TABLE S1: Primers used in this study

Primer #	Sequence 5' – 3'	Target/Use
P1	GTGAAGGCAAGATGCCATGCATG	Upstream *upp* gene
P2	CCGTC**TACTCATCGCGGAGGTGTCAGTAGTACACTTTAGGCACGT**GGTTGTCTCCTTGGACGTGG	Upstream *upp* gene
P3	CAACC**ACGTGCCTAAAGTGTACTACTGACACCTCCGCGATGAGTA**GACGGTATCCGCAATGGTGG	Dowstream *upp* gene
P4	ATTCGGGCGTGACGAAATTGGGCAC	Dowstream *upp* gene
P5	AAACGGAGAAGGTCAGCGTA	Internal *upp* gene
P6	GATGTAGCCGTGTTCGTTCA	Internal *upp* gene
P7	GGCACTCCATGTGGGAAC	Upstream of P1
P8	CGAAAAGGGTACGGTCGATA	Downstream of P4
P9	*GCGCCATGGCCAGTGTGCCGGTCTCCG*	Kan promoter
P10	GACGACGTAAACAGCCACGGTTGTC**TCCT***CATCCTGTCTCTTGATCAGAGC*	Kan promoter
P11	*GCTCTGATCAAGAGACAGGATG***AGGA**GACAACCGTGGCTGTTTACGTCGTC	*upp* gene
P12	GCCGGCACATTCACGATGCGCCG	*upp* gene
P13	CAGCAGAAAAAGGCGCTCATGCA	Upstream of *hsdR*, Southern probe
P14	GGCAGCATGGCGCATAAGTCGCGACGACTTGTT	Upstream of *hsdR*, Southern probe
P15	GCGACTTATGCGCCATGCTGCCAAGCTGGAAAGAAA	Downstream of DVU1702
P16	GCTGTTCCGTAATGAAGTATCGCCCAA	Downstream of DVU1702
P17	AAGGAATTCATTGACGGCAAGCCC	Internal *hsdR*
P18	GCTTGGACGCTGTGTTGAACTTGT	Internal *hsdR*
P19	GGATACACAAAGCGGGCTTATCGA	Screening for *hsdR* deletion & sequencing of *hsdR* deletion cassette
P20	GCACGGGTACATGGCATCCATTTA	Sequencing of *hsdR* deletion cassette
P21	CCGGTATATGACAGCCAACGAGTGT	Screening for *hsdR* deletion & sequencing of *hsdR* deletion cassette
P22	GTTATGGCTCGTTTGCCGATAGTCA	Sequencing of *hsdR* deletion cassette
P23	GCTATGACCATGATTACGCCAAGCTCAG	Screening inserts in pCR4Blunt-TOPO
P24	ACGGCCAGTGAATTGTAATACGACTCAC	Screening inserts in pCR4Blunt-TOPO
P25	AACGACGGCCAGTCTTAAGC	Screening inserts in pCR8/GW/TOPO
P26	AGACACGGGCCAGAGCTG	Screening inserts in pCR8/GW/TOPO
P27	GAATTCGACCCAGCTTTCTTGT	Screening inserts in EcoRV site of pCR8/GW/TOPO
P28	ATTTCTGTCCTGGCTGGTCTAGAGG	Screening inserts into EcoRV site of pCR8/GW/TOPO

TABLE S1. Continued

Primer #	Sequence 5’ – 3’	Target/Use
P29	CGGGCAGTGAGCGCAACGCAATT	Screening for pMO727
P30	GCTGAAGCGCATCGTGGACAAGCA	Screening for pMO727
P31	GCGAGCTCCCTCCAGCTTGAG	DVU3152 (Chromosomal screen)
P32	GCGAGCTCCGGTGTACATCCCA	DVU3152 (Chromosomal screen)
P33	AGAAAGGTAGCCATCTACGGCAAGG	*nifH* gene (pDV1 screen)
P34	TCTCAAGTTCTTCGACCTGAAGCG	*nifH* gene (pDV1 screen)
P35	CTCTCTGCAACCTGACGGCG	*fur* gene (Chromosomal screen)
P36	ACAGCAGTCTTCACATGCGCTT	*fur* gene (Chromosomal screen)

*Unique barcode sequence is indicated as: **Bold underline** base pairs

**Kan gene sequence is indicated as: *Italicized* base pairs

***AGGA common between kan promoter and *upp* gene is indicated as: **Bold** base pairs

Figure S1.

