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Supplemental Information

Specificity and Promiscuity at the Branch Point in Gentamicin Biosynthesis

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Figure S1, related to Figure 2. Sequence alignment of GenK (A) with homologous radical SAM-dependent methyltransferases and of GenQ (B) with homologous oxidoreductases from other aminoglycoside biosynthetic pathways

Black letters in gray boxes represent amino acid residues identical in all species, while white letters in gray boxes represent similar amino acid residues. The red box in **A** indicates the consensus CX_3CX_2C binding motif for the FeS cluster.

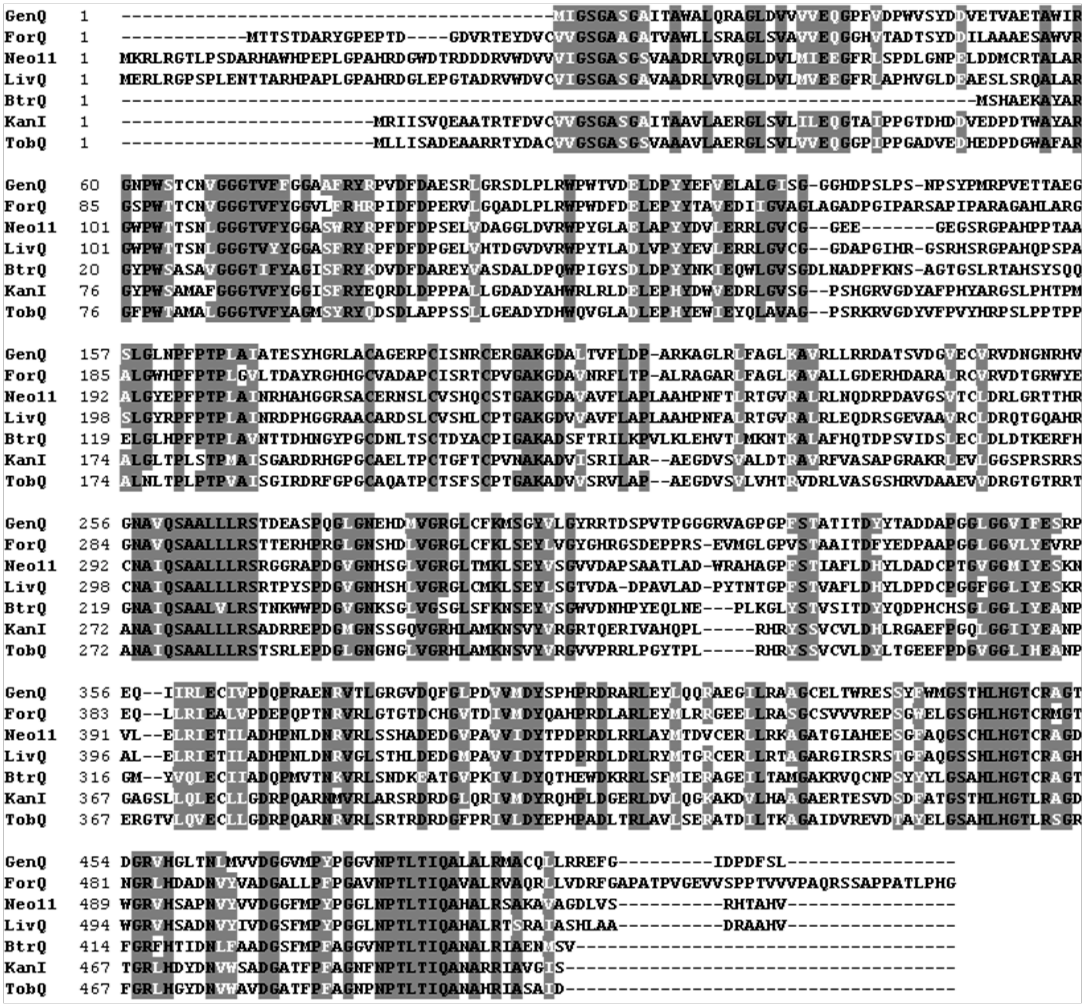
A

ForK (accession no. CAF31547.1): from *Micromonospora olivasterospora*;

Fom3 (accession no. ACG70826.1): from *Streptomyces fradiae*;

GenD1 (accession no. CAF31434.1): from *Micromonospora echinospora*.

B



Neo11 (accession no. CAH58694.1): from *S. fradiae*;

BtrQ (accession no. BAE07079.1): from *Bacillus circulans*;

LivQ (accession no. CAG38701.1): from *S. lividus*;

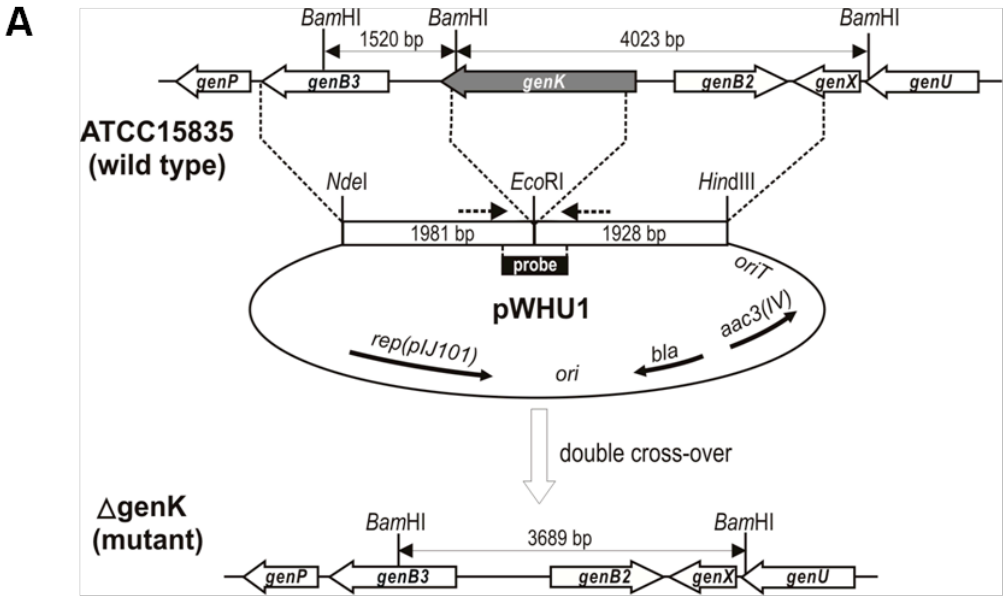
KanI (accession no. CAF60534.1): from *S. kanamyceticus*;

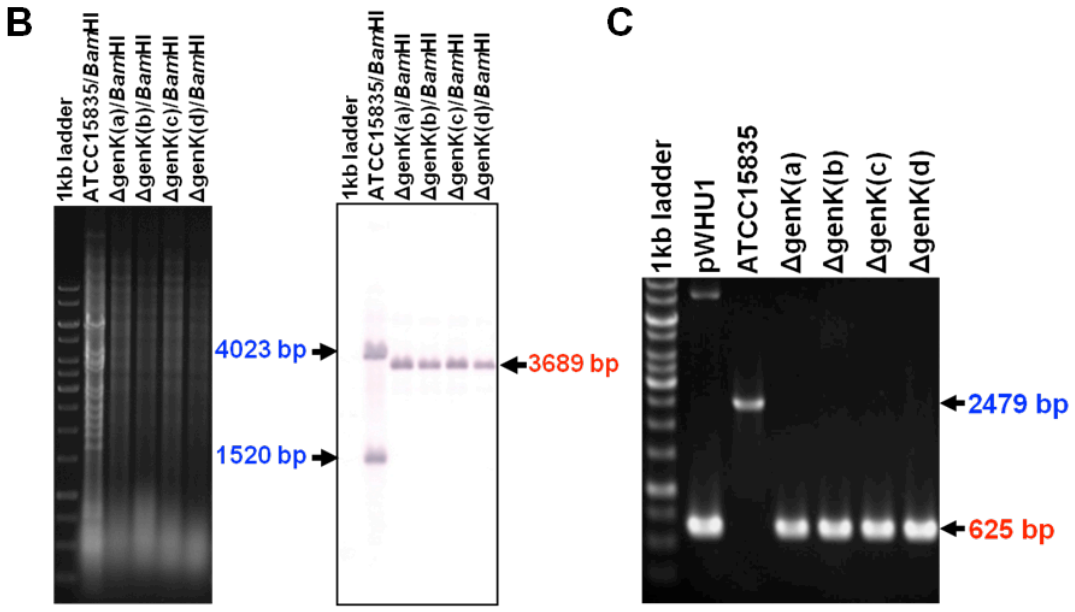
TobQ (accession no. CAH18553.1): from *Streptoalloteichus tenebrarius*;

ForQ (accession no. CAF31536.1): from *M. olivasterospora*.

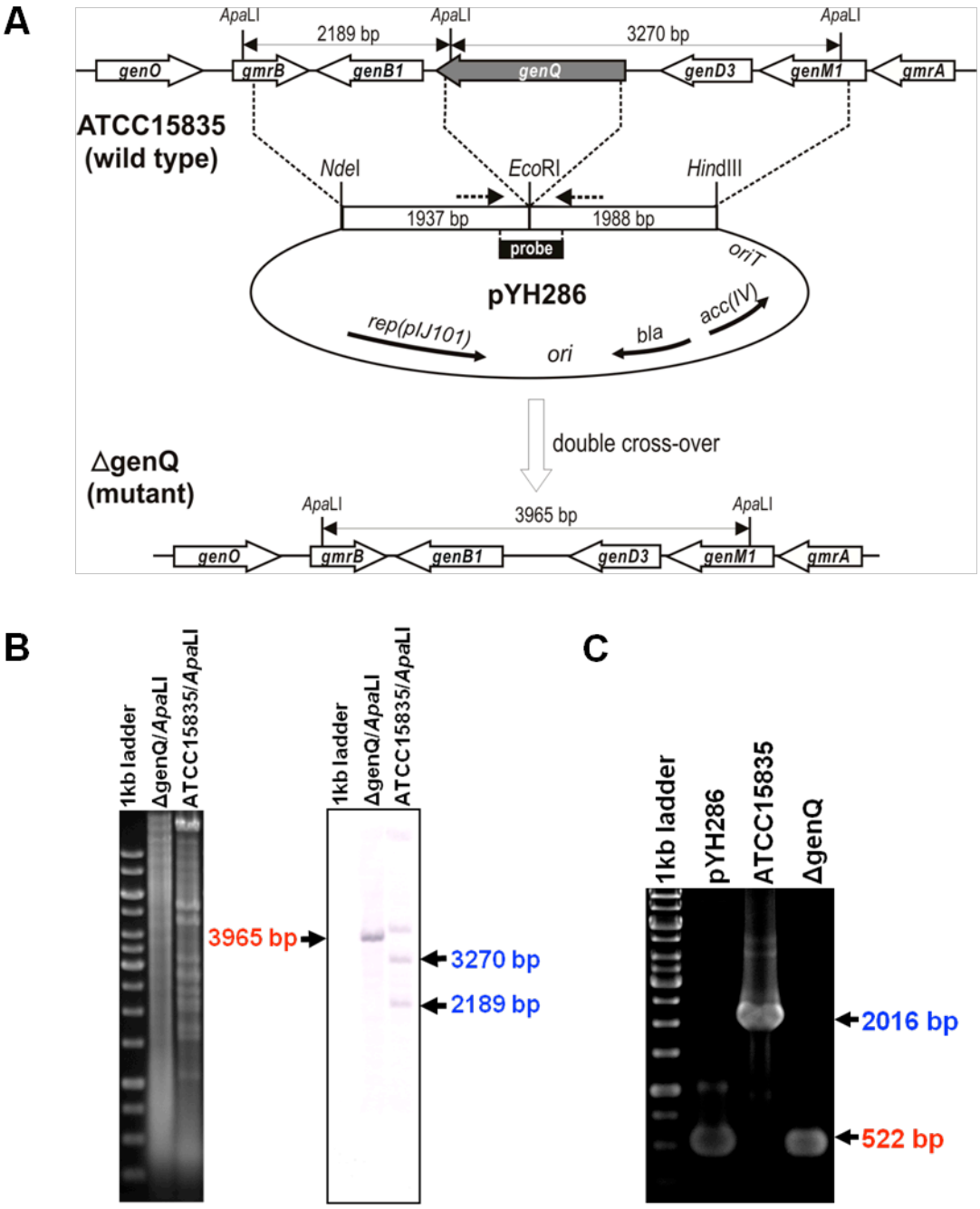
Figure S2, related to Figure 2. In-frame deletion of *genK* and *genQ* in *M. echinospora* ATCC15835, and complementation of ΔgenK and ΔgenQ
(A) Schematic representation of the in-frame deletions; (B) Confirmation by Southern blot; (C) Confirmation by PCR. The arrows indicate the expected size of the PCR fragments in the wild type and mutants. The dash arrows show the location of PCR primers for check mutants.

