Applied Microbiology and Biotechnology

Construction of a plasmid addiction system using *grpE* as selection marker in *Escherichia coli* and application in phloroglucinol biosynthesis

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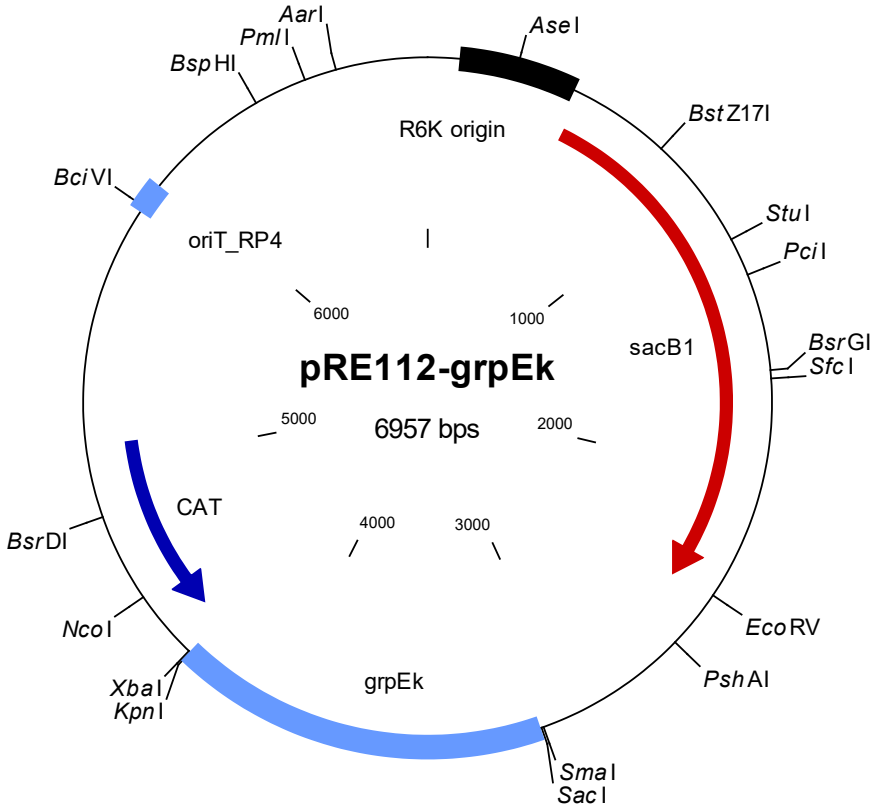
Supplemental Table S1 Primers used in this study *

Primers	**Sequences (5'→3')**
$grpE_k$-F	GGTACCTCATATGCGCCACTTTGCCTGGATG (*Kpn*I)
$grpE_k$-RV0	CGAAAGCAGAAATTACTACATGAATTTCTCCGCGTTT
$grpE_k$-F0	TCATGTAGTAATTTCTGCTTTCGTAATAATTCAC
$greE_k$-RV	GAGCTCGCCATCAGTATGCTGCGAGTACGTG (*Sac*I)
pmf-F	GCGGCCGCTCGATCTCGATCCCGCGAAATTAAT (*Not*I)
pmf-RV	CTTAAGCTAGCTGTTGTAATGATTTAATGGAT (*Afl*II)
$grpE_e$-F	TTAATTAAATAATAAGCGAAGTTAGCGAGATG (*Pac*I)
$grpE_e$-RV	CTCGAGAACGGCCCGGCATTCGCATGCAGGG (*Xho*I)
gck-F	CAGATCGCTGAGAAAACGCAAAGAT
gck-RV	CACTCACCTCACGGGCGCATCGT
birA-F	AGGCCTAAAGGAGATATACAATGAAGGATAACACCGTGCCA CTG (*Stu*I)
birA-RV	GCGGCCGCTTATTTTTCTGCACTACGCAGGGATATTTC (*Not*I)

* The underlined bases encode restriction endonucleases indicated in the brackets at the ends of each primer. The primers gkc-F/gkc-RV were located 50 bps upstream or downstream of $grpE_k$-F/$grpE_k$-RV, respectively (Fig. 1a).

a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BspHI
AseI
BstZ17I
R6K origin
BciVI
oriT_RP4
StuI
PciI
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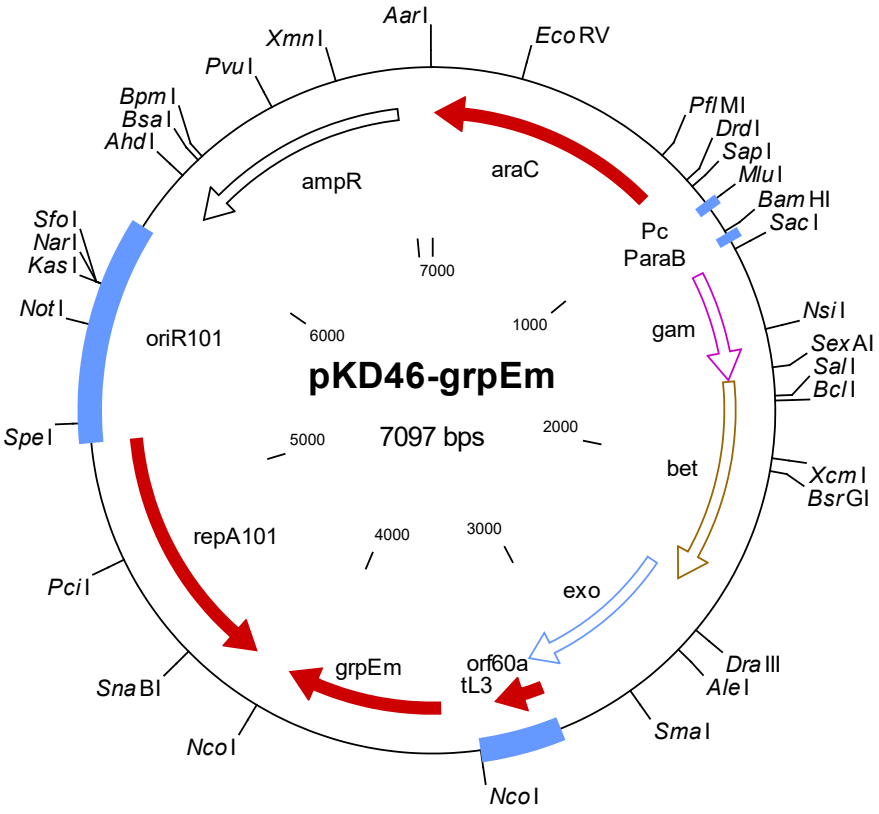
1000
BsrGI
SfcI
sacB1
pRE112-grpEk
5000
6957 bps
2000
CAT
4000
3000
BsrDI
EcoRV
NcoI
PshAI
XbaI
KpnI
grpEk
SmaI
SacI

