

Figure S1. Related to Figure 2: Sequences of essentializer degenerate library. Green = the antitoxin ORF and regulatory regions, Red = the toxin ORF and regulatory regions. Grey = the binding regions for cl and Cro, * indicating it has been modified to increase binding efficiency according to previously published work (Sarai & Takeda, 1989; Takeda et al., 1989).

1 19 2p 39 4P sp ep
 TTACCAGTCCCTGTTCTCATCGGAAAAGAGGCCGTTCAATTAAACCGGGCGACCTCAGCCA
 T

70 80 90 100 110 120 130
CCCTTCCTGATTTCCTGCTTCCAGCGTCGGCACGCAGACGACGGGCTTCATTCTGCATGGTGG
ccdA (Antitoxin)
 T

140 150 160 170 180 190
 GCTTACCAGACCGGAGATATTGACATCATATGCCTTGAGCAACTGATAGCTGTCACTGTCACGGT
ccdA (Antitoxin)

210 2 2 .
 GACTGT AATGCGCTGCTTCATACGTCNNCCTC
 ATTATTCCACACAYYATACGAGGGAAGCATA
- An ti toxin -10
 Antitoxin RBS

. . 3
 AAGTGTAAAGCATCTAAC ACCGTGCGTGTGACWATTTACCACTGGCGGTG
ATAATGGTTGTATC
-4j111 ≤ OR2 1 ≤ OR1 - 1 OR3-
 4111
 Antitoxin -35 Toxin -35--. Toxin -10--.

3 3 . .
 ACCGCMGGDGATAGTATGCAGT
AAGGTTACACCTATAAAAGAGAGAGGCCGTTATCGTCTGTT
OR3 ccdB (Toxin)

. . .
 GT GGAT GT CCAGAG TGACATT
 ATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGCGCC
ccdB (Toxin)

• • 5
CGCCTACTGTCAGACAAAGTCTCCGTGAGCTT
ACCCGGTGGTGCATGTCGGGGATGAAAGCTGG
ccdB (Toxin)

5 5
CGCATGATGACCACCGATATGCCAGTGTGCCGGTCTCGTTATCGGGG
AAGAAGTGGCCGATCTC
ccdB (Toxin)

600 610 620 630 640 651
AGCCACCGCGAAAATGACATCAAAACGCCATTAACCTGATTTCTGGGAATAA
ccdB (Toxin)