(1) ΔgenK

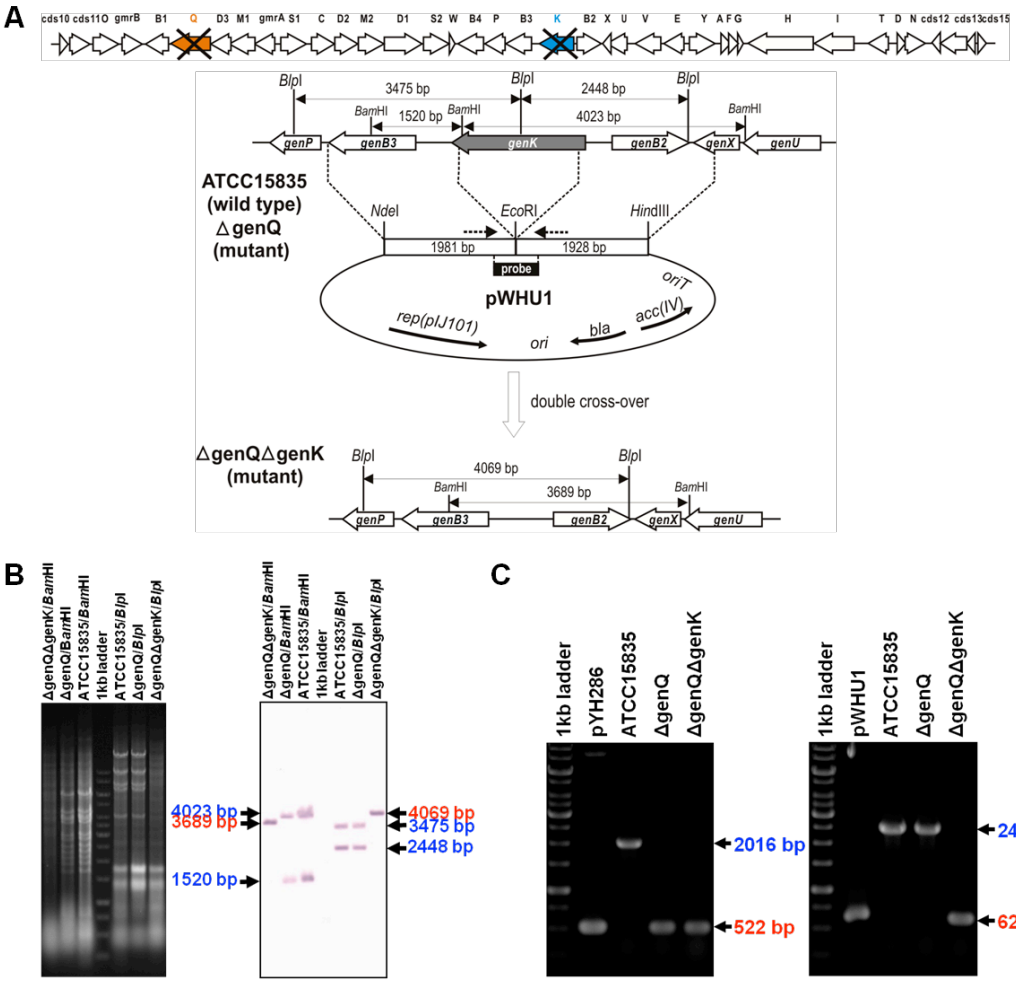




(2) ΔgenQ


A
ApaLI
2189 bp
ApaLI
3270 bp
ApaLI
genO
gmrB
genB1
genQ
genD3
genM1
gmrA
ATCC15835
(wild type)
NdeI
EcoRI
HindIII
1937 bp
1988 bp
probe
oriT
pYH286
rep(pIJ101)
ori
bla
acc(IV)
double cross-over
ΔgenQ
(mutant)
ApaLI
3965 bp
ApaLI
genO
gmrB
genB1
genD3
genM1
gmrA
B
1kb ladder
ΔgenQ/ApaLI
ATCC15835/ApaLI
3965 bp
3270 bp
2189 bp
C
1kb ladder
pYH286
ATCC15835
ΔgenQ
2016 bp
522 bp

(3) ΔgenQΔgenK



(4) Complementation of ΔgenK and ΔgenQ

Confirmation of complementation strains (A) ΔgenK::*genK*, (B) ΔgenQ::*genQ* and (C) ΔgenQ::*neo11* by PCR. The arrows indicate the expected size of the PCR fragments in the wild type and mutants. (D) Map of genetic complementation vector. The dash arrows indicate the location of PCR primers for check complementation strains.

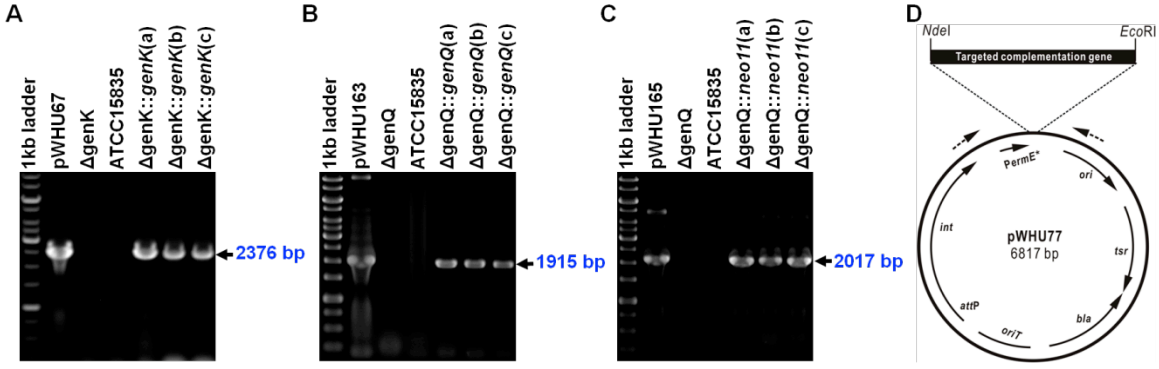
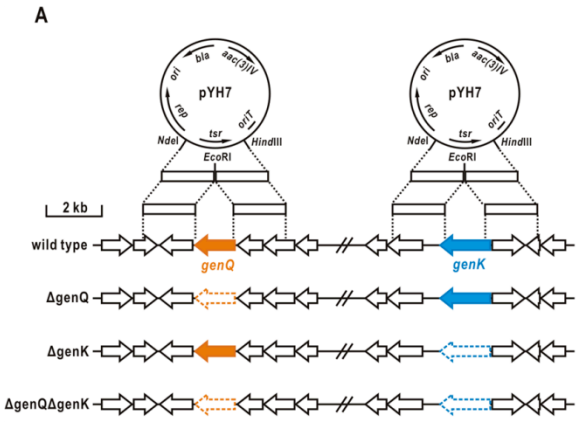


Figure S3, related to Figure 2. LC-HRMS analysis of production of gentamicin C complex and intermediates by the wild type, ΔgenK, ΔgenQ, and ΔgenQΔgenK mutants of *M. echinospora* ATCC15835

(A) Schematic representation of the in-frame deletion of *genQ* and *genK*. Deleted genes are shown in dotted arrows. (B) Production of gentamicin C complex and intermediates analyzed by LC-ESI-HRMS and bioassay using *Bacillus pumilus* as indicator strain. -: no production, +: production, ++: increased by about 10-fold compared with wild type, +++: increased by about 100-fold compared with wild type.



B

Production								Bioassay
C1a	C2	C2a	C2b	C1	X2	JI-20A	G418	JI-20B	
+	+	+	+	+	+	+	+	+	
−	−	−	−	−	+	−	+++	−	
++	−	−	++	−	++	++	−	−	
−	−	−	−	−	+++	−	−	−	

Figure S4, related to Figure 3. (A) Sequence alignment of GenB1, GenB2, GenB3 and GenB4 with their homolog Neo18 from the neomycin biosynthetic pathway. The red box in (A) indicates the putative catalytic lysine; (B) Schematic representation and confirmation by Southern blot of in-frame deletion of *genB1, genB2, genB3 and genB4.* (1) ~ (4): Single mutants; (5) ~ (10): Double mutants; (11) ~ (14): Triple mutants; (15): Quadruple mutant.