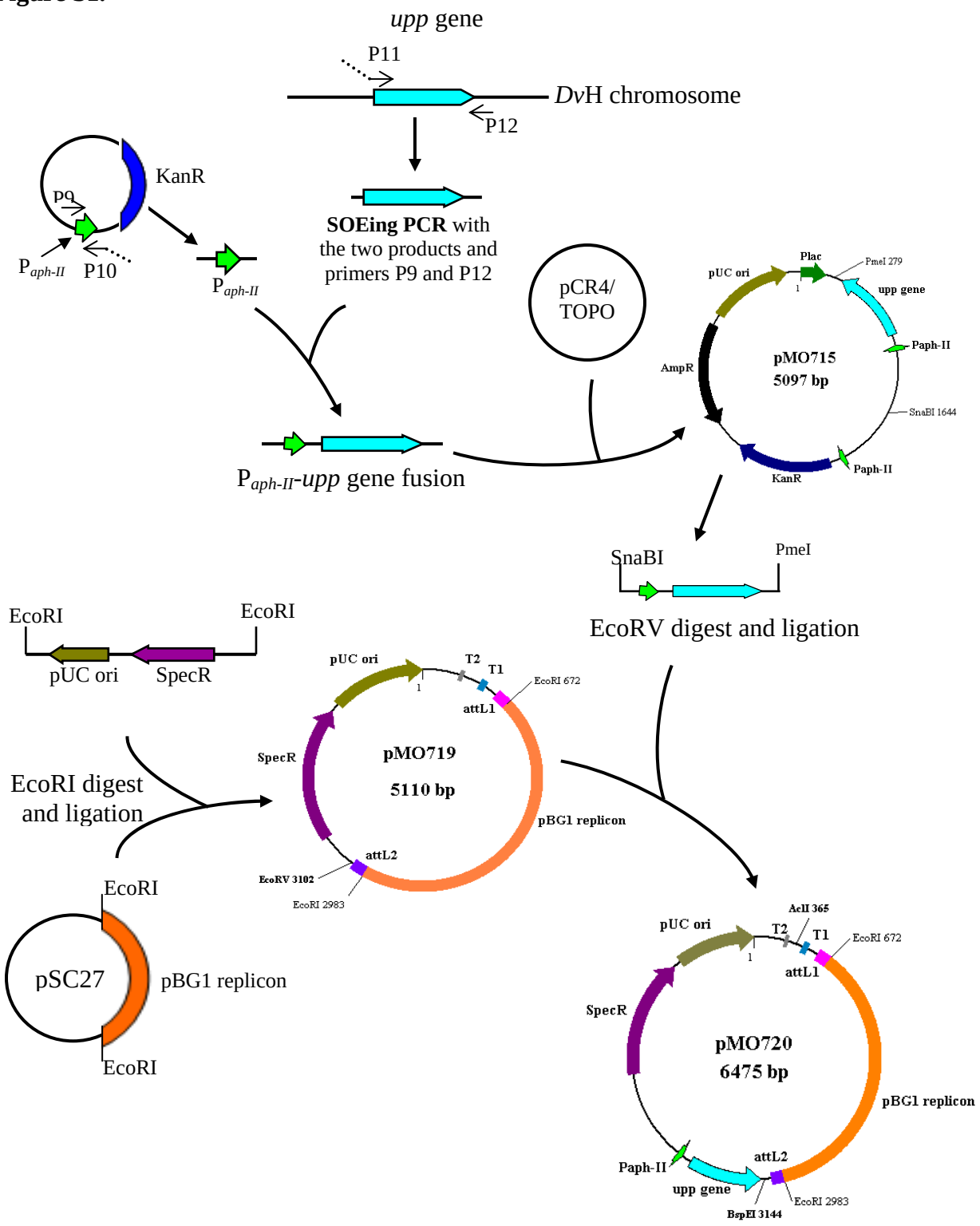

upp gene
P11
DvH chromosome
P12
KanR
P9
P aph-II
P10
P aph-II
SOEing PCR with the two products and primers P9 and P12
pCR4/ TOPO
P aph-II-upp gene fusion
pUC ori
Plac
PmeI 279
upp gene
Paph-II
pMO715
5097 bp
AmpR
SnaBI 1644
Paph-II
KanR
SnaBI
PmeI
EcoRV digest and ligation
EcoRI
EcoRI
pUC ori
SpecR
EcoRI digest and ligation
pUC ori
T2
T1
EcoRI 672
attL1
SpecR
pMO719
5110 bp
pBG1 replicon
attL2
EcoRV 3102
EcoRI 2983
EcoRI
pSC27
pBG1 replicon
EcoRI
pUC ori
AclI 365
T2
T1
EcoRI 672
attL1
SpecR
pMO720
6475 bp
pBG1 replicon
attL2
Paph-II
upp gene
EcoRI 2983
BspEI 3144

Figure S1: Construction of pMO715, pMO719, and pMO720. The *aph*-II promoter (the promoter for the kanamycin resistance gene in Tn*5*) was fused to the wild-type *upp* gene and captured in the pCR4Blunt-TOPO plasmid, creating pMO715. To construct pMO719, the pBG1 SRB replicon was excised from pSC27 by EcoRI digest. A ligation of the pBG1 replicon fragment with the pCR8/GW/TOPO bounded EcoRI fragment resulted in the generation of the plasmid pMO719. To construct pMO720, the constitutively expressed *upp* gene was excised from pMO715 by a SnaBI/PmeI double digest and then cloned into the EcoRV site of pMO719. (Not drawn to scale)

Figure S2.

