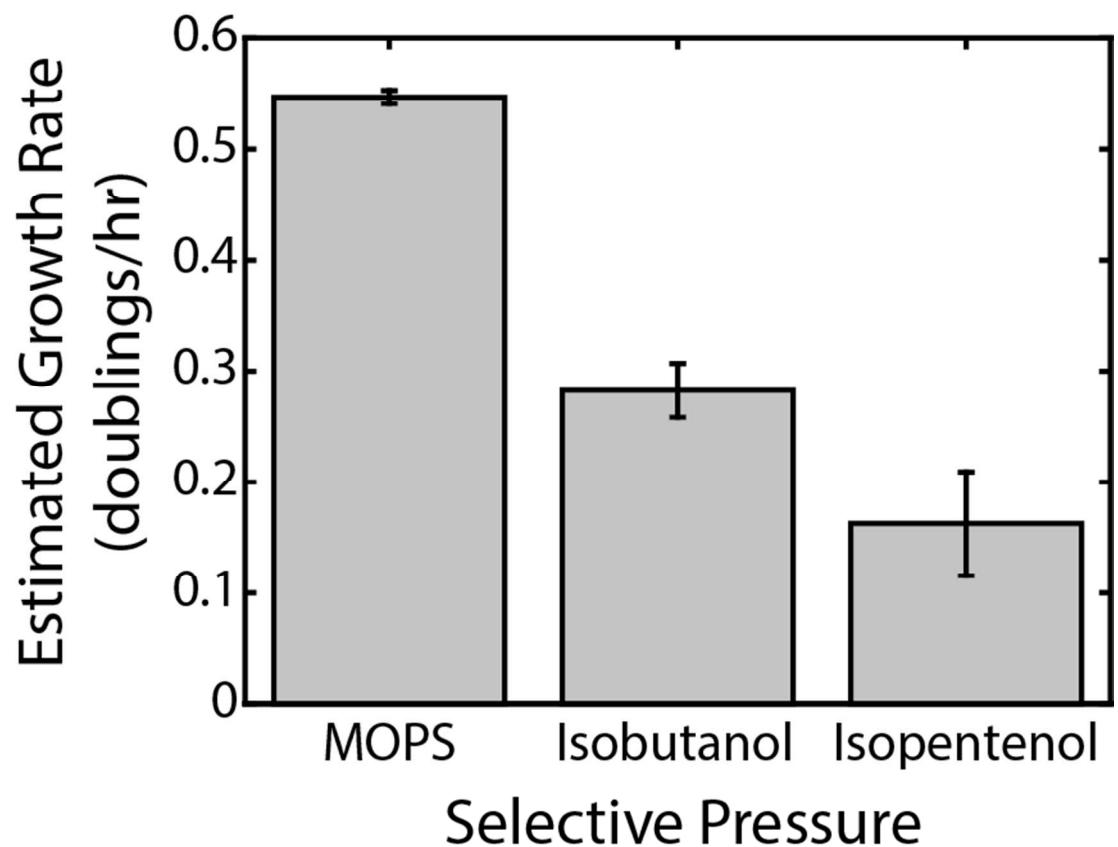
Supplemental Figure 6: Mutation rate for each site in the 31-site library.



Supplemental Figure 7: Representation of gentotypes in the filtered 31-sites library.



Supplemental Figure 8: Barcode frequencies between between three technical replicates. These replicated were measured from three overnight cultures (harvested from a single freezer stock) and grown in MOPS minimal media for 24-hours. N = 1121 genotypes, i. Replicate 2 versus Replicate 1 ($r^2 = 0.86$, $p << 10^{-6}$); ii. Replicate 1 versus Replicate 2 ($r^2 = 0.88$, $p << 10^{-6}$); iii. Replicate 3 versus Replicate 2 ($r^2 = 0.97$, $p << 10^{-6}$).



Supplemental Figure 9: Growth data of three conditions and three selection technical replicates apiece. Growth rate is calculated as the number of doublings the population underwent versus the amount of time elapsed before the population was harvested.