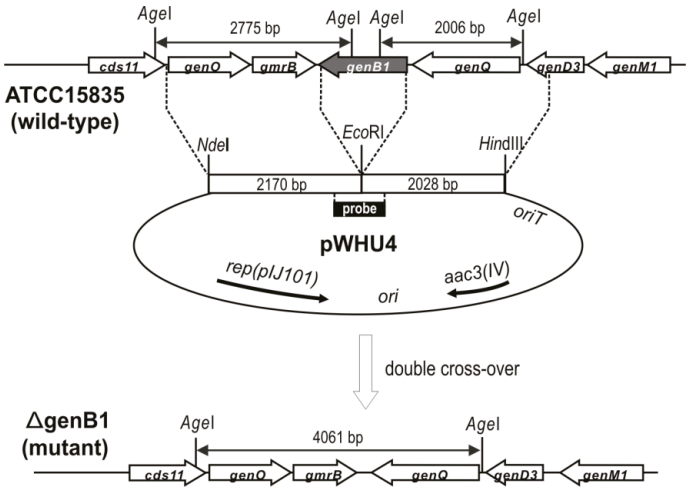
A

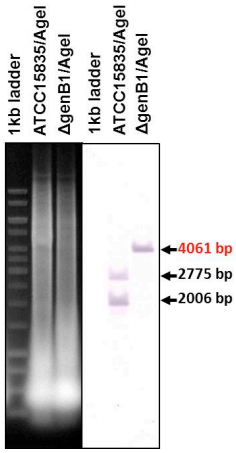
```
Neo18          ----------------------------------------MTKNS---SLLAEF 11
GenB1          ----------------------------------------MTIDIGAGKLLAQE 14
GenB2          ----------------------------------------MIIANA 6
GenB3          MAVADHRSSEPSWRAGRTARRRSRWHSWARVKSAREGSQDMDSANLTNRGLVERARRVTA 60
GenB4          ----------------------------------------MNYRELIERARRTTA 15

Neo18          PTCPRDEKDRPRVFTAASGAWLTDESGFRWIDFDNARGSILLGHGDPVVAEAVARAAT-- 69
GenB1          PTCPRDADGRPRVFVEGSGAYLTDPDGRRWIDFDNARGSVVLGHGDEEVAEAIARAAR-- 72
GenB2          DGCTPYEVARGVTIVRGEGAYVYDAEGRGLIDLSNSFGSVMLGHQDPVVTEAVLKTVR-- 64
GenB3          AENYDIGTRFSAMIQSGEGAWLTDVEGNRYVDLTASSGTIILGHRNQAVTEAITRQIRDF 120
GenB4          AEEYDISGRYPSVIAHAEGAWMTDLSGNRYVDLTGADAAVILGYRHPAVNEAITRQIRDY 75

Neo18          GADGTATGWSRRVDAVLERLHALCGGEVVGLFRSGTAAVRAAVLAVREATGRPLLLSAGY 129
GenB1          GRSGVGTAWSPVLDSLLGQLQEVCGGDVVGLYRTGTAALRSVTCAVRDARDRSIVLSSGY 132
GenB2          SGVPAAASLDLQNHLAEQIAGDLPGDQRVAFFKTGTAATRAAASAARQVTGKRLIASCGY 124
GenB3          GTAFASTLSVPRVELAERLCERYECAEKVVFHKTGSEGTAMAARLARAATGRELILSCGY 180
GenB4          GTTFASTLSVPRVELAERMCERYECAEKVVFHKTGTEGTAMAVRLARAATGRELVLSSGY 135

Neo18          HGYDPMWYPSEAPLEPNADGVVDFFFDLGLLRELLRA-PERVAAVVVSPDHMHLSPGWYR 188
GenB1          HGYDPMWH-CDEPFTPNQHGIVEFLFDLDVLAEWLSR-PEQVAAVVISPDHMHLGERWYT 190
GenB2          HGYDLMWE-FTPPGQPNSEDVLHCYHLPELIDQVLDKHAHELAAVIIAPDYIHVSPEYIA 183
GenB3          HGWHEWQLAGETFGYQQTTGVVGFGYNEKALAKMLEAFGNEVAGVLISPELLYFDVEFYQ 240
GenB4          HGWHEWQMAGEEFGYQQSTGVVGFGYNEKALAKMLEAFGEQVAGVIVSPEVLYFDLDHYR 195

Neo18          ELRRLCSAAGVVLVADEVKVGLRYAPGLSTAE-LLAPDVWVVAKGMANGHAVSAVGGSRR 247
GenB1          EFTRLTKEADVPVIADEVKVGLRYRAGLSTP--LLDPAVWIVAKCLANGSPVAAVGGDAH 248
GenB2          DLFERCERVGVVTIADEVKHGYRLRQGASVTEASVVADMYTYAKGISNGWPLSCVAGDER 243
GenB3          RMYALCARYDVPFMMDEVYTGFRAGP-KGVHGLGVPADVVVVSKGLANGHSLAAVMGRRD 299
GenB4          RMSALCARYDVPFMLDEVYTGFRAGP-KGVHGLGVPADVVVLGKGLANGHSLAAVMGRRD 254

Neo18          LLKP--LKEVSFTSFFEPTILAAADAALARVATGEPQRAVREAGDRFLRHARKALDDASL 305
GenB1          LLAA--LEDVSFTSYFEPTAMAAATTTLRRMATGEPQQAIRAAGDRFIAHTRAAFANAGV 306
GenB2          FLKP--LAEFVSTLTFEAPSFAAASATLDRLAELDVQAQLAIDGARFVSEAAKMISTRDL 301
GenB3          IIDAYDVSGIQGTYTREVPPMAAAMAVLDVLDTPGVYEHAEAMGRRLADGMREILTGEGI 359
GenB4          IIDAYDVSGIQGTYTREVPPMAAALAVFEVLDTPGVYEHAEAMGRRLADGMREILTGEGI 314

Neo18          PVEIAGDGTFFQFVPATEELEEALYGAANAEGLLFYAGDNQGVSAAFDEAVLGEAERRFA 365
GenB1          PIDLAGNGNLFQFVCADDEVADAFHAAAAAEGLLFFEGDNQTPSAAFTDEVVEDACGRID 366
GenB2          PIEMAGTGAAFQFVCA-EEVEEVLLPHALAEGLILEPSDQQYPSACFRGEVVDDALERLD 360
GenB3          PNWVGGPALMFDTVLPNDDLGWEIYKTAHDFGVYFEDSGTQLVTTAFDEAAVDHALTAFR 419
GenB4          PNWVGGPALMFDVVLPNDDLGWEIYKTAHDFGVYFEDSGTQLVTAAFDEAAVDHALTAFR 374

Neo18          RVCERLAPY---AGGEPVGDAARYRVAWNVMDGLRQAPRDREETTGLLARLLDD------ 416
GenB1          RVSAALTGR---FTDRELTEESWYASAWGAMDGLADRPRTREETTAIVERLWED------ 417
GenB2          RALTTMAAARPDLVGREVTQLDRVNAAFCQMDGLPGRPDGWSLDQCVEYVTAQL------ 414
GenB3          KATRQVIADRPDIAPTSGGELTEERKLDFAEEAFGGLLRDDERTNALIDETIEKVVNRDR 479
GenB4          KATRQVVADRPDIAPTSGGELTEERKLDFAEEAFGGLLRDDERTNALIDETIEKVVNRDR 434

Neo18          ----------
GenB1          ----------
GenB2          ----------
GenB3          SIKPVLIPAQN 490
GenB4          SIKPVLFPAQN 445
```

B

(1) ΔgenB1

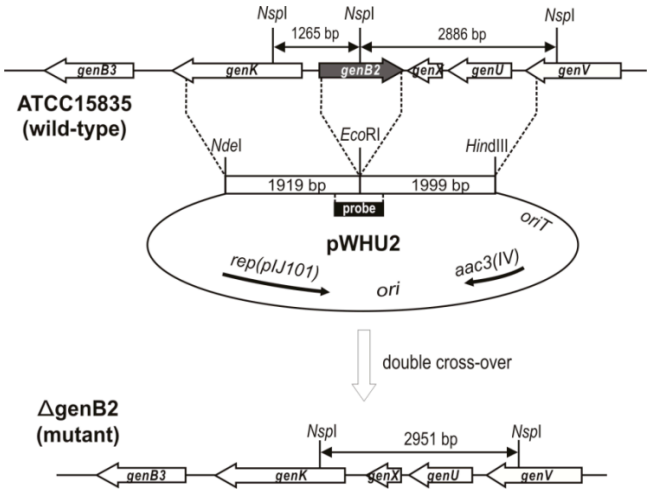
The arrows indicate the expected size of the fragments from the wild type and mutant chromosomal DNA, respectively, hybridized after digestion with *Age*I with a 581 bp probe (PCR-amplified from pWHU4 using oligonucleotide primers genB1-CK1 and CK2).

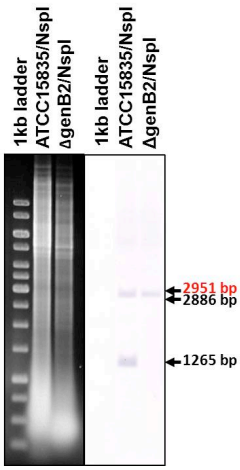




(2) ΔgenB2

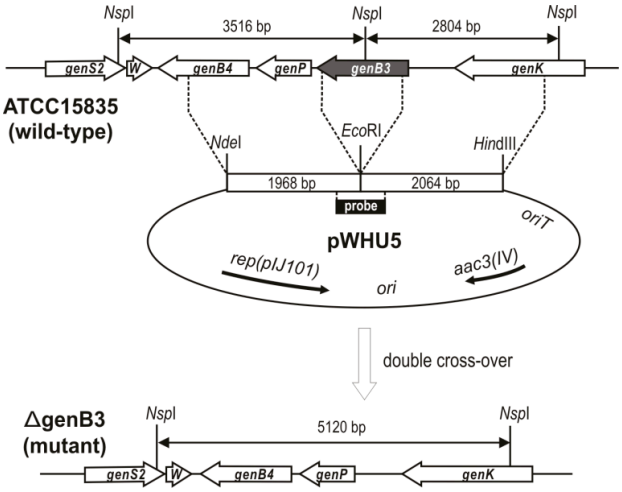
The arrows indicate the expected size of the fragments from the wild type and mutant chromosomal DNA, respectively, hybridized after digestion with *Nsp*I with a 587 bp probe (PCR-amplified from pWHU2 using oligonucleotide primers genB2-CK1 and CK2).

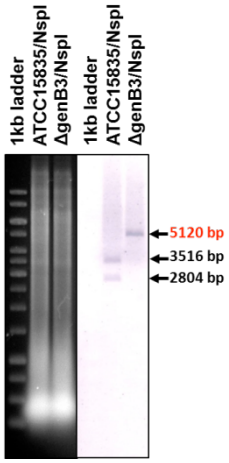




(3) ΔgenB3

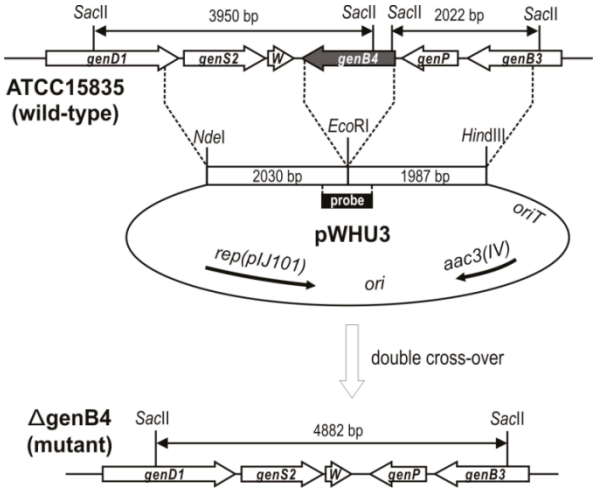
The arrows indicate the expected size of the fragments from the wild type and mutant chromosomal DNA, respectively, hybridized after digestion with *Nsp*I with a 658 bp probe (PCR-amplified from pWHU5 using oligonucleotide primers genB3-CK1 and CK2).