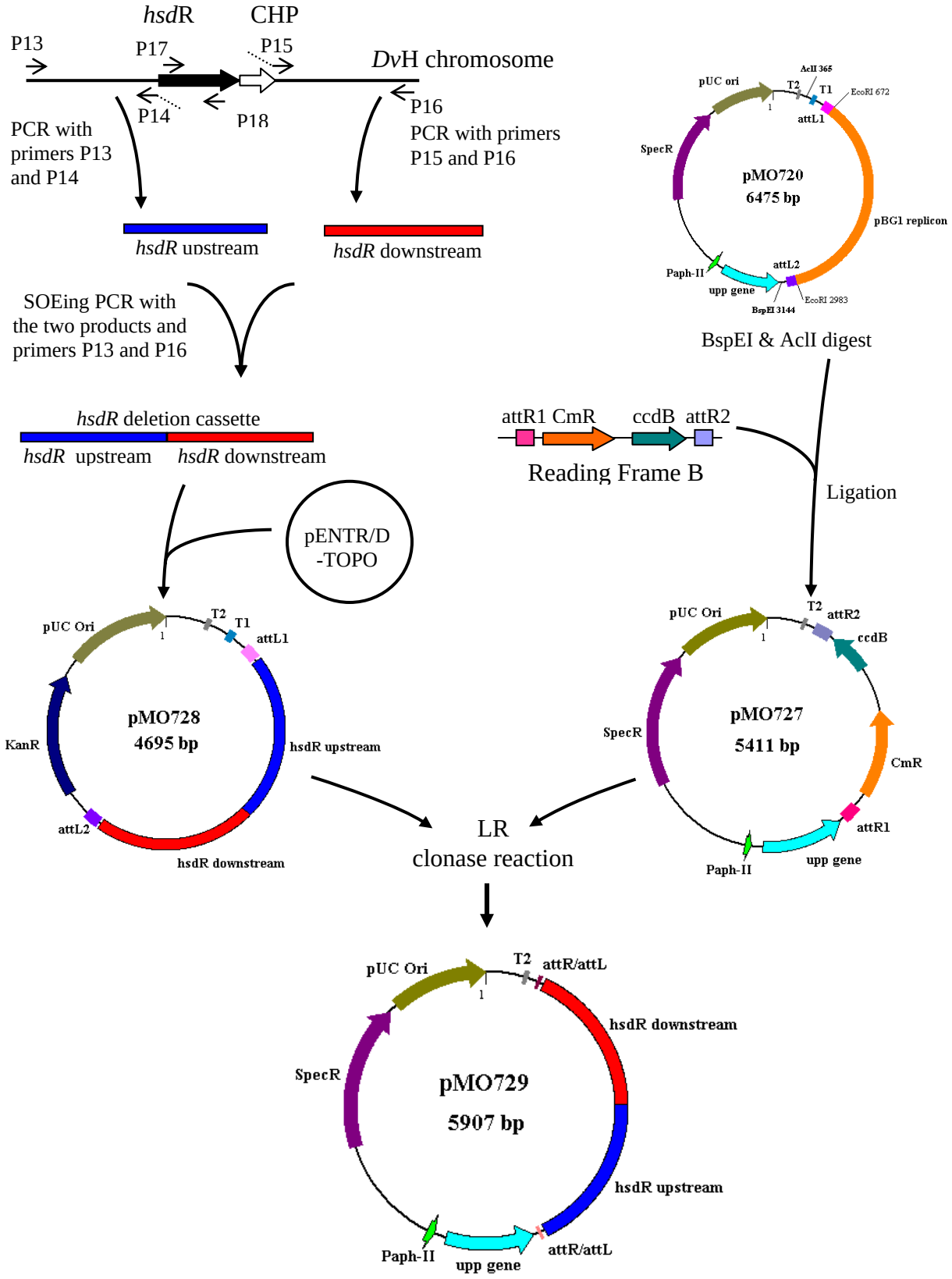