Software Output

Universal Barcode Forward Primer:
→ TTCAGGCTGCGCAACTGTTGG

Barcode Reverse Primers:
→ TCCCTAGGTGCCCTGACATTACTCGGCCTTGTGGAATTGTGAGCGGATAACA
→ TGACGTAGCAGACGAGGAGTGCAGAGGATTGTGGAATTGTGAGCGGATAACA

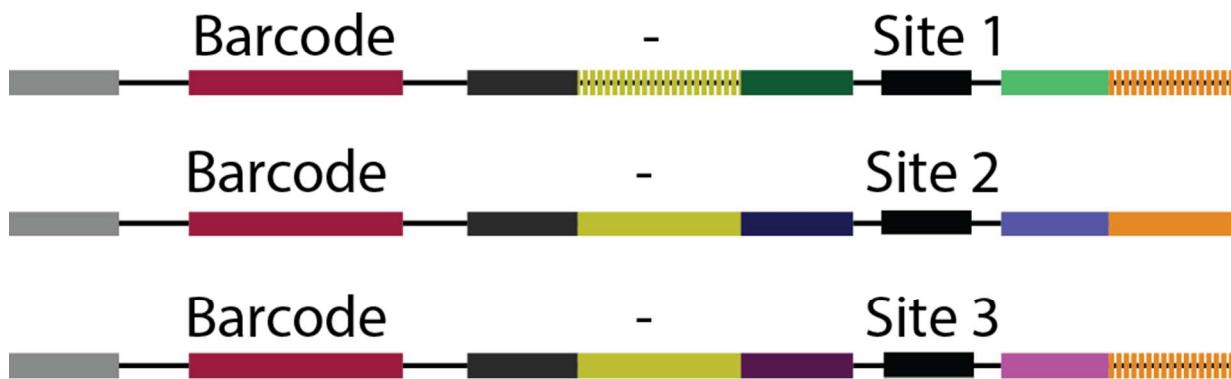
Site Primers

Primer Set 1
→ F: TCCTCTCGCACTCCCTCGTCTGCTACGTCAGGCTTCTGCTAGAATCCGAATAATT
→ R: GCAGAACCGCAGGTTCCCGATAGGATGGTAAGGCTCAGGCCGATAAGAATGG

Primer Set 2
→ F: GGCGAAGTAATGTCAGGGCACCTAGGGAGTGAATGTATGTACCATAGCACGACGA
→ R: AGTAGACGAACGAGTACCGCCAGTGTCCGAGCATCAGAACGACC GCCAGA

Primer Set 3
→ F: GGCGAAGTAATGTCAGGGCACCTAGGGATAACCAGCAAGCCGCAAGCG
→ R: GCAGAACCGCAGGTTCCCGATAGGATGGTATAATGGCGATCACCCACGCAA

Universal Reverse Adapters
→ GCAGAACCGCAGGTTCCCGATAGGATGGT
→ AGTAGACGAACGAGTACCGCCAGTGTCCG



Supplemental Figure 10: Graphical representation of the output from the bTRACE primer design software. In the case of assembling three sites, two linkers are designed and two universal end sequences are designed to assemble three barcode-site constructs in a single reaction.

Table 1: Predicted Genotypes from Colony PCR Sequencing versus barcode index number. Mutant sites are highlighted in red and genotyped colonies are highlighted in yellow. Index represents the location of the barcode in the filtered high-throughput sequencing data.

Site Sequence							
#	Index	<i>murB</i>	<i>murF</i>	<i>lpcA</i>	<i>ompT</i>	<i>ygiH</i>	<i>slt</i>
1	1686	ATCAAGAACGGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGATGCGCTT
2	271	ATCAGGAAAGCAGCT	GCTAGAGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAAGGAGCCAGCTT	TTAGAGGATGCGCTT
3	686	ATCAGGAAAGCAGCT	GCTAGAGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAAGGGGCCAGCTT	TTAGAGGATGCGCTT
4	1581	ATCAGGAAAGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GCGCGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGATGCGCTT
5	507	ATCAGGAAAGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GAGCGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGATGCGCTT
6	1113	ATCAGGAAAGCAGCT	GCTAGGGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGATGCGCTT
7	1307	ATCAGGAAAGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAAGGGGCCAGCTT	TTAGAGGATGCGCTT
8	175	ATCAGGAAAGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GCGCGCATGGAGAAC	CGAATTTCAAGCTT	TTAGAGGATGCGCTT
9	868	ATCAGGAAAGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGAGGGCCTT
10	1596	ATCAAGGACGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGATGCGCTT
11	1390	ATCAGGAAAGCAGCT	GCTAGAGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAAGGAGCCAGCTT	TTAGAGGATGCGCTT
12	1866	ATCAGGAAAGCAGCT	GCTGGGGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAACGGAACAGCTT	TTAGAGGATGCGCTT
13	1912	ATCAGGAAAGCAGCT	GCTGGAGGTGATTGC	CTGAAGGATATCCTC	GATTGAATGGAGAAC	AAAAGGGGCCAGCTT	TTAGAGGATGCGCTT