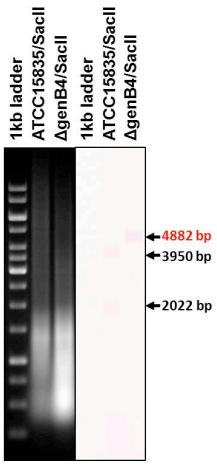




(4) ΔgenB4

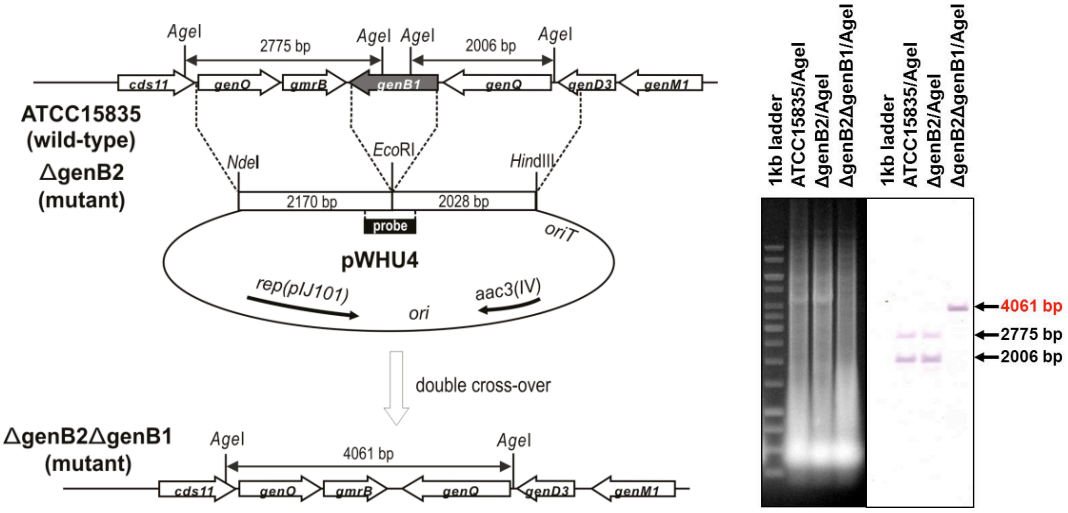
The arrows indicate the expected size of the fragments from the wild type and mutant chromosomal DNA, respectively, hybridized after digestion with *Sac*II with a 595 bp probe (PCR-amplified from pWHU3 using oligonucleotide primers genB4-CK1 and CK2).





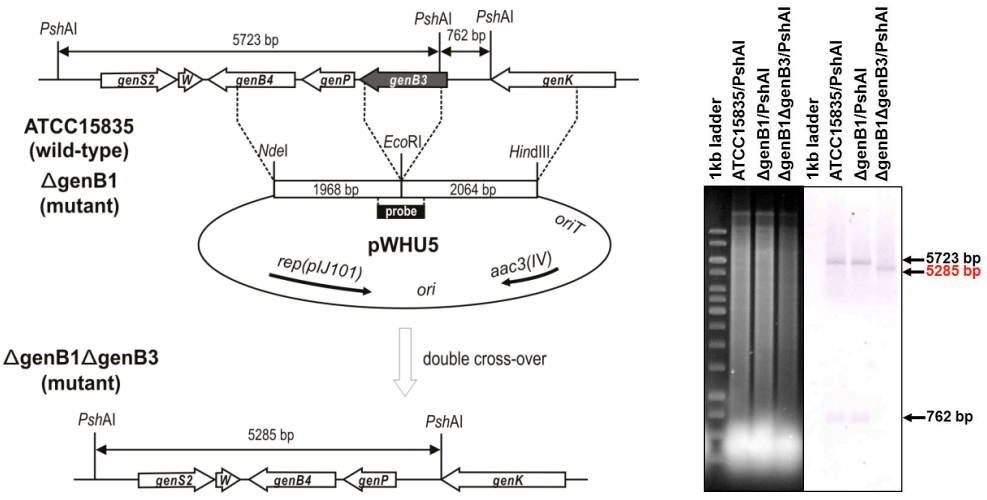
(5) ΔgenB2ΔgenB1

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Age*I with a 581 bp probe (PCR-amplified from pWHU4 using oligonucleotide primers genB1-CK1 and CK2).



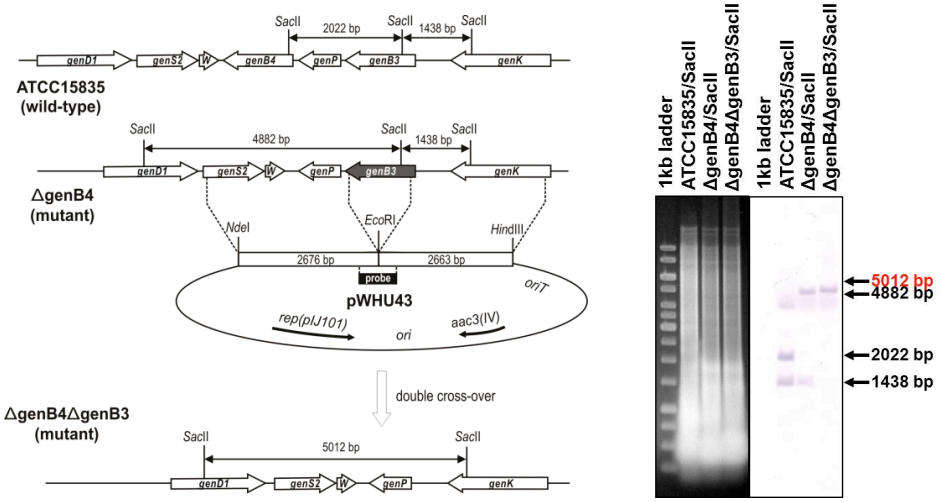
(6) ΔgenB1ΔgenB3

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Psh*AI to a 658 bp probe (PCR-amplified from pWHU5 using oligonucleotide primers genB3-CK1 and CK2).



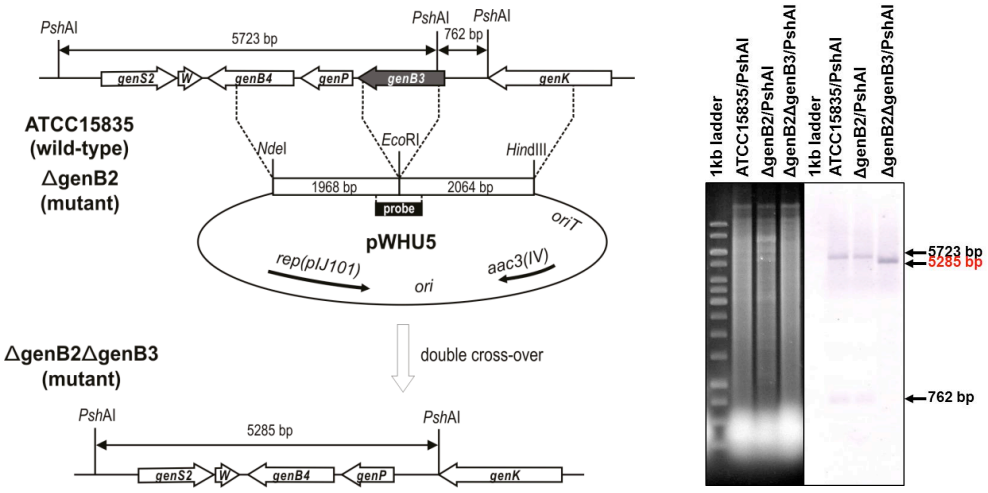
(7) ΔgenB4ΔgenB3

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Sac*II with a 741 bp probe (PCR-amplified from pWHU43 using oligonucleotide primers genB4-genB3-CK1 and CK2).



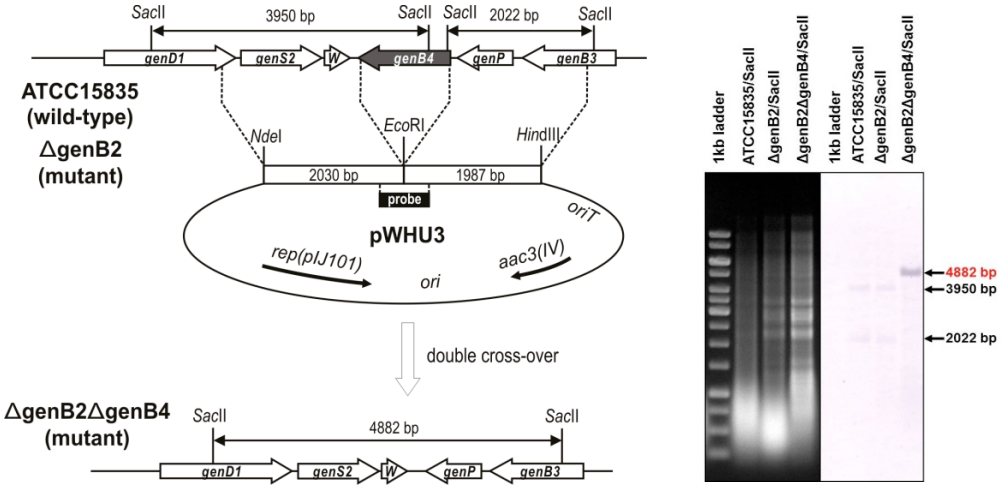
(8) ΔgenB2ΔgenB3

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Psh*AI with a 658 bp probe (PCR-amplified from pWHU5 using oligonucleotide primers genB3-CK1 and CK2).