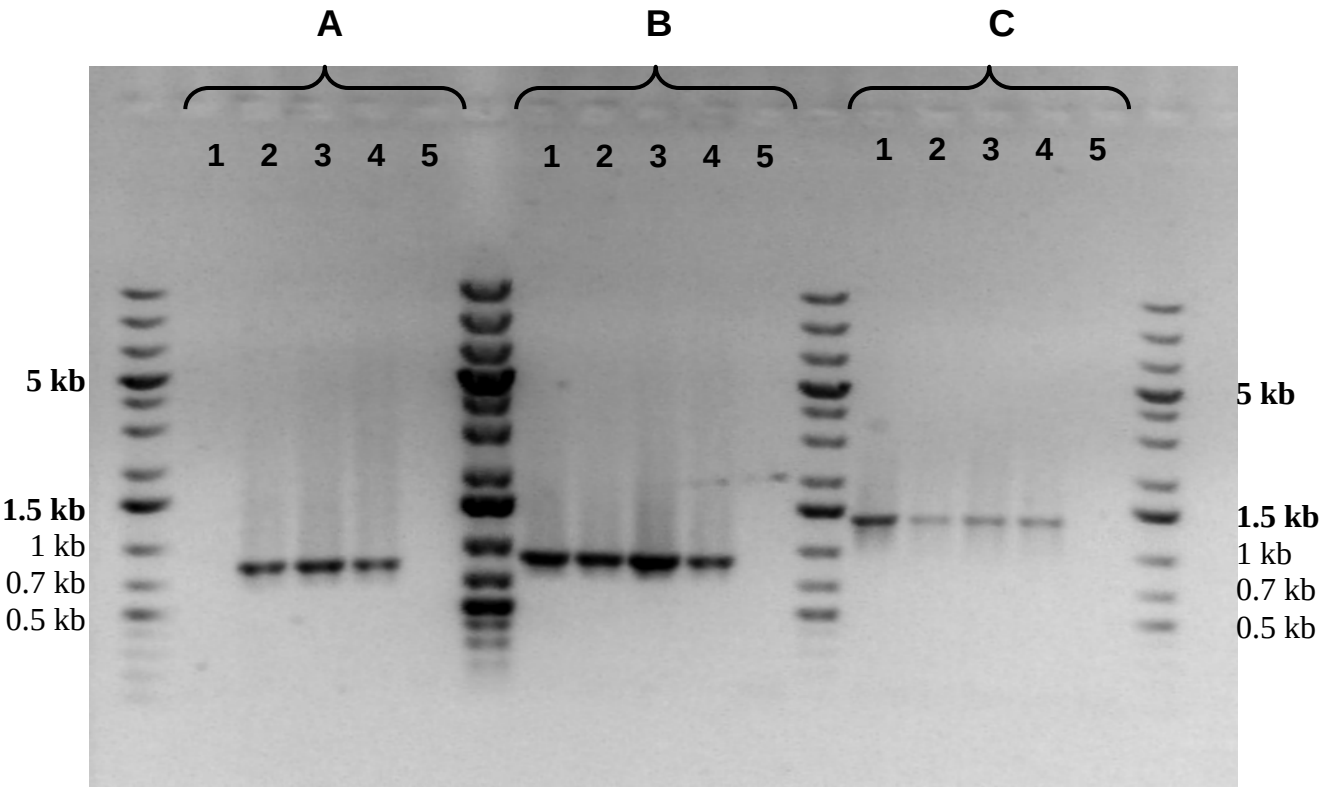