Table 2: RBS Gene targets for 31-site library

membrane	metabolism	membrane transport	stress response
<i>marB</i>	<i>fadR</i>	<i>acrA</i>	<i>groS</i>
<i>marC</i>	<i>gatC</i>	<i>acrB</i>	<i>groL</i>
<i>marR</i>	<i>gatY</i>	<i>feoA</i>	<i>clpB</i>
<i>murB</i>	<i>gatZ</i>	<i>emrB</i>	<i>ibpB</i>
<i>murF</i>	<i>glgB</i>	<i>tolC</i>	<i>ibpA</i>
<i>ompT</i>	<i>gltD</i>	<i>envR</i>	
<i>slt</i>	<i>mdh</i>	<i>emrA</i>	
<i>ygiH</i>	<i>rpmH</i>		
<i>yhjH</i>	<i>sad</i>		
<i>lpcA</i>			

Table 3: Commonly used primers across all libraries

Name	Primers (5'-3')
BCF	TTGTACTGAGAGTGCACCATATGNNNNNNNNNNNNNGATCCGGGCCGTCAGTCAGAGGCCTGCAT GCAAGCTTGGCGTAATC
BCR	GATTACGCCAAGCTTGCATGCAGG
LinF	GTTGTAAAACGACGCCAGTTGACTGAGAGTGCACCATATG
LinR	CACAGGAAACAGCTATGACCGATTACGCCAACGCTTGCATGC
HiSeq-pA-F	ACACTCTTCCCTACACGACGCTCTCCGATCTGCCAGTTGACTGAGAGTGCACC
HiSeq-pA-R	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCGATTACGCCAACGCTTGCATGCAGGCC
HiSeq-A-F	AATGATA CGGC ACCACCGAGATCTACACTCTTCCCTACACGACGCTCTCCGATCT
HiSeq-A-R	CAAGCAGAAGACGGC NNNNNNAGATATCACGGTACTGGAGTT CAGACGTGCTCTCCGATC
pA-F	TCTATCACGCCGAACCGTATTGCAGCAGCTTGGCAAAGGGGGATGTG
MiSeq-A-F	AATGATA CGGC ACCACCGAGATCTACACTATGTAATTGTTCTATCACGCCGAACCGTATTGCAGCAGCT
MiSeq-A-R	CAAGCAGAAGACGGCATA CGAGATNNNNNNNNNAGTCAGTCAGCCTCTGCCGGCG
Read 1	TCTATCACGCCGAACCGTATTGCAGCAGCT
Read 2	ACGGGATAATACCGCGCCACATAGCAG
Index Read	CGTATTGACGCCGGCAAGAGGCTGACTGAC