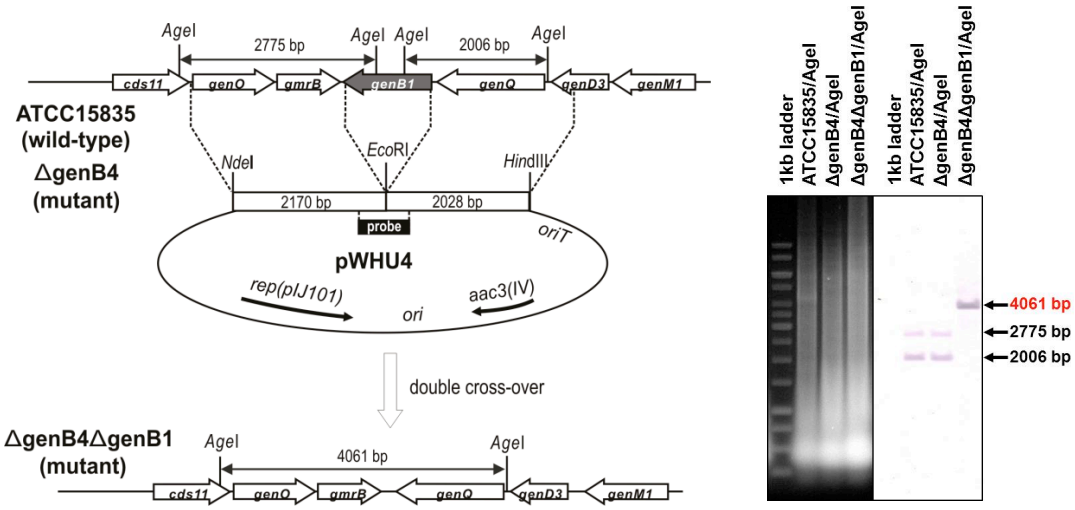
(9) ΔgenB2ΔgenB4

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Sac*II with a 595 bp probe (PCR-amplified from pWHU3 using oligonucleotide primers genB4-CK1 and CK2).



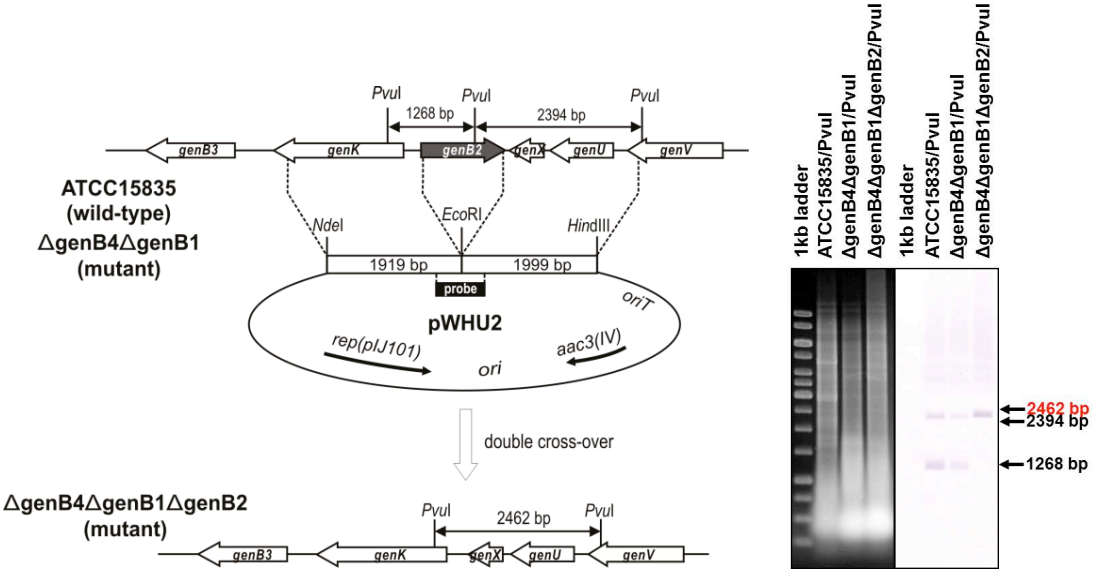
(10) ΔgenB4ΔgenB1

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Age*I with a 581 bp probe (PCR-amplified from pWHU4 using oligonucleotide primers genB1-CK1 and CK2) respectively,.



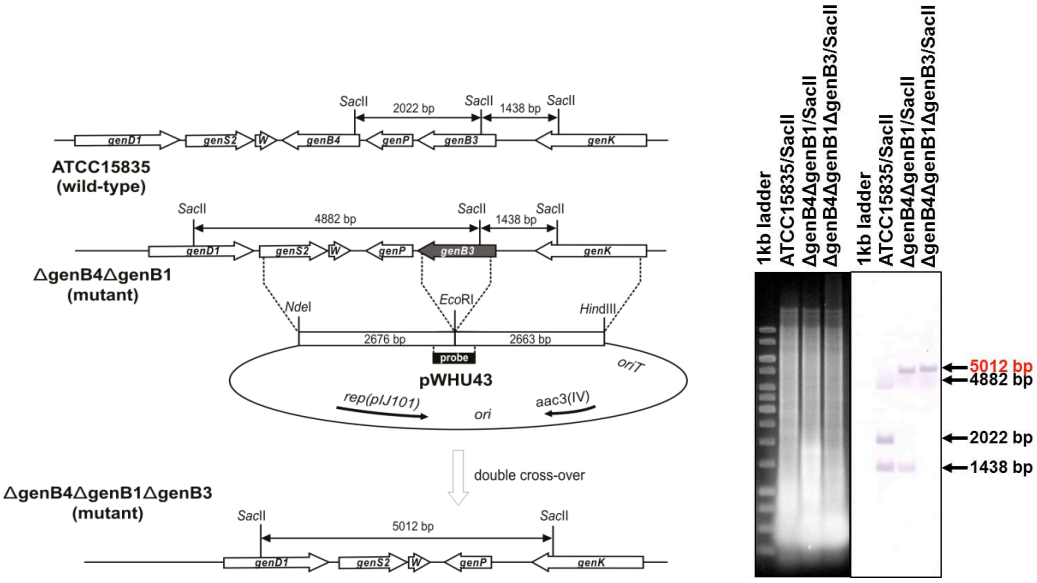
(11) ΔgenB4ΔgenB1ΔgenB2

The arrows indicate the expected size of the fragments from the wild type and mutant schromosomal DNA, respectively, hybridized after digestion with *Pvu*I with a 587 bp probe (PCR-amplified from pWHU2 using oligonucleotide primers genB2-CK1 and CK2).



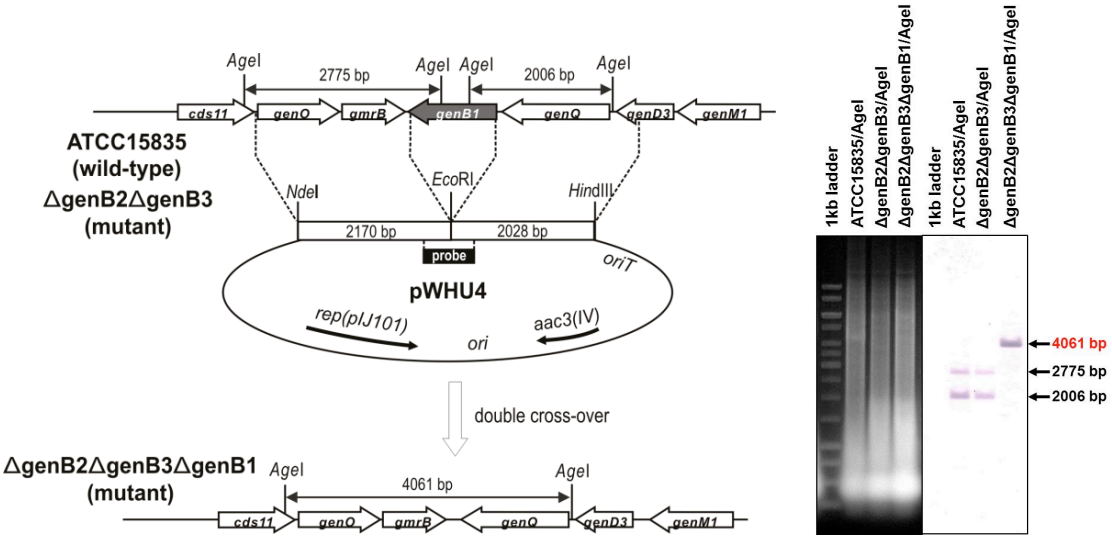
(12) ΔgenB4ΔgenB1ΔgenB3

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Sac*II with a 741 bp probe (PCR-amplified from pWHU43 using oligonucleotide primers genB4-genB3-CK1 and CK2).



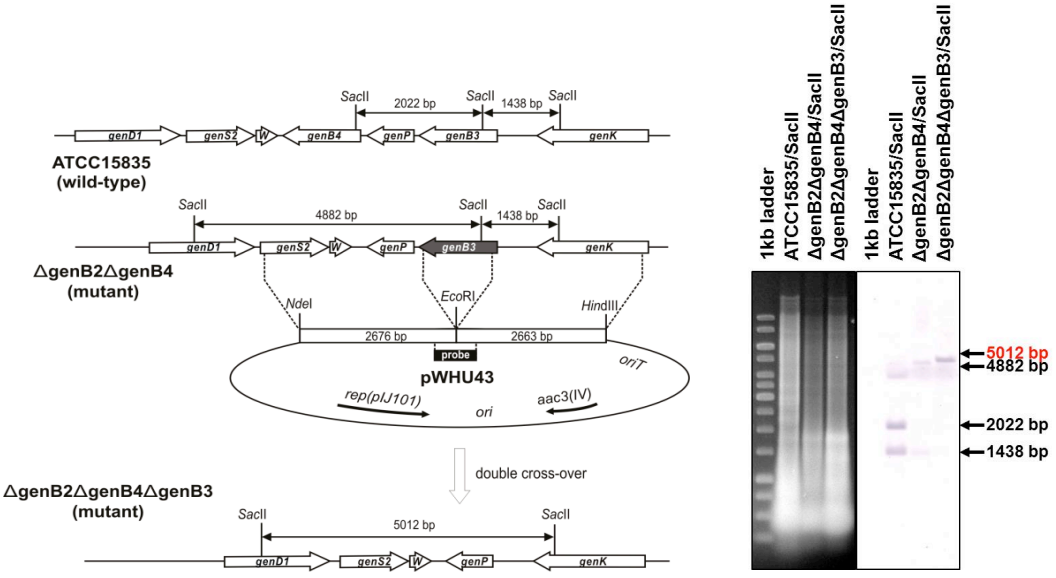
(13) ΔgenB2ΔgenB3ΔgenB1

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Age*I with a 581 bp probe (PCR-amplified from pWHU4 using oligonucleotide primers genB1-CK1 and CK2).



(14) ΔgenB2ΔgenB4ΔgenB3

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Sac*II with a 741 bp probe (PCR-amplified from pWHU43 using oligonucleotide primers genB4-genB3-CK1 and CK2).



(15) ΔgenB4ΔgenB1ΔgenB3ΔgenB2

The arrows indicate the expected size of the fragments from the wild type and mutants chromosomal DNA, respectively, hybridized after digestion with *Pvu*I with a 587 bp probe (PCR-amplified from pWHU2 using oligonucleotide primers genB2-CK1 and CK2).

