DNA manipulation.

PCR was performed with 2x Taq PCR MasterMix (Solarbio®, Beijing, China). Plasmid DNA was isolated with the TIANprep Mini Plasmid Kit (TIANGEN, Bejing, China). DNA fragments were purifified from agarose gels by using the Universal DNA Purufucation Kit (TIANGEN, Bejing, China) or the Monarch® DNA Gel Extraction Kit (NEB). DNA sequencing and primer synthesis were carried out by Sangon Biotech (Shanghai, China).

Plasmid construction. The plasmids and primers used in this study are listed in the supplemental material.

1) To construct pTarget-luxAB, the coding regions of luxAB, p15A, and AmpR are amplifified from the genome of Fg-1, a plasmid from 2021OUC-China iGEM and Cloned UpB_4A3m with three different primer pairs 138F/2488R, p15A-R/p15A-F, AmpR-R/AmpR-F, respectively. The J23119 promoter is added to PCR products of luxAB by primers138F and J23119R, resulting in J&luxAB. Oligonucleotides are designed to contribute flanking homologous regions to adjacent DNA fragments of 20-25bp in length, resulting in p15A-homo and AmpR-Homo.

2) A new pEvolvR plasmid expressing enCas9-PolI3M-TBD with gRNA targeting luxAB is constructed. Three fragments pEA, pEB and pEC with flanking homologous regions to adjacent DNA fragments of 20-25bp are amplifified from pEvolvR-enCas9-PolI3M-TBD with primer pairs 11615R/8425F, 8450R/2601F, 2627R/11662F, respectively. Double-stranded gRNA is gained after DNA annealing, and it is modified by the promoter with the primer pair——. Similarly, then homologous regions are added to produce J&gRNA-Homo.

3) Two plasmids were both constructed using the Seamless cloning Master Mix provided by Sangon Biotech (Shani, China). The plasmids pTarget-luxAB and pEvolvR were cotransformed into E. coli strainDH5α, following the specification steps provided.

The supplemental material

Oligonucleotides used in this study	
138F	ATCTTGGATTACTTTTTGTTCTTTA
2488R	CAAATTGAAAATGAATCTCTCGAACTT
p15A-R	CCAGGAAGATACTTAACAGGG
p15A-F	GGTGCTACATTTGAAGAGATA
AmpR-R	TGTCCCTAGTGCTTGGATTCT
AmpR-F	AAATGTGCGCGGAACCCCTAT
AmpR-R-Homo2	ACCTAGGACTGAGCTAGCTGTCAATGTCCCTAGTGCTTGGATTCT
p15A-R-Homo2	AACAAATAGGGGTTCCGCGCACATTTCCAGGAAGATACTTAACAGGG
p15A-F-Homo	GGAATTTAAAGAACAAAAAGTAATCCAAGATGgtgctacatttgaagaga
J23119R	TTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCtttctcctctttCAAATTGAAAATGAATCTCTCGAACTT
11615R	CGAAGGTGAGCCAGTGTGAC
8425F	AGCGTCAGACCCCGTAGAAAA
8450R	TGATCTTTTCTACGGGGTCTGA
2601F	ATCAACAGGCTCTCAGACTACGAC
2627R	CACGTCGTAGTCTGAGAGCCTGT
11662F	AGCAAGTTAAAATAAGGCTAGTCC
gRNA protospacers used in this study	
gRNA1	
J&gRNA1-Homo	

Plasmids used in this study	