Table 4: Degenerate MAGE Oligomers used

Site	Library	Oligomer Sequence (5'-3')
slt	6 site	C*T*T*GTTGGCTTTCCACAAGCGCCTSCWCTAAGTGTAAATACATCAATGCAGCGTCATGCCCGTA ATAGATTATGCTAACCGAGTC
murF	6 site	C*A*C*CTTGCAGTTCACCGTTGAGAATGTCGTAAGTTGGCTAAGGGTAGCCTAATCATGCAATCACCCY CYMGCAGACGCCACCGTGAC
murB	6 site	G*T*G*TTCCAGGGTTAACCGAGTGGTTCATAGCTGCSTYYTTGATGCAAAAACGAGGCTAGTTACCGT ATCTGTGGGGGGATGGCTTGTA
ygiH	6 site	A*T*T*ATCCACAGATTCATCGTTAACACGAATTTCAAAAKGVGSCAGCTTATGAGTGCAATCGCGCT GGAATGATCCTCATCGCGTACC
lpcA	6 site	T*A*G*CCAGCGTTCCGCCGCTTCGTTAGTCGTTACGAATAAGATCCTGGTACATGAGGAYCTYMTTC AGCATAAATGTAATAGACAAAAA
ompT	6 site	G*A*C*TCATTAAGTTAGATATAAAAAATACATATTCAATCATTAAACGMSCGMATGGAGAACTTTATG CGGGCGAAACTTCTGGGAATAG
acrA	31 site	G*C*A*GCAATGGGTTATTAACCTTGACCATTGACCAATTGAAATCGGACACTSGRAGKTTACATATG AACAAAAACAGAGGGTTACGC
acrB	31 site	A*T*A*ACCAGCAAGCCCAAGCGGTGCTCAGCCTGAAACAGTCCAAGTCTTAACCTAAACAGSAKGTGYTA AGACATGCCTAATTCTTATC
clpB	31 site	A*A*T*TTATTAGTAAGACGATCCAGACGCATAACTCCTCCCATAACGGATCAAATATGVKCCTGGAGATT AAATGAGGTCTCCCTCAATTAA
emrA	31 site	G*A*T*GAGTTAAGAAGATCGAGKRGACAATATGAGCGCAAATGCGGAGACTCAAACCCCGCAGCAACCG GTAAAGAAGAGCGGAAACGTA
emrB	31 site	A*C*C*TGTCAATAAACTGATCGACGATATCGTAAAGCTAACGCTGGCTAATAAGGHSGTGCCTGTGATG CAACAGCAAAACCGCTGGAAAG
envR	31 site	G*C*C*GGGTCTTCAGAGCTCGGCTTGGTTCTTTGCCATGATTAATTATCMTKAGAYAAATATATT CGACACAGAGTGAGAAAATAGC
fadR	31 site	G*G*G*CTTGCGCTTAATGACCATAGTGAGACYTCHTKAACACAGCAAAACAAAGTTGGACTCATCATA CCAGAGGGCTAACTACGCTGAT
feoA	31 site	C*T*G*TCAAACGCCCATGAGGTAGTTATCCAGTTAATGAGAAACAAGKRGKCCCTATGCAATAACACTC CAGATACTCGTGGAAAATCAC
gatC	31 site	A*T*A*ACCGATGACTTCTGAAAACATAASTMMCSCTGTAAGATAGTCAGAATTATTTGTAATGCTT CGATACCGATACCAGAAATAAA
gatY	31 site	G*T*T*CAGCATCTGCTTGTGATACCACGTACATTTCACSTCMCSTCGTTGTTGATTTCAAAT ATAATGAAATTATTTGTTTTA
gatZ	31 site	G*A*T*ATGTTACCGAGCTTATGCCGGCAATTACGTTTCTSCYCATTAAATGGCAGGTG CGTTATGCCCTGCCCTCGCAGC
glgB	31 site	T*C*A*CGTCTCTATCGATACGATCGGACATGCTCGACHTSCTGTTTATTGTGTACCCCCATCCAATTGG ATTTTGACTTCTGGTTGTGAC
gltD	31 site	G*G*G*TCACCGTAGTCGAGCGCAGCTGAGTTGCGCGTGCWGCMKTAGTAAGGGTAGCAACAAATGAGTC AGAATGTTATCAATTATCGA
groL	31 site	A*T*T*TTACGTCTTAGCTGCCATTATCTWMCTMCTTAAATTCTGTTAGTCAGTGTGCGCGGATT CGCTTCAACAATTGCCAGAATG

groS	31 site	T*T*G*ACGATCACCGCATGCAATGGACGAATTCTTGTAACTCTCCWTATGDAAGTCCGTATC TGTTATGGGTGACGCCGGAGCT
ibpA	31 site	A*C*G*GTAAGCGGGATAAAATCAAAGTTACGCATAATCAATACCTSCTKAAATCAGCGAGAATGTAAGA CCTTCCACAAATGGACAGGTAG
ibpB	31 site	A*T*T*GACGCATCAGTGGGATAAAATCGAAGTTACGCATAATCASWYCTCCTCTAAGAAGCGAGTAAGT ACCTGCAAATCCGAAGATTGCG
lpcA	31 site	C*G*C*CGCTTCGTTCAAGTACGAATAAGATCCTGGTACATGAKGAYCTSCTCAGCATAATGTAAG TAGACAAAATGCAGTGTACCGG
marB	31 site	A*T*T*AAATCATTACAAACAGCTAGTTGAAAACGTGACAACGTCAASGWRGTAATCATGAAACCAC TCCGCAATAGCAGCTGCGCTTA
marC-1	31 site	C*A*C*CACCAGCCCCAGGCCATTGTTAAACAAATCTAACATTGGTGACWGAMCTCCTGTATCTGG GTTATCAGCGAAAAGTATAAGG
marC-2	31 site	C*A*C*CACCAGCCCCAGGCCATTGTTAAACAAATCTAACATTGGTGGTTATCCTGTATMCTG GKTATCAGCGAAAAGTATAAGG
marR	31 site	T*G*G*GCAATATTATCCCCTGCAACTAATTACTTTTHASGACAACTAATGTGAAAAGTAC CTTCATGAAATTATTCCATTGG
mdh	31 site	C*T*G*GCCAATACCGCCAGCAGGCCGAGGACTGCGACTTCATTCTRRCSTCCTTATTATATTGATA CTAAGATATGTTGCTCCGCTGC
murB	31 site	T*G*C*CAAATGTGTTCCAGGGTTAAAGGAGTGGTTCATAGCTRCMTGATGCAAAAACGAGGCTAG TTTACCGTATCTGTGGGGGAT
murF	31 site	C*G*T*TGAGAATGTCGGTAAGTGGCTAAGGGTTACGCTAACATGCAATCASCWCSCGCAGAC CCGTGACGCGATCGGAGTAGTC
ompT	31 site	T*A*T*GTAATTGACTCATTAAGTTAGATATAAAACATATTCAATCATTAAAACGATTATWWGKAGA ACTTTATGCGGGCGAAACTTC
rpmH	31 site	T*T*C*GGCGGATATCCAATAAAGCCATTGAATTATTCAAGTTAGGKAGRKATGCCATGAAAC CTTCACCGTCTGTACTGAAGC
sad	31 site	C*G*A*AATTGCAAGGTTGCCGGAGTAATGGTCATGGGTAYCTCMTCTATGAGTCATGGTATGAAG ACGCAGATTACTCTGCTTTA
slt	31 site	C*A*G*GTAACTTGGCTTTCCACAAGCGCMTCWCAAAGTGTAAATACATCAATGCAGCGTCATG CCCGCTAATAGATTATGCTAA
tolC	31 site	A*T*T*TTACAGTTGATCGCGCTAAATACTGCTTACCCACAAGGRGDKTAATGAAGAAATTGCT TTCTTATCGGCCTGAGCCTTC
ygiH	31 site	C*G*A*AATGTGCGTATTATCCACAGATTACATGTTGAACACGAATTTCAGGASGTRACAG GCAATCGCGCCTGGAATGATCC
yhjH	31 site	T*C*A*GGGTTGCTTATTGCTGGATAACCTGCCTTATCATCAWASACSTCCTGTGTTAAAAGT GATGCTGCCGGACTCGACAAAGATTAT