b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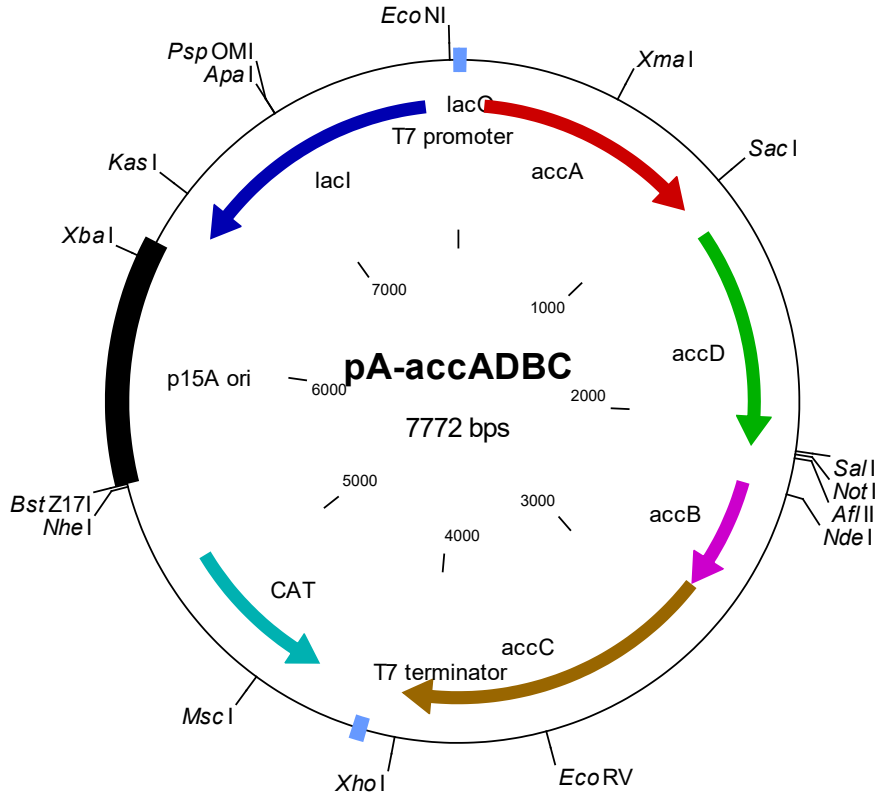
EcoRV
PvuI
PflMI
BpmI
BsaI
DrdI
AhdI
SapI
ampR
araC
MluI
BamHI
SacI
SfoI
NarI
Pc
ParaB
KasI
7000
NsiI
NotI
6000
1000
gam
oriR101
SexAI
SalI
pKD46-grpEm
BclI
SpeI
5000
7097 bps
2000
bet
XcmI
BsrGI
repA101
4000
3000
PciI
exo
orf60a
tL3
DraIII
AleI
grpEm
SnaBI
SmaI
NcoI
NcoI

c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XmaI
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SacI
KasI
lacI
accA
XbaI
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accD
p15A ori
6000
pA-accADBC
2000
7772 bps
SalI
NotI
AflII
NdeI
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BstZ17I
NheI
accB
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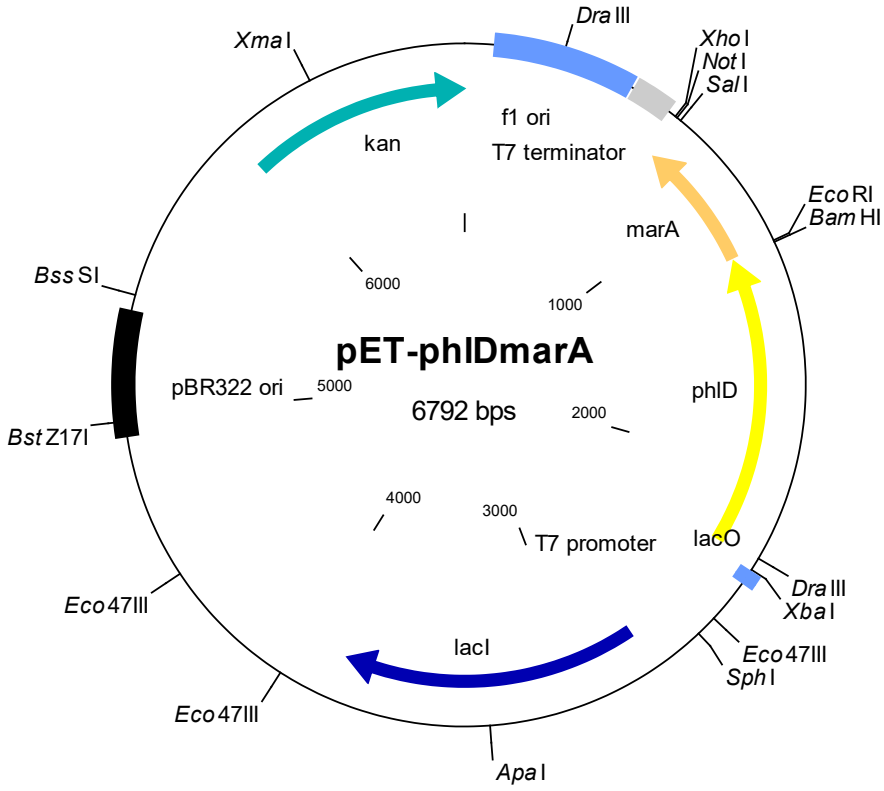
CAT
accC
T7 terminator
MscI
XhoI
EcoRV