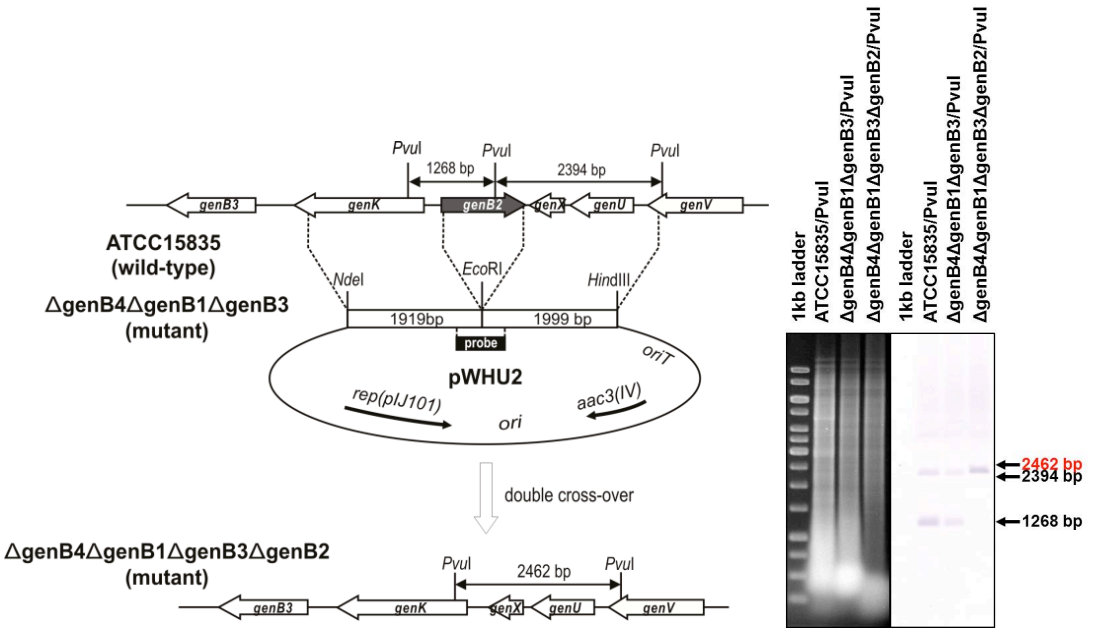


Figure S5, related to Figure 3. Confirmation of C2a production in ΔgenB2 and ΔgenB2ΔgenB1 mutants

(A) Comparison of product mixture from strain ΔgenB2 with gentamicin C2 standard; (B) Comparison of product mixture from strain ΔgenB2ΔgenB1 with gentamicin C2 standard; (C) Comparison of product mixture from strain ΔgenB2 with gentamicin C2a standard; (B) Comparison of product mixture from strain ΔgenB2ΔgenB1 with gentamicin C2a standard; (E) Comparison of product mixture from strain ΔgenB2 with gentamicin C1 standard; (F) Comparison of product mixture from strain ΔgenB2ΔgenB1 with gentamicin C1 standard.

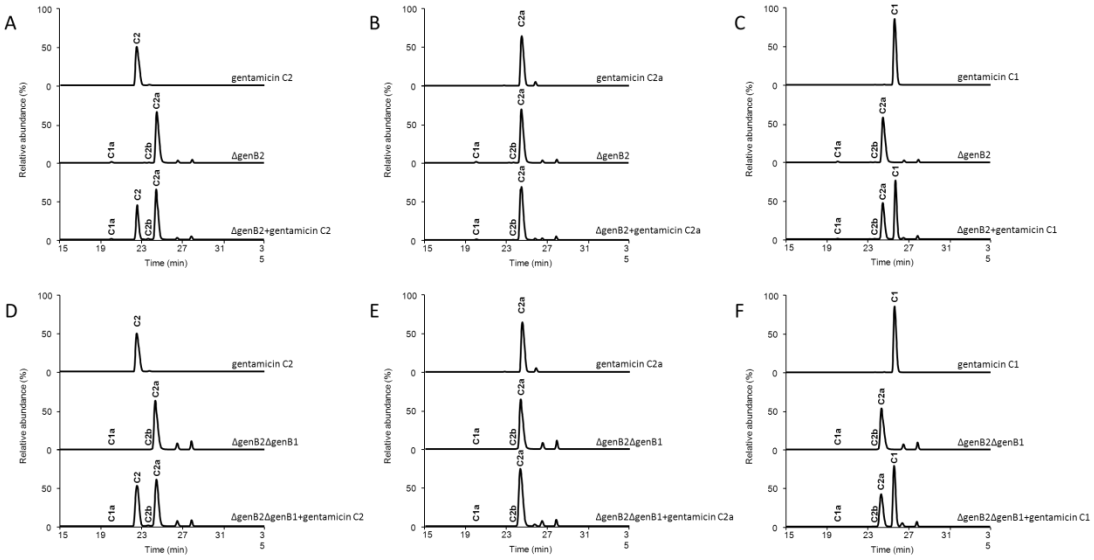


Figure S6, related to Figures 4, 5 and 6. Cloning and characterization of GenQ, GenB1, GenB2, GenB3 and GenB4

(A) Cloning *genQ* with alternative start codons. Positions of the original start codon of *genQ* and the new start codons in LM_*genQ* and VM_*genQ* are indicated in red boxes; (B) SDS-PAGE gel of VM_GenQ, GenB1, GenB2, genB3 and GenB4; (C) UV-Vis spectra of VM_GenQ and FAD standard; (D) UV-Vis spectra of GenB proteins.

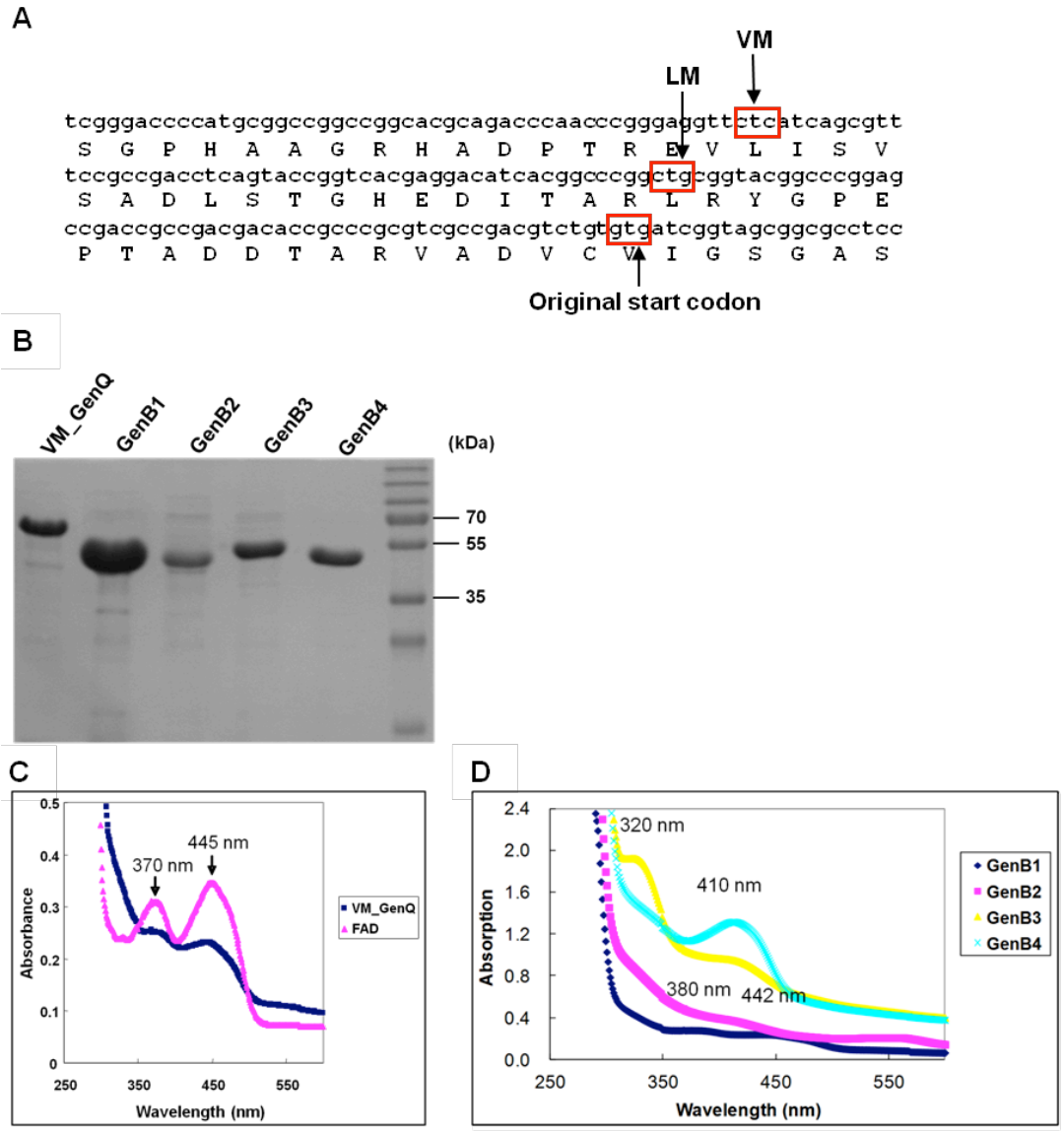
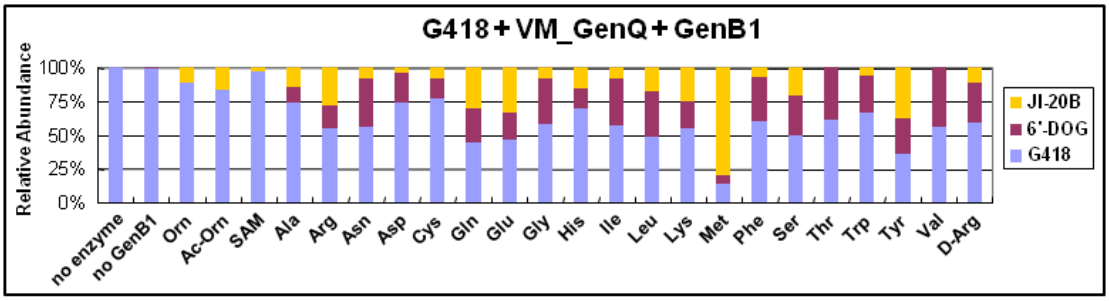


Figure S7, related to Figures 4 and 5. LC-MS analysis of activity assays of GenB1, GenB2, GenB3 and GenB4 (GenB)

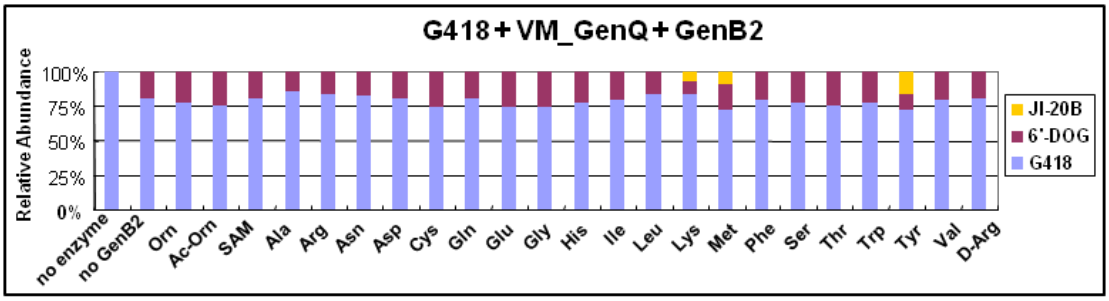
(A) Test of different amino donors on the conversion of G418 to JI-20B (i - iv) and of gentamicin X2 to JI-20A(v - viii) catalysed by VM_GenQ coupled with GenB1, GenB2, GenB3, and GenB4 by LC-MS analysis. The percentages of G418, gentamicin X2, 6'-DOG, 6'-DOX, JI-20B and JI-20A present after 16 h of incubation, based on the abundance of corresponding ions detected, are shown as stacked plots. (B) LC-MS analysis of isomerase activity of GenB enzymes on gentamicin C2 and C2a. Selective ion monitoring $[M+H]^+$ (*m*/*z* 464) and $[M+Na]^+$ (*m*/*z* 486) ions of C2 and C2a.