Table 5: Assembly Primers for the 6-Site Library

Name	Linker	Primers (5'-3')
Assembly-BCR-1	BCR-1	TCAGCCTATGGTGGGAGGGTCGAAATCTTGTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-2	BCR-2	GGGTTTGCATGGTGGCTAGGCAGAACATCAGTTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-3	BCR-3	ATTAACGACGGGTCGGTGGTACTGGACGATTGTGGAATTGTGAGCGGATAACA
Assembly-CR-1	CR-1	TGCAAAGCGAAGAGGGACTGAGACTGCTG
Assembly-CR-2	CR-2	CAGGATACGACCGACCGCTGTACTCCCGTT
Assembly-CR-3	CR-3	GCAATGACGGGTAAAGCAGGGCCGAACAT
murB-F	BCR-2	CTGATTTGCCCTAGCCACCATGCAAACCCCTCCCTGTCGTATCTACAAGCCATC
murB-R	CR-1	TGCAAAGCGAAGAGGGACTGAGACTGCTGTATGATCAATGCCAATGTGTTCCAGG
slt-F	CR-3	AGATTTCCGACCCCTCCACCATAGGCTGACGGCATGACGCTGCATTGATG
slt-R	BCR-1	GCAATGACGGGTAAAGCAGGGCCGAACATCAACAGCCGCCAGGTAACCTGT
murF-F	BCR-2	CTGATTTGCCCTAGCCACCATGCAAACCCCAGCGTCTGGACTACTCCGATC
murF-R	CR-1	TGCAAAGCGAAGAGGGACTGAGACTGCTGTTGAGAATGTCGTAAGTTGGCTAAGG
ompT-F	BCR-3	TCGTCCAGTACCAACCACCGACCCGTCGTTAAATAACAGATAGCGCATTGTGATAATCATTCAA
ompT-R	CR-2	CAGGATACGACCGACGCTGTACTCCGTTAGGACTATTCCCAGAAGTTCGCC
lpcA-F	BCR-1	AGATTTCCGACCCCTCCACCATAGGCTGAGCGGTATCCGGTACACTGCATTT
lpcA-R	CR-3	TGCAAAGCGAAGAGGGACTGAGACTGCTGTCGTTACGAATAAGATCCTGGTACATGAG
ygiH-F	BCR-2	CTGATTTGCCCTAGCCACCATGCAAACCCGTGCGTATTATCCACAGATTGATCGTTG
ygiH-R	CR-2	CAGGATACGACCGACGCTGTACTCCGTTGAGGATCATTCCAGGCGCGAT

Table 6: Assembly Primers for the 31-Site Library (Set A)