hsdR
CHP
P13
P17
P15
DvH chromosome
P14
P18
P16
PCR with primers P15 and P16
PCR with primers P13 and P14
hsdR upstream
hsdR downstream
SOEing PCR with the two products and primers P13 and P16
hsdR deletion cassette
hsdR upstream
hsdR downstream
pENTR/D -TOPO
pUC Ori
T2
T1
attL1
pMO728
4695 bp
KanR
hsdR upstream
attL2
hsdR downstream
pUC ori
AclI 365
T2
T1
EcoRI 672
attL1
SpecR
pMO720
6475 bp
pBG1 replicon
Paph-II
attL2
upp gene
BspEI 3144
EcoRI 2983
BspEI & AclI digest
attR1 CmR
ccdB
attR2
Reading Frame B
Ligation
pUC Ori
T2
attR2
ccdB
SpecR
pMO727
5411 bp
CmR
attR1
Paph-II
upp gene
LR
clonase reaction
pUC Ori
T2
attR/attL
hsdR downstream
SpecR
pMO729
5907 bp
hsdR upstream
Paph-II
upp gene
attR/attL

Figure S2: Construction of the destination vector, pMO727; entry clone for *hsdR*, pMO728; and the mutagenic plasmid for the construction of Δ*hsdR*, pMO729. Reading Frame B from the Gateway® Vector Conversion (Invitrogen) was cloned into pMO720 following the removal of the pBG1 replicon and *att*L1/*att*L2 sites, generating the destination vector, pMO727. A markerless deletion cassette with ~1 kb of the region upstream of *hsdR* was fused to directly to ~1 kb of the region downstream of DVU1702 and cloned into pENTR/D producing the entry vector, pMO728. The markerless deletion suicide vector for the deletion of the *hsdR* was generated by a LR clonase reaction between pMO727 and pMO728, creating pMO729. (Not drawn to scale)

Figure S3.


A
B
C
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
5 kb
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1 kb
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0.5 kb
5 kb
1.5 kb
1 kb
0.7 kb
0.5 kb

Figure S3: Determination that the increased transformation efficiency of JW7035 is not due to loss of the native plasmid, pDV1. Three separate PCR screens were performed, (A) *nifH* gene (DVUA0015) located on native pDV1, 793 bp; (B) histidine kinase gene (DVU3152) located on the chromosome, 868 bp; and (C) *pep* and *fur* genes (DVU0941 and DVU0942, respectively) located on the chromosome, 1241 bp. The template DNAs used were: (1) JW801, (2) *D. vulgaris*, (3) JW710, (4) JW7035, and (5) primers only, no DNA template added. Standards are the GeneRuler™ 1 kb Plus DNA Ladder (Fermentas).