A

i



ii



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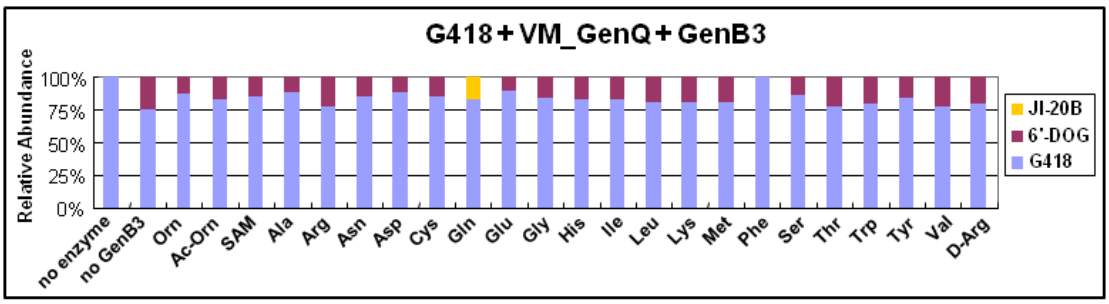

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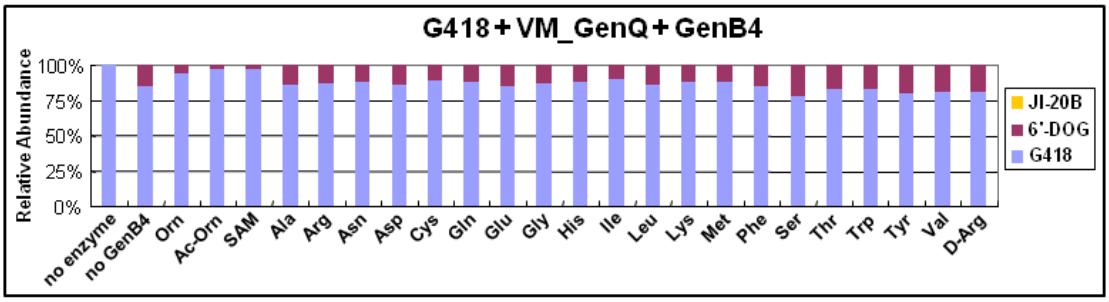
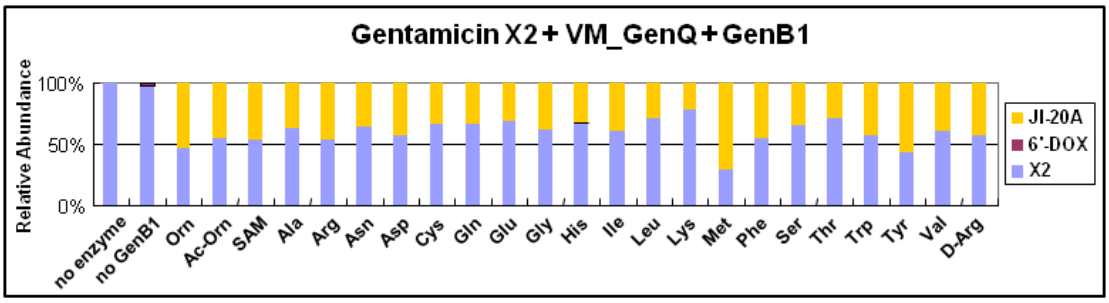
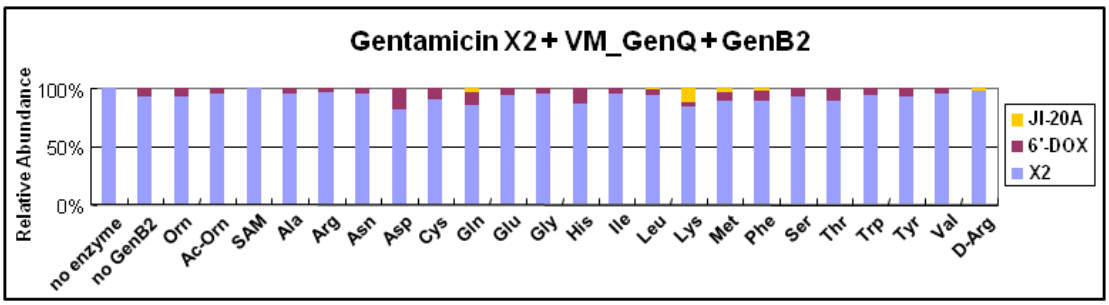


v



vi



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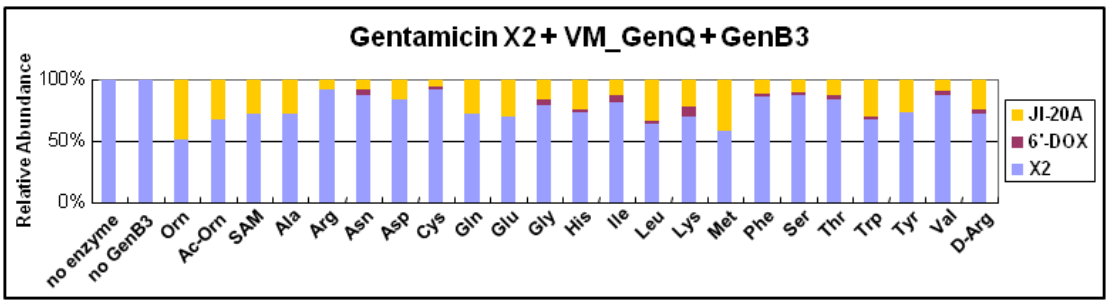
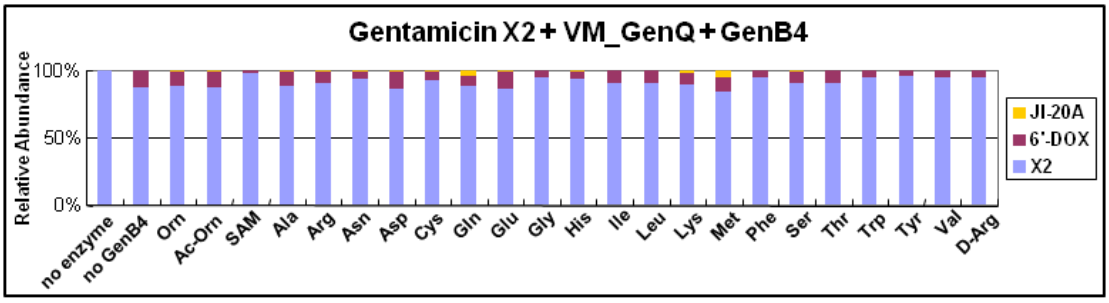
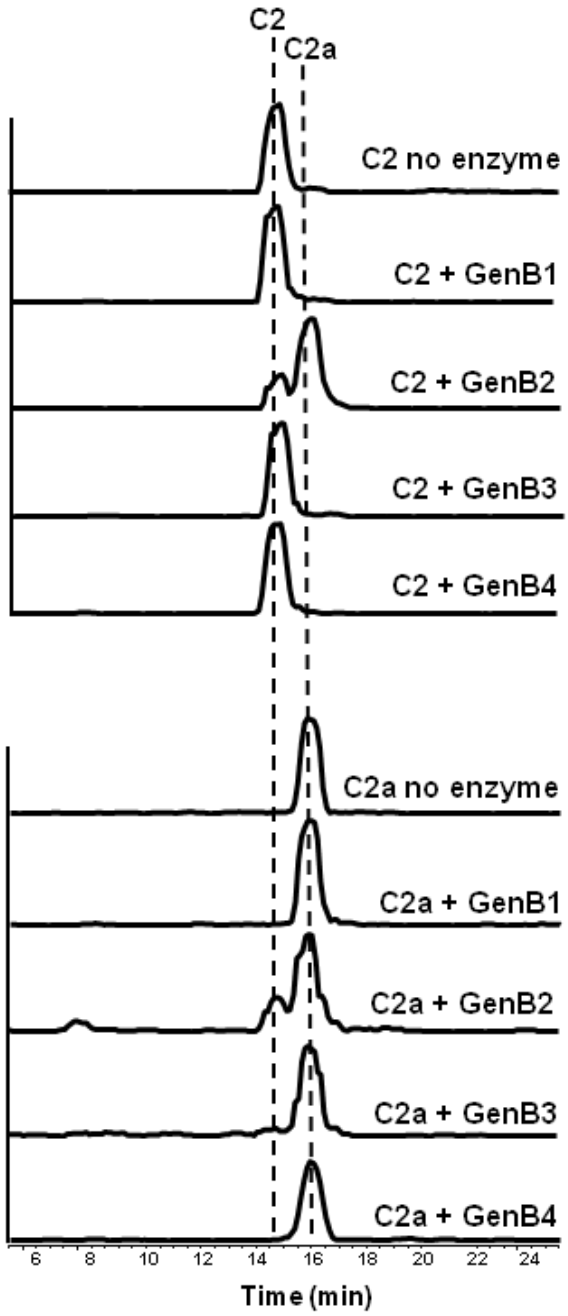


viii



B


C2
C2a
C2 no enzyme
C2 + GenB1
C2 + GenB2
C2 + GenB3
C2 + GenB4
C2a no enzyme
C2a + GenB1
C2a + GenB2
C2a + GenB3
C2a + GenB4
6
8
10
12
14
16
18
20
22
24
Time (min)

List of oligonucleotide primers used in this study, related to Experimental Procedures