Name	Linker	Sequence (5'-3')
<hr/>		
Assembly-BCR-1		GGCACGGATAAACCAAGAGCGGCACGAAACTTGTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-2		CTATACCGTCCTGGGTGTGGTGGCAGTTGTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-3		CTCTCTGTCTTGGGTGTCGTGGTGGCTGATTGTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-4		TCATTGCTAGGTGTCCCAGGGAACGCATCTTGTGTGGAATTGTGAGCGGATAACA
Assembly-CR-1		CACAGTAAGGTGAAAGGTGTCCGGTGCCTGCGT
Assembly-CR-2		TAATTCGCGGCACGAGGAGCAAGCAACA
Assembly-CR-3		TACCCGCACTAGCCATGTTGCATGTGGAG
Assembly-CR-4		CCC GTTAGGGAGCACCAGCTTCAATGGA
Assembly-CR-5		GAGGGCCTTAGGGACTTCCTGCTCGGATT
Assembly-CR-6		TGCTGCTGTCGGTGGCTGCAATTAGAC
tolC-F	BCR-2	ACTGCCACCCACACCAAGGACGGTATAGGCCTCTGCTAGAATCCGCAATAATT
tolC-R	CR-1	CACAGTAAGGTGAAAGGTGTCCGGTGCCTGCGTAAAGGCTCAGGCCGATAAGAATGG
acrA-F	BCR-4	GATGCGTCCCTGGGACACCTAGCAATGAGTGAATGTATGTACCATAGCACGACGA
acrA-R	CR-3	TACCCGCACTAGCCATGTTGCATGTGGAGAGCATCAGAACGACCGCCAGA
acrB-F	BCR-3	TCAGCCACCCACGACACCAAGACAGAGAGATAACCAGCAAGCCGCAAGCG
acrB-R	CR-5	GAGGGCCTTAGGGACTTCCTGCTCGGATTATAATGGCGATCACCACGCCAAA
emrB-F	BCR-4	GATGCGTCCCTGGGACACCTAGCAATGAGTCCACTCCGGTAGCGGTAAG
emrB-R	CR-2	TAATTCGCGGCACGAGGAGCAAGAACACCTGCATGAATGTCGCCAGTGA
feoA-F	BCR-2	ACTGCCACCCACACCAAGGACGGTATAGCATCTGGTCTCATGTCGCTGTCA
feoA-R	CR-1	CACAGTAAGGTGAAAGGTGTCCGGTGCCTGCGTAAACATGCCAAGAGAAAGCAGTT
slt-F	BCR-3	TCAGCCACCCACGACACCAAGACAGAGAGGCATAAAATCTATTACGCGGCATGACG
slt-R	CR-1	CACAGTAAGGTGAAAGGTGTCCGGTGCCTGCGTAAACAGCCGCCAGGTAACTTGT
murF-F	BCR-4	GATGCGTCCCTGGGACACCTAGCAATGAAATCAGCGTCTGGACTACTCCGA
murF-R	CR-1	CACAGTAAGGTGAAAGGTGTCCGGTGCCTTGAGAATGTCGGTAAGTTGGCTAAGG

murB-F	BCR-1	GTTTCTGTGCCGCTCTGGTTATCCGTGCCCTCCTGTCGTATATCTACAAGCCATC
murB-R	CR-5	GAGGGCCTTAGGGACTTCCTGCTCGGATTAATTGTTGTTCGTCTCGGCACATACA
ompT-F	BCR-3	TCAGGCCACCACGACACCCAAGACAGAGAGAAACAGATAGCGCATTGTGATAATCATTCAA
ompT-R	CR-3	TACCCGCACTAGCCATGTTGCATGTGGAGACTATTCCCAGAAGTTCGCCCG
ygiH-F	BCR-3	TCAGGCCACCACGACACCCAAGACAGAGAGATATCCATCACGTCCGTGCTGCT
ygiH-R	CR-1	CACAGTAAGGTGAAAGGTGTCCGGTGCATCATCCAGGCGCGATTGCA
lpcA-F	BCR-1	GTTTCTGTGCCGCTCTGGTTATCCGTGCCACCTGCCGTACTTCTCGCTT
lpcA-R	CR-4	CCCGTTAGTGGAGCACCAGCTTCAATGGATTCCGCCGCTTCGTTCAGTTC
marC-F	BCR-2	ACTGCCACCCACACCCAAGGACGGTATAGTCATGCGATGCAAGGCTAGCTAA
marC-R	CR-2	TAATTCGCGGCACGAGGAGCAAGAACATAACGGCAGCAACACCACAG
marB-F	BCR-3	TCAGGCCACCACGACACCCAAGACAGAGAGGCGCATAAACCGGATGACCA
marB-R	CR-6	TGCTGCTGTCGGTGGGCTGCAATTAGACGAAGTAACAACACTGGCTGCGTGGT
groS-F	BCR-4	GATGCGTCCCTGGGACACCTAGCAATGAAAACACGTAAGCTCCGGCGT
groS-R	CR-4	CCCGTTAGTGGAGCACCAGCTTCAATGGACGCGATCATGCAATGGACGAATATT
groL-F	BCR-3	TCAGGCCACCACGACACCCAAGACAGAGAGAATTGTTGAAGCGTAATCCGCGCA
groL-R	CR-4	CCCGTTAGTGGAGCACCAGCTTCAATGGACACACGAGCGTCGTTACCGAA
ibpA-F	BCR-1	GTTTCTGTGCCGCTCTGGTTATCCGTGCCGCTACCTGACCTGTCCATTGTGG
ibpA-R	CR-2	TAATTCGCGGCACGAGGAGCAAGAACAAATCCAATAGCAGAACGGTAAAGCGG
ibpB-F	BCR-1	GTTTCTGTGCCGCTCTGGTTATCCGTGCCACATCTCCATGCTCGCCGTCA
ibpB-R	CR-2	TAATTCGCGGCACGAGGAGCAAGAACAAACCGATCCATTGACGCATCAGTG
clpB-F	BCR-2	ACTGCCACCCACACCCAAGGACGGTATAGAGACGATCCGGTACGCGTGAT
clpB-R	CR-1	CACAGTAAGGTGAAAGGTGTCCGGTGCATCGGCAAGAGCAAGCTGGAATT