d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XmaI
XhoI
NotI
SalI
f1 ori
T7 terminator
kan
EcoRI
BamHI
marA
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BssSI
pET-phlDmarA
pBR322 ori
5000
6792 bps
phlD
2000
BstZ17I
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DraIII
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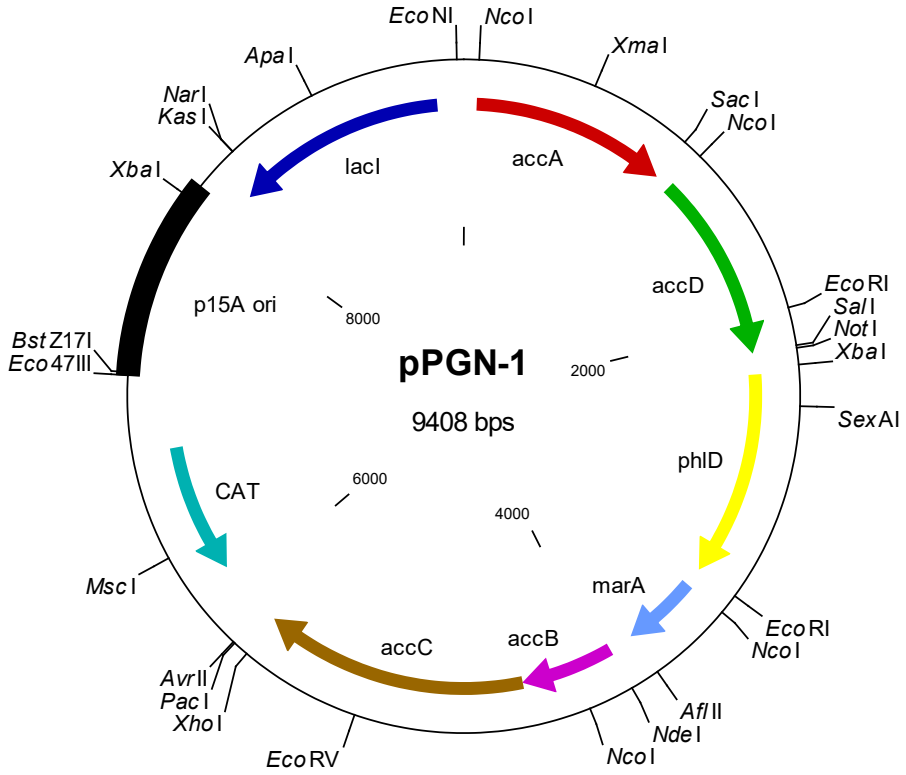
lacI
Eco47III
SphI
Eco47III
ApaI

e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XmaI
SacI
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EcoRI
SalI
NotI
XbaI
SexAI
phlD
marA
EcoRI
NcoI
AflII
NdeI
NcoI
EcoRV
XhoI
PacI
AvrII
MscI
CAT
BstZ17I
Eco47III
XbaI
NarI
KasI
ApaI
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accD
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accC
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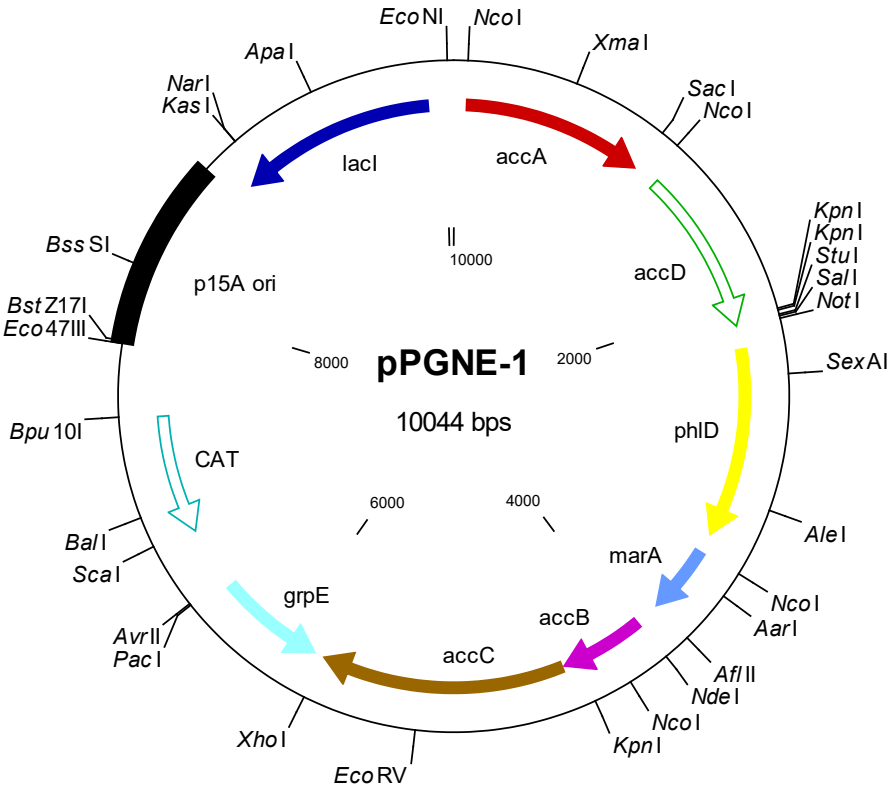
6000
8000