Primer	Oligonucleotide sequences (5' to 3')	Restriction site
genQ-L1	CTG**CATATG**CTCACCGCCCGGC	*Nde*I
genQ-L2	CCC**GAATTC**TCCCTGTGACCTGTCG	*Eco*RI
genQ-R1	ACC**GAATTC**GCCGCTACCGATCACAC	*Eco*RI
genQ-R2	GGC**AAGCTT**GATCGGAACCATCCGG	*Hin*dIII
genQ-CP1	CCTCCTCGTCACCGTGG	-
genQ-CP2	CAGGTGCTCAGCGTCCG	-
genQ-EP1	GTC**CATATG**ATCGGTAGCGGCGCC	*Nde*I
genQ-EP2	CGA**GAATTC**ACAGGGAGAAATCGG	*Eco*RI
neo11-EP1	CGC**CATATG**AAGCGCCTTCGAGGC	*Nde*I
neo11-EP2	CCG**GAATTC**TCAGACGTGCGCGGTGTG	*Eco*RI
genK-L1	GCG**CATATG**AGAACCGGCTTGATGC	*Nde*I
genK-L2	GCG**GAATTC**CACTGACCCTCGTCCC	*Eco*RI
genK-R1	GAG**GAATTC**CTTCACTTGATTACC	*Eco*RI
genK-R2	CAC**AAGCTT**CACCGGCGAGACG	*Hin*dIII
genK-CP1	CGGGCGAACCTTCGGGATA	-
genK-CP2	CCGTCAGCGTTGGCAATAA	-
genK-EP1	CGC**CATATG**AACGCGCTGGTGGCA	*Nde*I
genK-EP2	CCG**GAATTC**TCAGTGGGAAACCGCCTC	*Eco*RI
genB1-L1	GCA**CATATG**CTGGAGAATCCGTCTG	*Nde*I
genB1-L2	GTC**GAATTC**CTCTGGGAGGACTGAG	*EcoR*I
genB1-R1	ACC**GAATTC**GATGGTCATCGTGTCG	*EcoR*I
genB1-R2	GAA**AAGCTT**GACCGTCAACCTGGCG	*Hin*dIII
genB1-CK1	GAGTCGTCGTACTTCTGGATGGG	-
genB1-CK2	GCTGACCTGGGGTTGCTGC	-
genB2-L1	GCG**CATATG**TCACCGGCGAAGCGGC	*Nde*I
genB2-L2	CGT**GAATTC**GTCAGCGTTGGCAATA	*EcoR*I
genB2-R1	GTG**GAATTC**GTGACCGCTCAGCTCT	*EcoR*I
genB2-R2	GTC**AAGCTT**GTTGCAGGCGGTCCAG	*Hin*dIII
genB2-CK1	GCCGACTTCGACCTCTTCC	-
genB2-CK2	ATCCGCTGACCCCGTGCC	-

genB3-L1	CGT**CATATG**GCAACACCACGTCG	*Nde*I
genB3-L2	GAC**GAATTC**ATCGAGAAGGTGGTC	*Eco*RI
genB3-R1	CGA**GAATTC**GGTCCCGATGTCGTAG	*Eco*RI
genB3-R2	CTT**AAGCTT**TGGCGAGGGGCTCTGC	*Hin*dIII
genB3-CK1	CGCGTTACGGAAAGTAAAATCAC	-
genB3-CK2	CATCGAGGGCCACCACC	-
genB4-L1	CGC**CATATG**CAGCGGTTCAAGGGCG	*Nde*I
genB4-L2	GTC**GAATTC**CCGGCACAGAACTGAC	*Eco*RI
genB4-R1	GAT**GAATTC**ACGGTAGTTCATGTGC	*Eco*RI
genB4-R2	CGG**AAGCTT**TGTGTGAGCGGTACGG	*Hin*dIII
genB4-CK1	TGACTTCTGCCTCGACAACG	-
genB4-CK2	AAGCTCTACCTGGAGACCTTCC	-
genB4-genB3-L1	GGC**CATATG**CGGTGGCGACCC	*Nde*I
genB4-genB3-L2	CGC**GGTACC**AAGCCGGTTCTCA	*Kpn*I
genB4-genB3-R1	CAA**GGTACC**AGAATCCATGTCC	*Kpn*I
genB4-genB3-R2	AAT**AAGCTT**GGGAATTAGTCCAG	*Hin*dIII
genB4-genB3-CK1	CAGCGTTCCTCCGGTTTC	-
genB4-genB3-CK2	GGTAAGTGATCCGCAGTTGG	-
pGenQ-LM_up	ATCACGGCC**CATATG**CGGTACGGCCCGGAG	*Nde*I
pGenQ-VM_up	CCCAACCCGG**CATATG**CTCATCAGCGTTTC	*Nde*I
pGenQ-dn	TTGCCGGCACC**GAATTC**GATGGTCATCGTG	*Eco*RI
pGenB1-up	AATCGAGCGAC**CATATG**ACCATCGACATCG	*Nde*I
pGenB1-dn	AGGACCTGGTGG**GGATCC**ACAAGGAGTGAA	*Bam*HI
pGenB2-up	CGGAGGTTTCG**CATATG**ATTATTGCCAACG	*Nde*I
pGenB2-dn	CGGCCCCTGCC**GGATCC**GTCAGAGCTGAGC	*Bam*HI
pGenB3-up	TTG GGGATAG**CATATG**GCAGTCGCCGACCA	*Nde*I
pGenB3-dn	ATCTTCACCGGCCCTG**GAATTC**CTACGCC A	*Eco*RI
pGenB4-up	CGTGGTGGG**CATATG**AACTACCGTGAGTTG	*Nde*I
pGenB4-dn	CGGCCCTCGAACCG**GAATTC**CTCAAGGTCA	*Eco*RI

Primer pairs for amplification of left- or right-flanking fragments of a target gene, for PCR/sequencing confirmation, and for cloning target genes for over-expression are marked with suffixes –L1/–L2, -R1/-R2, CK1/CK2, or –up/-dn, respectively.

Table S1, related to FIgure 3. LC-HRMS analysis of *genB* knock-out mutants

Strain	Gentamicin C complex production ($\times 10^5$)					Intermediates production ($\times 10^5$)			
	C1a	**C2b**	**C2**	**C2a**	**C1**	**X2**	**JI-20A**	**G418**	**JI-20B**
wild type	8.8	2.8	11.9	1.1	44.6	8.5	1.5	8.2	4.3
ΔgenB1	0.4	2.9	1.2	1.7	14.6	6.8	0.5	15.9	2.5
ΔgenB2	0.8	0.1	ND	7.6	ND	7.1	0.7	0.7	1.0
ΔgenB3	ND	ND	ND	ND	ND	7.2	19.1	10.8	75.4
ΔgenB4	ND	ND	ND	ND	ND	11.0	2.7	14.1	8.7
ΔgenB2ΔgenB3	ND	ND	ND	ND	ND	8.6	2.7	8.8	20.5
ΔgenB2ΔgenB4	ND	ND	ND	ND	ND	7.6	2.0	1.2	3.7
ΔgenB4ΔgenB1	ND	ND	ND	ND	ND	5.2	1.4	18.6	6.7
ΔgenB2ΔgenB1	0.3	0.1	ND	15.3	ND	4.0	0.5	10.7	2.9
ΔgenB1ΔgenB3	ND	ND	ND	ND	ND	1.5	0.1	43.0	1.4
ΔgenB4ΔgenB3	ND	ND	ND	ND	ND	9.7	1.1	17.0	6.3
ΔgenB2ΔgenB3ΔgenB1	ND	ND	ND	ND	ND	3.4	0.1	12.8	0.1
ΔgenB4ΔgenB1ΔgenB2	ND	ND	ND	ND	ND	1.9	0.1	40.3	3.4
ΔgenB4ΔgenB1ΔgenB3	ND	ND	ND	ND	ND	3.3	0.1	20.8	0.8
ΔgenB2ΔgenB4ΔgenB3	ND	ND	ND	ND	ND	7.4	2.5	20.8	35.2
ΔgenB4ΔgenB1ΔgenB3ΔgenB2	ND	ND	ND	ND	ND	2.3	ND	142.0	ND

Table S2, related to Experimental Procedures. Plasmids used in this study

Plasmid	Description	Reference
pUC18	Sub-cloning vector	Sambrook and Maniatis
pET28a(+)	Vector for protein expression	Invitrogen
pIB139	Site-specific integrative vector with P*ermE** promoter	Wilkinson et al., 2002; Del Vecchio et al., 2003
pYH7	*E. coil* - *Streptomyces* shuttle vector	Sun et al., 2008
pWHU77	pIB139 derivative with Tsr^R	This study
pYH286	*genQ* in-frame deletion construct	This study
pWHU1	*genK* in-frame deletion construct	This study
pWHU67	ΔgenK complementation construct containing *genK* under control of P*ermE** promoter	This study
pWHU163	ΔgenQ complementation construct containing *genQ* under control of P*ermE** promoter	This study
pWHU165	ΔgenQ complementation construct containing *neo11* under the control of the P*ermE** promoter	This study
pWHU4	*genB1* in-frame deletion construct	This study
pWHU2	*genB2* in-frame deletion construct	This study
pWHU5	*genB3* in-frame deletion construct	This study
pWHU3	*genB4* in-frame deletion construct	This study
pWHU43	*genB3* and *genB4* double in-frame deletion construct	This study
pET28/LM_genQ	pET28a(+) vector with VM_*genQ* gene insert	This study
pET28/VM_genQ	pET28a(+) vector with LM_*genQ* gene insert	This study
pET28/genB1	pET28a(+) vector with *genB1* gene insert	This study
pET28/genB2	pET28a(+) vector with *genB2* gene insert	This study
pET28/genB3	pET28a(+) vector with *genB3* gene insert	This study
pET28/genB4	pET28a(+) vector with *genB4* gene insert	This study

Table S3, related to Experimental Procedures. In-frame deletion mutants and complemented strains used in this study

Gene knockout mutant	Parent strain	Plasmid used to achieve deletion
ΔgenK	wild type	pWHU1
ΔgenQ	wild type	pYH286
ΔgenQΔgenK	ΔgenQ	pWHU1
ΔgenB1	wild type	pWHU4
ΔgenB2	wild type	pWHU2
ΔgenB3	wild type	pWHU5
ΔgenB4	wild type	pWHU3
ΔgenB2ΔgenB1	ΔgenB2	pWHU4
ΔgenB1ΔgenB3	ΔgenB1	pWHU5
ΔgenB4ΔgenB3	ΔgenB4	pWHU43
ΔgenB2ΔgenB3	ΔgenB2	pWHU5
ΔgenB2ΔgenB4	ΔgenB2	pWHU3
ΔgenB4ΔgenB1	ΔgenB4	pWHU4
ΔgenB4ΔgenB1ΔgenB2	ΔgenB4ΔgenB1	pWHU2
ΔgenB4ΔgenB1ΔgenB3	ΔgenB4ΔgenB1	pWHU43
ΔgenB2ΔgenB3ΔgenB1	ΔgenB2ΔgenB3	pWHU4
ΔgenB2ΔgenB4ΔgenB3	ΔgenB2ΔgenB4	pWHU43
ΔgenB4ΔgenB1ΔgenB3ΔgenB2	ΔgenB4ΔgenB1ΔgenB3	pWHU2
Complemented mutant	**Parent strain**	**Plasmid for complementation**
ΔgenK::*genK*	ΔgenK	*genK* gene in pWHU77
ΔgenQ::*genQ*	ΔgenQ	*genQ* gene in pWHU77
ΔgenQ::*neo11*	ΔgenQ	*neo11* gene in pWHU77