Table 7: Assembly Primers for the 31-Site Library (Set B)

Name	Linker	Sequence (5'-3')
Assembly-BCR-1		CGCGTATGCTGCCGGACCTCTAACAGTTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-2		CAGTGAAAGTGCAGTCCGTGAGGGCATTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-3		GGCACGGATAAACCAAGAGCGGCACGAAACTTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-4		ACGACGGCCTGTACGACCTAGATAGACCCTTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-5		GACGGCCCTGGAAAGTTCTCCTGGATGATTGTGGAATTGTGAGCGGATAACA
Assembly-CR-1		GAGGGTAGAGGAGGTTCCCGATTGGAGAG
Assembly-CR-2		CTGGTTGAGCGAACAGGGTTAGACCCGAA
Assembly-CR-3		GGCCATGCGATGTTGTGGAACCTTC
Assembly-CR-4		TCAGCAAACCTCACACCTGCAAGCCTGTC
marR-F	BCR-5	TCATCCAAGGAGAACTTCCCAGGGCGTCCGTGGCATCGTCATTCAATTCTATT
marR-R	CR-2	CTGGTTGAGCGAACAGGGTTAGACCCGAAACAGATACTCGTTAAGCAGGCGATC
gatY-F	BCR-2	TGCCCTCACGGACTCGCACTTACACTGTTGCCTTCACTCCTCGAATAATTTCAT
gatY-R	CR-1	GAGGGTAGAGGAGGTTCCCGATTGGAGAGTGTGCGTTGTCAGCATCTGCTT
gatZ-F	BCR-5	TCATCCAAGGAGAACTTCCCAGGGCGTCAGTGATTGCCATTGGCTGC
gatZ-R	CR-2	CTGGTTGAGCGAACAGGGTTAGACCCGAAAGCAGAACAGACTGAACATATGCCGA
mdh-F	BCR-3	GTTTCGTGCCGCTCTGGTTATCCGTGCCCTTGCCTGACTACACATTGAGATG
mdh-R	CR-1	GAGGGTAGAGGAGGTTCCCGATTGGAGAGTTAACAGTAGTGCAAGCGCCTGG
gatC-F	BCR-4	GGGTCTATCTAGGTCGTACAGGCCGTCGTCTTTATTCGGTATCGGTATCGAAGC
gatC-R	CR-1	GAGGGTAGAGGAGGTTCCCGATTGGAGAGCGAGGTCGAGAATATAACGCATGACT
gltD-F	BCR-2	TGCCCTCACGGACTCGCACTTACACTGGAACCTGGTCAACCTCGCCACTAA
gltD-R	CR-1	GAGGGTAGAGGAGGTTCCCGATTGGAGAGCGCTGCAGGTCGATAAATTGATAAAC
sad-F	BCR-1	CTGTTTAAGAGGTCCGGGCAGCATAACGCGCAGATCCATAGCATTCTGATTGAG
sad-R	CR-1	GAGGGTAGAGGAGGTTCCCGATTGGAGAGCATGAGTTGCCGGAGTAATGGT
fadR-F	BCR-4	GGGTCTATCTAGGTCGTACAGGCCGTCGTAGTTAGCCCTCTGGTATGATGAG

