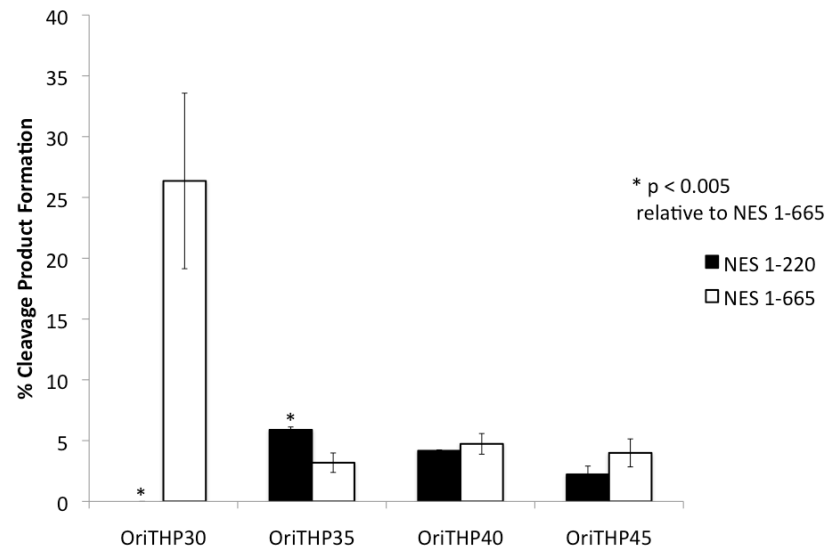
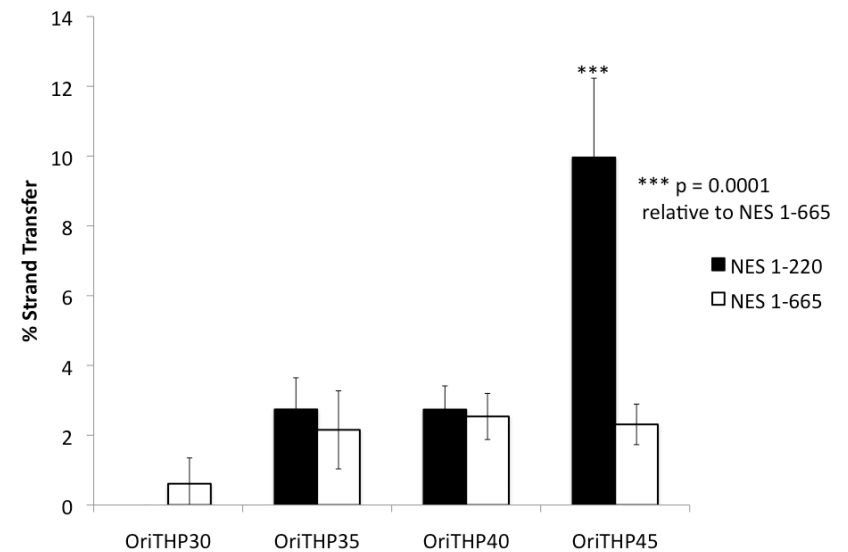


Figure S1

A.



B.

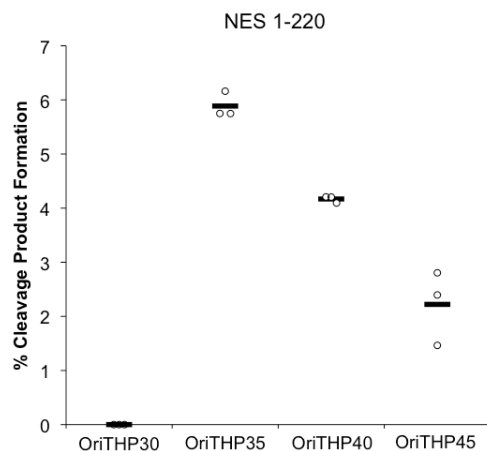


Supplemental Figure S1. Cleavage and Strand Transfer by Relaxase Domain and Full-length NES.

