

Table 2. Plasmids and primers used in this study.

Plasmid	Description
pACYC-Duet1	Cam <sup>R</sup> expression vector, P15A origin
pK09	<i>pylB</i> PCR fragment (using primers PylBNdeI and PylBBglII) cloned into TOPO 2.1 NcoI site removed from <i>pylB</i> (using primers pylBNcoImut1 and pylBNcoImut2)
pK11	Nde I / Bgl II <i>pylB</i> fragment from pK09 cloned into Nde I / Bgl II-cut pACYC-Duet1
pK12	Nco I / BamH I-cut <i>pylCD</i> PCR fragment (using primers PylCDNcoI and PylCDBamHI) cloned into Nco I / BamH I-cut pK11
pK13	Nco I site removed from <i>pylC</i> (using primers pylCNcoImut1 and pylCNcoImut2) in pK12
pK14	Nco I / BamH I-cut <i>pylD</i> PCR fragment (using primers PylDNcoI and PylCDBamHI) cloned into Nco I / BamH I-cut pK11
pK15	Nco I / BamH I-cut <i>pylCD</i> PCR fragment (using primers PylCDNcoI and PylCDBamHI) cloned into Nco I / BamH I-cut pACYC
pK16	Mlu I / BamH I-cut <i>pylC</i> PCR fragment (using primers PylCMluI and PylCDBamHI) cloned into Mlu I / BamH I-cut pK11
Primers	Sequence
PylBNdeI	<b>CATATGATCAAAAAAATGGCAACCGAGGACC</b>
PylBBglII	<b>AGATCTTTAGCACCTCAGGACAGTTTCG</b>
PylCDNcoI	<b>CCATGGAAACCATATGCCTTATAGGCGGG</b>
PylCDBamHI	<b>GGATCCTTACAGGATAGAATACAGCATGGA</b>
PylCBamHI	<b>GGATCCTTACACAGCCGCTCCGAAG</b>
PylDNcoI	<b>CCATGGCACTTTTAACCCCGGAAG</b>

PylCMluI	<b>ACGCGTTGCGCGAGAAGATTGTGC</b>
pylBNcoImut1	CATGATCGACCTGACGATGGGAGAAGACCC
pylBNcoImut2	GGGTCTTTCTCCCATCGTCAGGTCGATCATG
pylCNcoImut1	CTTTAATAAGGAGATATACCATGAAAACCATATGCCTTATAGGCGG
pylCNcoImut2	CCGCCTATAAGGCATATGGTTTT <b>C</b> ATGGTATATCTCCTTATTAAAG
pBADF	CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGGCCGTCAATTG TCTGATTCGTT
pBADR	<b>CATATGTGTATATCTCCTGCTAGCCCAAAAAACGGGTA</b>
UTAGF	GGGCGAACAGTTCCTGATTAACCACTAGCCGTTCTACTTTACTGG
UTAGR	CCAGTAAAGTAGAACGGCTAGTGGTTAATCAGGAACTGTTCGCCC

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Bolded sequence indicates sequence introducing restriction site into the resulting PCR product.