fadR-R	CR-4	TCAGCAAACCTCACACCTGCAAGCCTGTATAATGTAACCTTCCGCAGAACCCG
glgB-F	BCR-4	GGGTCTATCTAGGTGCGTACAGGCCGTGAGAGGATGCACAGAGTGCTGC
glgB-R	CR-3	GGCCATGCGATGTTGTGGACTCCTGCAGCTCTCGATGCTTGCTTCAGG
yhjH-F	BCR-5	TCATCCAAGGAGAACTTCCCAGGGCCGTCCGCAGCGATAATCTTGTGAG
yhjH-R	CR-3	GGCCATGCGATGTTGTGGACTCCTGCAGCTCTCGATGCTTGCTTCAGG
rpmH-F	BCR-5	TCATCCAAGGAGAACTTCCCAGGGCCGTGGCTTCAGTACAGACGGTTG
rpmH-R	CR-3	GGCCATGCGATGTTGTGGACTCCTGCCTGCGCTTCAGTACAGACGGTTG
envR-F	BCR-3	GTTTGTGCCGCTCTGGTTATCCGTGCCGATTATAGCAACTCAATATTAGCCTCTCG
envR-R	CR-2	CTGGTTGAGCGAACAGGGTTAGACCCGAATCTGCTTACGCCATGCTGCG
emrA-F	BCR-5	TCATCCAAGGAGAACTTCCCAGGGCCGTATAACCAGCAAGCCGCAAGCG
emrA-R	CR-4	TCAGCAAACCTCACACCTGCAAGCCTGTATAATGGCGATCACCCACGCAAA

Table 8: Assembly Primers for the 31-Site Library (Set C)

Name	Linker	Sequence (5'-3')
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Assembly-BCR-1		GTAGTCTGTGGCCAAGGCCTTCGTAGTCGTTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-2		TGTGACTAGGTCGCGTTCGCGTAAAGGTTGTGGAATTGTGAGCGGATAACA
Assembly-BCR-3		TCGTCCCTCATCGGGAAATCTCCTCGGTTGTGGAATTGTGAGCGGATAACA
Assembly-CR-1		GTCGGCACAGGGCTAGAAGTCTCCGAA
Assembly-CR-2		CCCAGCTGTGCTTAACACGGACATCCCAT
Assembly-CR-3		AGTCAAGAGTTCGAATGATCGGCCAAC
acrA-F	BCR-3	GCCGAAGGAGATTCCCGATGAAGGACGAGTGAATGTATGTACCATAGCACGACGA
acrA-R	CR-1	GTCGGCACAGGGCTAGAAGTCTCCGAAAGCATTGAGAACGACCGCCAGA
emrA-F	BCR-1	CGACTACGAAGGCCTGGCCACAGACTACAGCTAACGCGTCATCTGCTCA
emrA-R	CR-3	AGTCAAGAGTTCGAATGATCGGCCAACAGGAGGAGCAGACGCTTACGTT
envR-F	BCR-2	ACCTTCACCGAACCGGACCTAGTCACAGATTAGCAACTCAATTAGCCTCTCG
envR-R	CR-1	GTCGGCACAGGGCTAGAAGTCTCCGAAATCTGCTTACGCCATGCTGCG
gatY-F	BCR-2	ACCTTCACCGAACCGGACCTAGTCACAGCACTTGCTACGGTTCCCTAT
gatY-R	CR-3	AGTCAAGAGTTCGAATGATCGGCCAACATGTGCGTTGTTAGCATCTGCTT
glgB-F	BCR-3	GCCGAAGGAGATTCCCGATGAAGGACGAGAGAGGATGCACAGAGTGC
glgB-R	CR-2	CCCAGCTGTGCTTAACACGGACATCCCATACGCTCTATCGATACGATCGGACA
groL-F	BCR-3	GCCGAAGGAGATTCCCGATGAAGGACGAGACATTCTGGCAATTGTTGAAGCGTAA
groL-R	CR-1	GTCGGCACAGGGCTAGAAGTCTCCGAAACACAGCGCTCGTTACCGAA
ompT-F	BCR-1	CGACTACGAAGGCCTGGCCACAGACTACCCACACAGTAAACCGAAATATGAAACAATAAC
ompT-R	CR-2	CCCAGCTGTGCTTAACACGGACATCCCATATTCCGAAAGTTCGCCGC
tolC-F	BCR-3	GCCGAAGGAGATTCCCGATGAAGGACGACGACGTTGACTGCCGTTGAG
tolC-R	CR-2	CCCAGCTGTGCTTAACACGGACATCCCATCTGCATCAGGTTCTCGGCCT