pEvolvR-enCas9-PolI3M-TBD	acatcattaattcctaatttttgttgacactctatcgttgatagagttatttttaccactccctatcagtgatagagaaaagaattcaaaagatctaaagaggagaaaagatctatggacaagaagtattctatcggactggctatcgggactaatagcgtcgggtgggccgtgatcactgacgagtacaaggtgccctctaagaagttcaaggtgctcgggaacaccgaccggcattccatcaagaaaaatctgatcggagctctcctctttgattcaggggaaaccgctgaagcaacccgcctcaagcggactgctagacggcggtacaccaggaggaagaaccggatttgttaccttcaagagatattctccaacgaaatggcaaaggtcgacgacagcttcttccataggctggaagaatcattcctcgtggaagaggataagaagcatgaacggcatcccatcttcggtaatatcgtcgacgaggtggcctatcacgagaaatacccaaccatctaccatcttcgcaaaaagctggtggactcaaccgacaaggcagacctccggcttatctacctggccctggcccacatgatcaagttcagaggccacttcctgatcgagggcgacctcaatcctgacaatagcgatgtggataaactgttcatccagctggtgcagacttacaaccagctctttgaagagaaccccatcaatgcaagcggagtcgatgccaaggccattctgtcagcccggctgtcaaagagccgcagacttgagaatcttatcgctcagctgccgggtgaaaagaaaaatggactgttcgggaacctgattgctctttcacttgggctgactcccaatttcaagtctaatttcgacctggcagaggatgccaagctgcaactgtccaaggacacctatgatgacgatctcgacaacctcctggcccagatcggtgaccaatacgccgacctttttccttgctgctaagaatctttctgacgccatcctgctgtctgacattctccgcgtgaacactgaaatcaccaaggcccctctttcagcttcaatgattaagcggtatgatgagcaccaccaggacctgaccctgcttaaggcactcgtccggcagcagcttccggagaagtacaaggaaatcttctttgaccagtcaaagaatggatacgccggctacatcgacggaggtgcctcccaagaggaattttataagttttatcaaacctatccttgagaagatggacggcaccgaagagctcctcgtgaaactgaatcgggaggatctgctgcggaagcagcgcactttcgacaatgggagcattccccaccagatccatcttggggagcttcacgccatccttcggcgccaagaggacttctacccctttcttaaggacaacagggagaagattgagaaaattctcactttccgcatcccctactacgtgggacccctcgccagaggaaatagccggtttgcttggatgaccagaaagtcagaagaaactatcactccctggaacttcgaagaggtggtggacaagggagccagcgctcagtcattcatcgaacggatgactaacttcgataagaacctccccaatgagaaggtcctgccgaaacattccctgctctacgagtactttaccgtgtacaacgagctgaccaaggtgaaatatgtcaccgaagggatgaggaagcccgcattcctgtcaggcgaacaaaagaaggcaattgtggaccttctgttcaagaccaatagaaaggtgaccgtgaagcagctgaaggaggactatttcaagaaaattgaatgcttcgactctgtggagattagcggggtcgaagatcggttcaacgcaagcctgggtacttaccatgatctgcttaagatcatcaaggacaaggattttctggacaatgaggagaacgaggacatccttgaggacattgtcctgactctcactctgttcgaggaccgggaaatgatcgaggagaggcttaagacctacgcccatctgttcgacgataaagtgatgaagcaacttaaacggagaagatataccggatggggacgccttagccgcaaactcatcaacggaatccgggacaaacagagcggaaagaccattcttgatttccttaagagcgacggattcgctaatcgcaacttcatgcaacttatccatgatgattccctgacctttaaggaggacatccagaaggcccaagtgtctggacaaggtgactcactgcacgagcatatcgcaaatctggctggttcacccgctattaagaagggtattctccagaccgtgaaagtcgtggacgagctggtcaaggtgatgggtcgccataaaccagagaacattgtcatcgagatggccagggaaaaccagactacccagaagggacagaagaacagcagggagcggatgaaaagaattgaggaagggattaaggagctcgggtcacagatccttaaagagcacccggtggaaaacacccagcttcagaatgagaagctctatctgtactaccttcaaaatggacgcgatatgtatgtggaccaagagcttgatatcaacaggctctcagactacgacgtggaccatatcgtccctcagagcttcctcgcagacgactcaattgacaataaggtgctgactcgctcagacaagaaccggggaaagtcagataacgtgccctcagaggaagtcgtgaaaaagatgaagaactattggcgccagcttctgaacgcaaagctgatcactcagcggaagttcgacaatctcactaaggctgagaggggcggactgagcgaactggacaaagcaggattcattaaacggcaacttgtggagactcggcagattactaaacatgtcgcccaaatccttgactcacgcatgaataccaagtacgacgaaaacgacaaacttatccgcgaggtgaaggtgattaccctgaagtccaagctggtcagcgatttcagaaaggactttcaattctacaaagtgcgggagatcaataactatcatcatgctcatgacgcatatctgaatgccgtggtgggaaccgccctgatcaagaagtacccagcactggaaagcgagttcgtgtacggagactacaaggtctacgacgtgcgcaagatgattgccaaatctgagcaggagatcggaaaggccaccgcaaagtacttcttctacagcaacatcatgaatttcttcaagaccgaaatcacccttgcaaacggtgagatccggaaggcgccgctcatcgagactaatggggagactggcgaaatcgtgtgggacaagggcagagatttcgctaccgtgcgcaaagtgctttctatgcctcaagtgaacatcgtgaagaaaaccgaggtgcaaaccggaggcttttctaaggaatcaatcctccccaagcgcaactccgacaagctcattgcaaggaagaaggattgggaccctaagaagtacggcggattcgattcaccaactgtggcttattctgtcctggtcgtggctaaggtggaaaaaggaaagtctaagaagctcaagagcgtgaaggaactgctgggtatcaccattatggagcgcagctccttcgagaagaacccaattgactttctcgaagccaaaggttacaaggaagtcaagaaggaccttatcatcaagctcccaaagtatagcctgttcgaactggagaatgggcggaagcggatgctcgcctccgctggcgaacttcagaagggtaatgagctggctctcccctccaagtacgtgaatttcctctaccttgcaagccattacgagaagctgaaggggagccccgaggacaacgagcaaaagcaactgtttgtggagcagcataagcattatctggacgagatcattgagcagatttccgagttttctaaacgcgtcattctcgctgatgccaacctcgataaagtccttagcgcatacaataagcacagagacaaaccaattcgggagcaggctgagaatatcatccacctgttcaccctcaccaatcttggtgcccctgccgcattcaagtacttcgacaccaccatcgaccggaaacgctatacctccaccaaagaagtgctggacgccaccctcatccaccagagcatcaccggactttacgaaactcggattgacctctcacagctcggaggggatggttctagtgaaaccccgggaacaagtgagtcggccacccctgaaggtggatcaggggtagcggatccgttcagatcccgcagaacccgctgattctggttgacggatctagttacctgtaccgtgcttaccatgctttcccgcctttgaccaattctgctggtgaacctacgggagctatgtacggagttctgaatatgttgcgttctttaattatgcagtacaagcctacccacgctgctgttgttttcgatgctaaaggtaagacgttccgcgacgagttattcgagcactataagtctcaccgtcctccgatgcctgatgacttacgcgctcagattgagccgctgcatgctatggtgaaggctatgggtttacctcttttggctgtcagcggtgttgaggctgatgatgtcattggcaccttagctcgtgaggctgagaaggctggtcgccctgttttgatttctaccggtgacaaggacatggctcaattggttaccccgaacatcaccctgatcaacaccatgaccaacacgattctgggtcctgaggaagttgttaacaaatatggtgttcctccggagttgattattgactttcttgctcttatgggcgattcttcagacaatatcccgggtgttccaggtgttggagagaagactgctcaagctctgcttcagggtctgggtggttggacacccttacgctgaaccggagaagatcgccggtctgtcttttcgcggtgctaagaccatggctgctaaactggaacagaataaggaggtcgcatacctgtcttatcaattggctaccatcaagacggatgtggagttagaacttacgtgcgagcagcttgaggttcaacagcctgctgctgaggaactgctgggtctttttaagaaatacgaatttaagcgttggaccgccgacgttgaggctggtaagtggctgcaagctaagggtgctaagccggctgctaaaccgcaagaaacgagtgtcgctgatgaggctccggaggttaccgctaccgttatctcttacgataattatgttacgattct

	ggacgaggaaaccttaaaggcttggatcgctaaattagagaaggctcctgttttcgctttcgacacggaaacggattctctggacaatattagtgcgaatcttgttggtctgagtttcgcaattg aaccgggtgttgctgcttacatccctgtggcacacgactacctggacgctccggaccagatttcacgtgaacgcgctctggaactgctgaagcctttattagaggacgagaaagctttgaaag ttggtcagaatttgaagtatgctcgtggaatcttagctaattatggtatcgagttgcgcggtatcgctttcgacacgatgttggaatcttatatcctgaactctgtcgctggtcgccatgacatgga ctctctggctgagcgctggctgaaacataagacgattaccttcgaggaaatcgcaggaaagggtaagaaccagctcacgttcaatcaaatcgctctggaggaagctggtcgctatgctgctg aggacgctgacgttactctgcaactgcacttgaagatgtggcctgacttgcagaagcataagggtccactgaatgtttttgaaaacattgagatgcctttggttccagttctgtctcgtatcgagc gcaatggcgttaaaattgacccaaaggttttacataaccactcagaggaactgacgctgcgcttagccgaattggagaaaaaggctaccgaaacgttcggctcgtggtatcagcctaaaggt ggcactgagatgttctgccatccgcgaacaggtaagccactacctaaataccctcgcattaagacacctaaagttggtggtatctttaagaagcctaagaacaaggcacagcgagaaggccg tgagccttgcgaacttgatacccgcgagtacgttgctggtgctccttacaccccagttgaacatgttgtgttcaatctgtcatctacgaaacaactgcagactatcctgttcgagaagcaaggtat