

Gibson assembly

MCS

TTCTGGACCAGTTGCGTGACGCGGCCGCCAGATCTTCCGGATGGCTCGAGTTTTTCAGCAAGAT

ATCTTACTAGAAGATCTCCTACAATATTCTCAGCTGCCATGGAAAATCGATGGTTGACAATTAA

Trc1O promoter

TCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTACACATTCTAGAGAAAG

AGGAGAAATACTAGATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT

CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC

ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTGGCCCA

CCCTCGTGACCACCTTCGGCTACGGCCTGCAATGCTTCGCCCCGCTACCCCGACCACATGAAGCT

GCACGACTTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAG

YFP

GACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCA

TCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAA

CTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTC

AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCC

CCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAG

CAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC

ACTCTCGGCATGGACGAGCTGTACAAGTAATAATACTAGTGCCAGGCATCAAATAAAACGAAAG

terminator

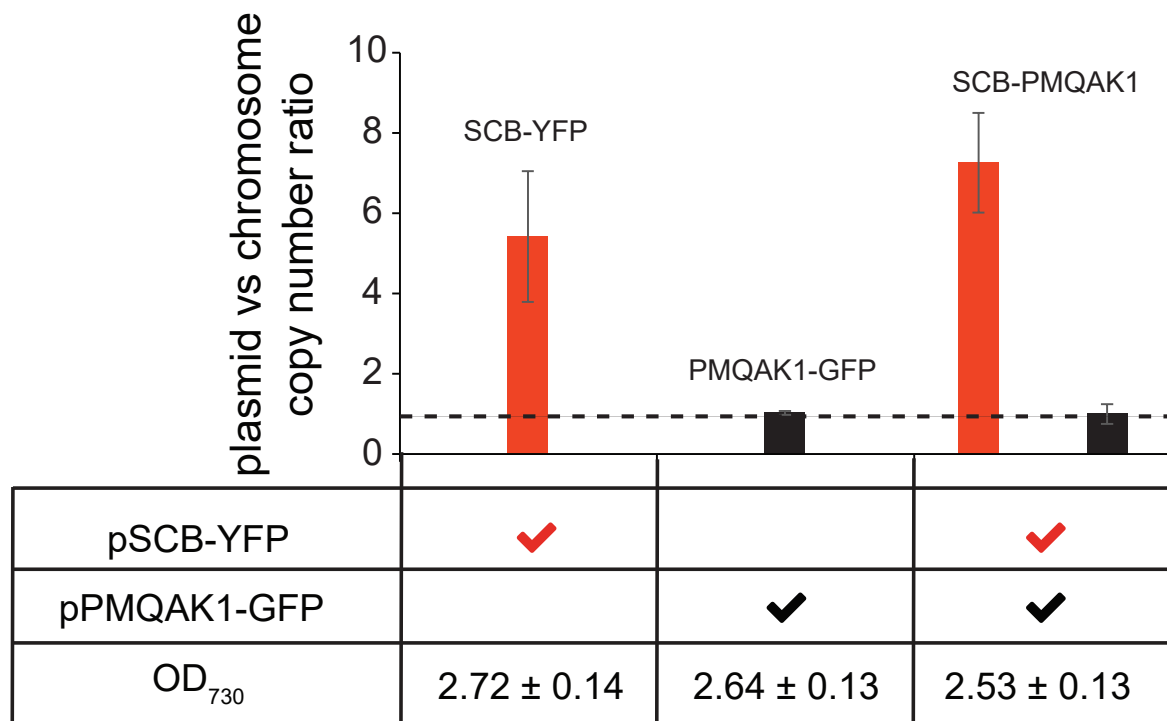
GCTCAGTCGAAAGACTGGGCCTTTCTGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTCTACTAGA

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GTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATAAACAGCGTGACAGCGACCG

### Supplementary Figure 1.

This is the pSCB-YFP synthesis fragment from IDT. There are two 20 bp gibbon assembly overlap sequence with either *aadA* gene or ORFb 5' UTR region. This synthesis fragment is consisting of MCS (black line); Trc1O promoter (blue line); YFP gene (yellow line); and a terminator downstream of YFP gene.



Supplementary Figure 2.

pSCB-YFP and pPMQAK1-GFP plasmids copy number ratio to 16sRNA gene which has two copies located on chromosome. The assay was conducted on all three different exconjugants, SCB-YFP, PMQAK1-GFP, and SCB-PMQAK1. All the cells were taken from stationary phase which OD<sub>730</sub> were around 2.5 to 2.7, the plasmid to chromosome copy number ratio was calculated as number on Y-axis. DB423 & DB424 amplicon from pSCB-YFP, and DB419 & DB420 amplicon from pPMQAK1-GFP were used as target and 16sRNA gene fragment from chromosome was used as reference. pPMQAK1-GFP plasmid has similar copy number as chromosome which the ratio around 1 (dash line), while pSCB-YFP plasmid copy number ratio to chromosome is around 5 to 7 times. Error bars represent standard deviations calculated from two biological and three technical replicates.