f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SacI
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KpnI
KpnI
StuI
SalI
NotI
SexAI
phlD
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NcoI
AarI
AflII
NdeI
NcoI
KpnI
EcoRV
XhoI
AvrII
PacI
grpE
BalI
ScaI
Bpu10I
CAT
BstZ17I
Eco47III
BssSI
NarI
KasI
ApaI
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marA
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p15A ori
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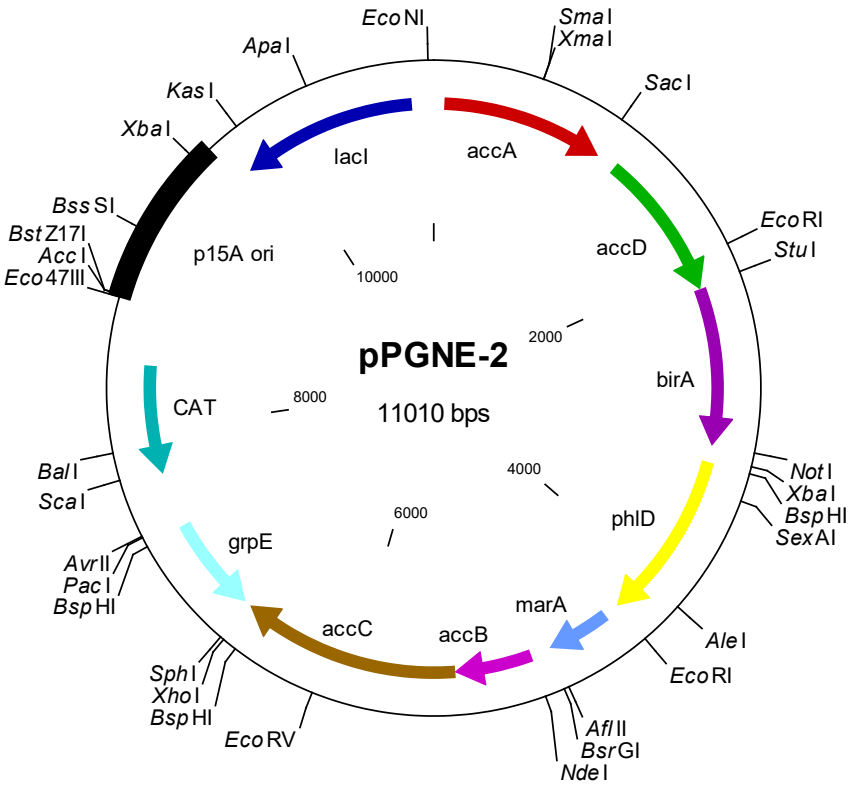
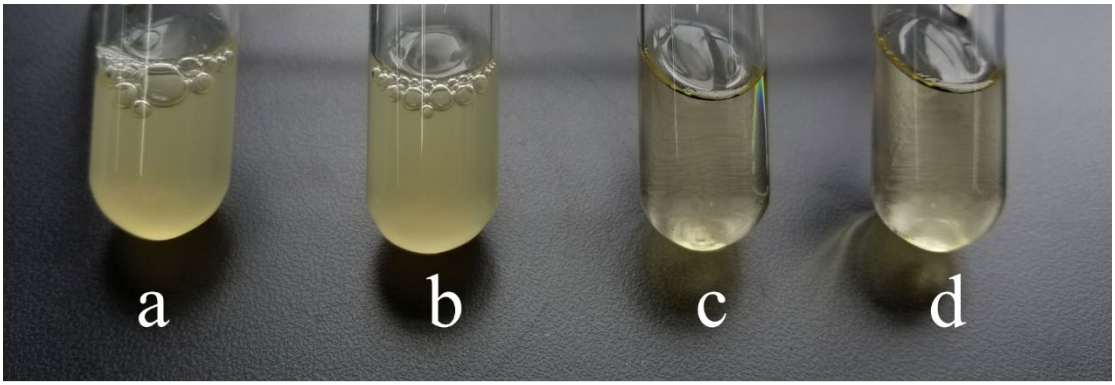
g