caagccattaaaaaagaccccctggcggtgctccgtctacctctgaggaagttttggaggagttagctttggattaccctctgccgaaggttatcttggaataccgcggtttggctaaattgaagt ctacttatacggataaacttcctttgatgattaatccaaagacgggtcgcgttcacacgtcgtaccatcaagctgttaccgctaccggtcgcctgtcttctacggatccgaatttacagaatattcct gtgcgcaatgaggagggccgccgcattcgtcaagcttttatcgctccggaagactacgttatcgtttctgctgattattctcaaaatgaattacgtatcatggctcacctgtctcgcgataagggt ctgttgacggcctttgctgagggtaaggacattcatcgtgctaccgctgctgaggttttcggcctgccgttggaaacggttacgtctgaacagcgtcgctctgctaagcgtattaatttcggctta atctacggtatgtctgcgtttggcttagctcgtcagctgaatatcccgcgcaaggaagctcaaaaatatatggatctgtattttgagcgttacccgggtgttttggaatacatggagcgtacgcg cgcgcaagctaaggaacaaggttatgtggaaaccttggatggtcgtcgcttgtacttgcctgacattaagtcttctaacggcgcccgccgcgctgctgccgagcgcgctgctatcaatgctcc gatgcaaggtactgctgctgatattattaagcgtgctatgatcgctgtggacgcttggctgcaagctgaacagcctcgcgttcgcatgattatgcaagttcatgacgagttggttttcgaggtgc ataaggacgacgtggacgctgttgctaaacaaatccaccagttgatggagaattgcacgcgcttagacgttccgctgctggttgaagttggttctggtgaaaactgggaccaggctcactaat aatggcctcggtaccaaagacgaacaataagacgctgaaaagcgtctttttttcgttttggtccgctgagcagttacagagatgttacgaaccactagtgcactgcagtacagtgttacaaccaa ttaaccaattctgattagaaaaactcatcgagcatcaaatgaaactgcaatttattcatatcaggattatcaataccatatttttgaaaaagccgtttctgtaatgaaggagaaaactcaccgaggc agttccataggatggcaagatcctggtatcggtctgcgattccgactcgtccaacatcaatacaacctattaatttcccctcgtcaaaaataaggttatcaagtgagaaatcaccatgagtgacg actgaatccggtgagaatggcaaaagcttatgcatttctttccagacttgttcaacaggccagccattacgctcgtcatcaaaatcactcgcatcaaccaaaccgttattcattcgtgattgcgcct gagcgaggcgaaatacgcgatcgctgttaaaaggacaattacaaacaggaatcgaatgcaaccggcgcaggaacactgccagcgcatcaacaatattttcacctgaatcaggatattcttct aatacctggaatgctgttttcccggggatcgcagtggtgagtaaccatgcatcatcaggagtacggataaaatgcttgatggtcggaagaggcataaattccgtcagccagtttagtctgacc atctcatctgtaacatcattggcaacgctacctttgccatgtttcagaaacaactctggcgcatcgggcttcccatacaatcgatagattgtcgcacctgattgcccgacattatcgcgagcccatttt atacccatataaatcagcatccatgttggaatttaatcgcggcctggagcaagacgtttcccgttgaatatggctcataacaccccttgtattactgtttatgtaagcagacagttttattgttcatg atgatatatttttatcttgtgcaatgtaacatcagagattttgagacacaacgtggctttgttgaataaatcgaacttttgctgagttgaaggatcaggagttcatgaccaaaatcccttaacgtga gttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttg ccggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcc tacatacctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggatAAggcgcagcggtcgggctgaacgg ggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatcc ggtaagcggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgct cgtcaggggggcggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggccttttgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgtat taccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgcctgatgcggtattttctccttacgcatctgtgcggt atttcacaccgcaatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgccccgacacccgccaacacccg ctgacgcgccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccttctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggcagctgc ggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaagcgggccatgttaagg gcggttttttcctgtttggtcactgatgcctccgtgtaagggggatttctgttcatccctgaattcgcatctagactgaactggccgataattgcagacgaacggatttactttacagctagctcagt cctaggtattatgctagcgctagcaaagaggagaaaagatctatggagaaaaaaatcactggatataccaccgttgatatatcccaatggcatcgtaaagaacattttgaggcatttcagtcag ttgctcaatgtacctataaccagaccgttcagctggatattacggccttttaaagaccgtaaagaaaaataagcacaagttttaaccggccttttattcacattcttgcccgcctgatgaatgctcat ccggaatttcgtatggcaatgaaagacggtgagctggtgatatgggatagtgttcacccttgttacaccgttttccatgagcaaactgaaacgttttcatcgctctggagtgaataccacgacg atttccggcagtttctacacatatattcgcaagatgtggcgtgttacggtgaaaacctggcctatttccctaaagggtttattgagaatatgtttttcgtttcagccaatccctgggtgagtttcacc agttttgatttaaacgtggccaatatggacaacttcttcgcccccgttttcaccatgggcaaatattatacgcaaggcgacaaggtgctgatgccgctggcgattcaggttcatcatgccgtttgt gatggcttccatgtcggcagaatgcttaatgaattacaacagtactgcgatgagtggcagggcggggcgtaaggatcctaactcgagctcggtaccaaattccagaaaagaggcctcccga aagggggcctttttcgttttggtccgctgccaaaccagatgtcaacacagctacaacgtttatagctagctcagcccttggtacaatgctagctgagacggaaagtgaaacgtgatttcatgc gtcattttgaacattttgtaaatcttatttaataatgtgtgcggcaattcacatttaatttatgaatgttttcttaacatcgcggcaactcaagaaacggcaggttcggatcttagctactagagaaag

	aggagaaatactagatgcgtaaaggcgaagagctgttcactggtgtcgtccctattctggtggaactggatggtgatgtcaacggtcataagttttccgtgcgtggcgagggtgaaggtga cgcaactaatggtaaactgacgctgaagttcatctgtactactggtaaactgccggttccttggccgactctggtaacgacgctgacttatggtgttcagtgctttgctcgttatccggaccatat gaagcagcatgacttcttcaagtccgccatgccggaaggctatgtgcaggaacgcacgatttcctttaaggatgacggcacgtacaaaacgcgtgcggaagtgaaatttgaaggcgatacc ctggtaaaccgcattgagctgaaaggcattgactttaaagaggacggcaatatcctgggccataagctggaatacaattttaacagccacaatgtttacatcaccgccgataaacaaaaaaat ggcattaaagcgaattttaaaattcgccacaacgtggaggatggcagcgtgcagctggctgatcactaccagcaaaacactccaatcggtgatggtcctgttctgctgccagacaatcactatc tgagcacgcaaagcgttctgtctaaagatccgaacgagaaacgcgatcatatggttctgctggagttcgtaaccgcagcgggcatcacgcatggtatggatgaactgtacaaatgaccagg catcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgctctctactagagtcacactggctcaccttcgggtgggcctttctgcgtttatacgt ctcagttttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgaagcttgggcccgaacaaaaactcatctcagaagag gatctgaatagcgccgtcgaccatcatcatcatcatcattgagtttaaacggactccagcttggctgttttggcggatgagagaagattttcagcctgatacagattaaatcagaacgcagaag cggtctgataaaacagaatttgcctggcggcagtagcgaggtggtcccacctgaccccatgccgaactcagaagtgaaacgccgtagcgccgatggtagtgtggggactccccatgcgag agtagggaactgccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgacagggctggatgcacacactggcttaagatgaca acgttaagacccactttcacatttaagttgtttttctaatccgcatatgatcaattcaaggccgaataagaaggctggctctgcaccttggtgatcaaataattcgatagcttgtcgtaataatggcg gcatactatcagtagtaggtgtttccctttcttctttagcgacttgatgctcttgatcttccaatacgcaacctaaagtaaaatgccccacagcgctgagtgcatataatgcattctctagtgaaaaa ccttgttggcataaaaaggctaattgattttcgagagtttcatactgtttttctgtaggccgtgtacctaaatgtacttttgctccatcgcgatgacttagtaaagcacatctaaaactttagcgttat tacgtaaaaaatcttgccagctttccccttctaaagggcaaaagtgagtatggtgcctatctaacatctcaatggctaaggcgtcgagcaaagcccgcttatttttacatgccaatacaatgtag gctgctctacacctagcttctgggcgagtttacgggttgttaaaccttcgattccgacctcattaagcagctctaatgcgctgttaatcactttacttttatctaatctag tetR/tetA promoters-KanR-ori-bom-rop-CmR-superfolder GFP-gRNA scaffold-TetR