Supplemental Fig. S1 Schematic representation of plasmids used in this study. (a) pRE112-grpE$_k$, plasmid carrying the grpEk disruption cassette, generated from the suicide plasmid pRE112. (b) pKD46-grpE$_m$, a disruption helper plasmid carrying the artificially muted *grpE* expression cassette, generated from pKD46, carrying the artificially mutated *grpE* expression cassette. (c) and (d) PG producing plasmids constructed in the previous work (Appl Microbiol Biotechnol 2011, 91(6), 1545-52). (e) PG producing plasmid propagated in *E. coli* BL21(DE3). (f) PG producing plasmid with *grpE* expression cassette inserted into pPGN-1. (g) PG producing plasmid with birA inserted into pPGNE-1.



Supplemental Fig. S2 Confirmation of pKD46-grpEm curing in *E. coli* PGNE-1 and PGNE-2 after being grown at 42℃. The strains could propagate in LB supplemented with Cm (a, PGNE-1; b, PGNE-2) but not with Amp (c, PGNE-1; d, PGNE-2), suggesting that pKD46-grpE$_m$, which confers Amp resistance to the host, has been successfully eliminated.

Supplemental Sequence S1

>$grpE_m$

CCATGG*CTTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACTT*

TCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTATCGAGATTTT

*CAGGAGCTAAGGAAGCTAAA*ATGAGCAGCAAAGAACAGAAAACCCCGGAAG

GCCAAGCTCCGGAAGAAATTATCATGGATCAGCATGAAGAAATTGAAGCAG

TTGAACCGGAAGCATCTGCTGAACAAGTCGATCCGCGCGACGAAAAAGTG

GCCAACCTGGAAGCACAGCTGGCGGAAGCCCAAACCCGCGAACGTGATG

GTATTCTGCGTGTGAAAGCCGAAATGGAAAATCTGCGTCGCCGTACGGAAC

TGGATATTGAAAAAGCTCACAAATTTGCGCTGGAAAAATTCATTAACGAAC

TGCTGCCGGTTATCGATTCACTGGACCGCGCCCTGGAAGTCGCTGATAAAG

CGAATCCGGACATGAGTGCAATGGTTGAAGGCATCGAACTGACCCTGAAAT

CCATGCTGGATGTGGTTCGTAAATTTGGTGTCGAAGTGATTGCCGAAACGA

ACGTGCCGCTGGACCCGAATGTTCATCAGGCAATCGCTATGGTGGAATCGG

ATGACGTTGCGCCGGGCAACGTCCTGGGTATTATGCAGAAAGGCTATACCC

TGAATGGTCGCACGATCCGTGCGGCGATGGTGACGGTCGCGAAAGCCAAA

GCATAA**CCTGTTTTACGTAAAAACCCGCTTCGGCGGGTTTCTAGCGGA**

GTCCATGG

The underlined bases encoded *Nco*I. The italy bases encode the promoter of chloramphenicol acetyltransferase. The bold bases encode the terminator of *rnpB*.