Cloned UpB_4A3m	ctagtagcggccgctgcagtccggcaaaaaagggcaaggtgtcaccaccctgccctttttctttaaaaccgaaaagattacttcgcgttatgcaggcttcctcgctcactgactcgctgcgctcg gtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatctcgaggttacattgtcgatctgttcatggtgaacagctttGaatgcaccaaaaactcgtaaaagctctgatgtatc tatctttttacaccgttttcatctgtgcatatggacagtttttcctttgatatGtaacggtgaacagttgttctacttttgtttgttagtcttgatgcttcactgatagatacaagagccatgaagaacctc agatccttccgtatttagccagtatgttctctagtgtggttcgttgtttttgcgtgagccatgagaacgaaccattgagatcatActtactttgcatgtcactcaaaaattttgcctcaaaactggtga gctgaatttttgcagttaaagcatcgtgtagtgtttttcttagtccgttaTgtaggtaggaatctgatgtaatggttgttggtattttgtcaccattcatttttatctggttgttctcaagttcggttacg agatccatttgtctatctagttcaacttggaaaatcaacgtatcagtcgggcggcctcgcttatcaaccaccaatttcatattgctgtaagtgtttaaatctttacttattggtttcaaaacccattggtt aagccttttaaactcatggtagttattttcaagcattaacatgaacttaaattcatcaaggctaatctctatatttgccttgtgagttttcttttgtgttagttctttttaataaccactcataaatcctcatag agtatttgttttcaaaagacttaacatgttccagattatattttatgaatttttttaactggaaaagataaggcaatatctcttcactaaaaactaattctaatttttcgcttgagaacttggcatagtttg tccactggaaaatctcaaagcctttaaccaaaggattcctgatttccacagttctcgtcatcagctctctggttgctttagctaatacaccataagcattttccctactgatgttcatcatctgagcgta ttggttataagtgaacgataccgtccgttctttccttgtagggttttcaatcgtggggttgagtagtgccacacagcataaaattagcttggtttcatgctccgttaagtcatagcgactaatcgcta gttcatttgctttgaaaacaactaattcagacatacatctcaattggtctaggtgattttaatcactataccaattgagatgggctagtcaatgataattacatgtccttttcctttgagttgtgggtat ctgtaaattctgctagacctttgctggaaaacttgtaaattctgctagaccctctgtaaattccgctagacctttgtgttttttttgtttatattcaagtggttataatttatagaataaagaaagaat aaaaaaagataaaaagaatagatcccagccctgtgtataactcactactttagtcagttccgcagtattacaaaaggatgtcgcaaacgctgtttgctcctctacaaaacagaccttaaaacccta aaggcttaagtagcaccctcgcaagctcgggcaaatcgctgaatattccttttgtctccgaccatcaggcacctgagtcgctgtctttttcgtgacattcagttcgctgcgctcacggctctggca gtgaatgggggtaaatggcactacaggcgccttttatggattcatgcaaggaaactacccataatacaagaaaagcccgtcacgggcttctcagggcgttttatggcgggtctgctatgtggt gctatctgactttttgctgttcagcagttcctgccctctgattttccagtctgaccacttcggattatcccgtgacaggtcattcagactggctaatgcacccagtaaggcagcggtatcatcaaca ggcttacccgtcttactgtccctagtgcttggattctcaccaataaaaaacgcccggcggcaaccgagcgttctgaacaaatccagatggagttctgaggtcattactggatctatcaacagga gtccaagcgagctcgtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcctgactccccgtcgtgtagataactacga tacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctccagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtc ctgcaactttatccgcctccatccagtctattaattgttgccgggaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtggtgtcacgctcgtc gtttggtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagttggccg cagtgttatcactcatggttatggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaatagtgtatgcggcgacc gagttgctcttgcccggcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctgttgaga tccagttcgatgtaacccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaaaaaagggaataagggcgaca cggaaatgttgaatactcatactcttcctttttcaatattattgaagcatttatcagggttattgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaaatagggggttccgcgcaca tttcccatggtgccacctgacgtctaagaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggcagaatttcagataaaaaaaatccttagctttcgctaaggatgatttct ggAATTCGCGGCCGCTTCTAGAGCCTAGTTGTCTTCATGCATGAAGACAAAATTAATACTAGAGGGACCAAAACGAAAAAAGACGCTCGAAAGC

	GTCTCTTTTCTGGAATTTGGTACCGAGCTCTAGTATTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGGAACTCGCTCG GGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGAGTTGATCGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCATCCGGGT GGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCCTCGCGCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGAC GCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATTGCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTAC CCGATTATCCATCGGTGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCAGATTTATCGCCAGCAGCTCCGAAT AGCGCCCTTCCCCTTGCCCGGCGTTAATGATTTGCCCAAACAGGTCGCTGAAATGCGGCTGGTGCGCTTCATCCGGGCGAAAGAACCCCGTATTG GCAAATATTGACGGCCAGTTAAGCCATTCATGCCAGTAGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTCGCGAGCCTCCGGATGAC GACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATATCACCCGGTCGGCAAACAAATTCTCGTCCCTGATTTTTCACCACCCCCTGACCG CGAATGGTGAGATTGAGAATATAACCTTTCATTCCCAGCGGTCGGTCGATAAAAAAATCGAGATAACCGTTGGCCTCAATCGGCGTTAAACCCGC CACCAGATGGGCATTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTTTTCATACTCCCGCCATTCAGAGAAGAAACCA ATTGTCCATATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGCATTCTGTA ACAAAGCGGGACCAAAGCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTTGCACGGCGTCACA CTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATA Rep101-pSC101 ori-lambda t0 terminator-AmpR-AmpR promoter-bacterial terminator-BioBrick prefix-L3S2P11-araC-araBAD promoter
pTarget-luxAB	Ggtgctacatttgaagagataaattgcactgaaatctagaaatattttatctgattaataagatgatcttcttgagatcgttttggtctgcgcgtaatctcttgctctgaaaacgaaaaaaccgcctt gcagggcggtttttcgaaggttctctgagctaccaactctttgaaccgaggtaactggcttggaggagcgcagtcaccaaaacttgtcctttcagtttagccttaaccggcgcatgacttcaaga ctaactcctctaaatcaattaccagtggctgctgccagtggtgcttttgcatgtctttccgggttggactcaagacgatagttaccggataaggcgcagcggtcggactgaacgggggttcgt gcatacagtccagcttggagcgaactgcctacccggaactgagtgtcaggcgtggaatgagacaaacgcggccataacagcggaatgacaccggtaaaccgaaaggcaggaacagga gagcgcacgagggagccgccaggggaaacgcctggtatctttatagtcctgtcgggtttcgccaccactgatttgagcgtcagatttcgtgatgcttgtcaggggggcggagcctatgga aaaacggctttgccgcggccctctcacttccctgttaagtatcttcctggcataaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccct gataaatgcttcaataatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaa aagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttct gctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggatgg catgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatggggg atcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaa ctacttactctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtg agcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctga gataggtgcctcactgattaagcattggtaactgtcagaccaagtttacgagctcgcttggactcctgttgatagatccagtaatgacctcagaactccatctggatttgttcagaacgctcggtt gccgccgggcgtttttttattggtgagaatccaagcactagggacaTTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCtttctcctctttCAAATTGAAAATGAATCT CTCGAACTTGCGTAAAGTTAGATAACTCATTATATAAAAGTAAAGGAAGATACTATGAAGTTTGGAAATATTTGTTTCTCATATCAGCCACCAGGT GAAACACATAAGGAAGTAATGGATCGTTTTGTTCGACTAGGGGTTGCCTCTGAAGAGCTTGGATTTGATACCTACTGGACTCTTGAGCACCATTTT ACTGAATTCGGTCTCACTGGCAATCTGTTTGTTGCGGCGGCAAATTTACTTGGTCGCACGAAAACATTAAATGTTGGAACAATGGGTGTAGTGATT CCAACAGCACATCCCGTGCGACAAACAGAAGATGTATTGTTACTAGATCAATTATCTAAAGGTCGTTTTAATTTTGGTGTTGTTCGAGGTTTGTATC ATAAAGACTTTCGTGTTTTTGGTGTCAATATGGAAGACTCACGTGGTATAACTCAAAATTTTCATAATATGATCATGACGAGTTTAGAAACGGGAA AAATAAGTTCAAACAGTGAGCATATTGAGTTTCCAGAAGTTAATATCTACCCTAAAGTATATTCAAAAAAAGTTCCGACATGTATGACTGCTGAGT CAGCTAGTACGACCGAATGGCTAGCAAAACAGGGGTTACCTATGGTTCTTAGTTGGATCATCGGAACGAATGAAAAGAAGGCTCAAATGGAGCT CTATAATGAAATAGCTTTGGAGCATGGTCATGATATTACTCAAATAGATCATTCAATGACATTTATATGCTCTGTTGACGATGTTGGAGATAAAGC GCGTGAAGTGTGCCGAATATTTTTAACTAATTGGTACGATTCATATGTTAATGCTACCAACATCTTTAAAGATAGTAATCAAACTCGTGGCTATGAC TATCATAAAGGACAATGGCGTGATTTTGTTTTACAAGGTCATACTAATACAAATAGACGAGTCGATTATAGCAATGAAATAAACCCCGTAGGGACT CCTGAGGAATGTATTGCAATTATCCAACGTGATATTGATGCAACAGGTATTAGCAATATTACTTGTGGTTTTGAAGCTAATGGCAGTGAAGAAGAA ATCGTGGCATCGATGGAGCGATTTATGAAACAAGTTGCTCCTTTCCTGAAAGACCCAAAGTAATTGTTTATACATAGTATTAAATACAACAAGGAT ATTTATTATGAAATTTGGCTTATTTTTTCTTAACTTCCAAAATAATAACGCTACTTCAGAAGATGTTTTAGATAATATGGTAAATACCGTGTCGTTCAT TGATTCAAAGAAATATCATTTTGATACCGTCTTTGTTAATGAACACCACTTTTCGACAAATGGTATTGTAGGTGCACCAATTACAGCTGCAGGTTTT TTATTGGGAATGACAACTCAACTTCAAATAGGGTCATTAAATCAAGTTATTACTACTCATCACCCGGTTCGAATTGCTGAGGAAGCAAGTTTATTA

	GACCAAATGTCTGAAGGACGCTTTATATTAGGACTGAGCGATAGCGAAAGTAATTTTGAAATGGAATTTTTTAAGCGTCATATCCCCTCTAAGCAG CAACAATTTGAGGCATGTTATGACATAATTAATGAAGCTTTAATCACTGGTTATTGCCATGCGCAAAATGATTTTTTTGACTTTCCTAAAGTGTCAAT AAACCCTCATTGTTTTAGTGTAAATGGGCCTAAACAGTATGTTACAGCTACCAGTAACGACATTGTTGTATGGGCTGCTAAAAAATCGTTGCCTTTA ATATTTAAATGGGAAGATAGCCTTGCTGTAAAAGAAAGCTACGCGACTCTTTATAATAATACAGCAGAACATTACGGTATCGATGTTTCAGAAGTA GATCATCAATTAACGATTATTGTTAATTTGAATGTTGATGGAGATATTGCTCGTGAAGAGGCTAGGCAATATTTGGAAAGTTATATTATAGAAACAT ATCCAAATATTAACCCGTTAGAAAAGATTAATTCCATAATTGAAGAAAATGCAGTTGGAACAGATTTCAATTATTATGATTCGACTAAATTAGCAA TAGAAAAAACAGGAGCTAAAAATATTCTTTTATCGTTTGAATCAATGGGAAGTCAAGATAATGTTAAGGCTGTTATTGATATGGTAAATCAGAAAA TACATAAAAACCTTGCATGATAATAAAATAATGACAGATTAACGCTGTCGTTATTTTTAAACTAGTTATTATATTGATAATATGGATTATATATGGA AATTCAAGTACCTATTGCAAAGAATAATATTATAGCAAGTTCAGAGATAGATGATCTTATATTTATGAGCTCACCAAAAGAATGGGAATTTAAAGA ACAAAAAGTAATCCAAGAT p15A-AmpR promoter-AmpR-lambda t0 terminator-J23119 promoter-luxAB
pEvolvR	