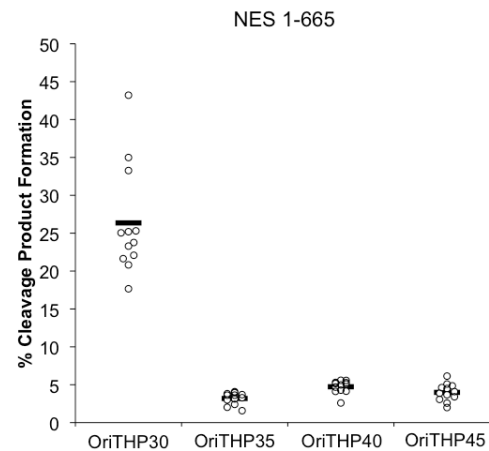
- Effect of oligonucleotide length on cleavage by NES 1-220 (relaxase domain) and NES 1-665 (full-length) protein. Data for NES 1-665 is the same as presented in Figure 2C.
- Effect of oligonucleotide length on strand transfer by NES 1-220 (relaxase domain) and NES 1-665 (full-length) protein. Data for NES 1-66 is the same as presented in Figure 2D.

Figure S2

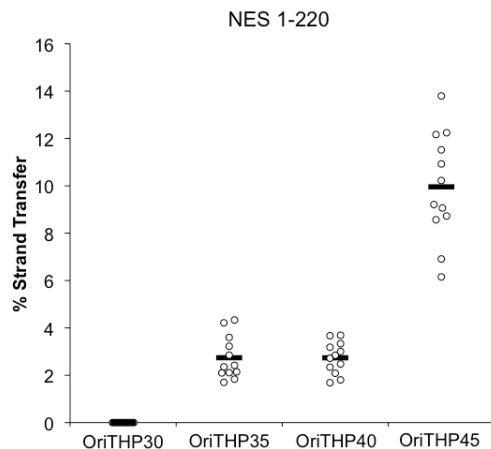
A.



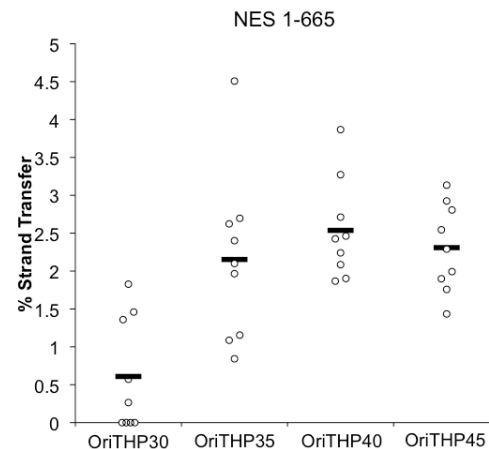
B.



C.



D.

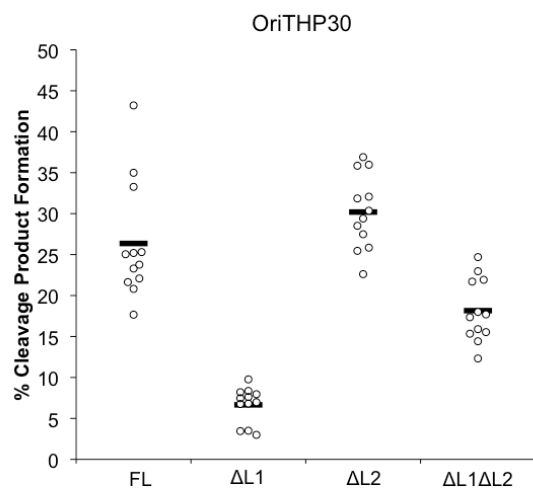


Supplemental Figure S2. Cleavage and Strand Transfer by Relaxase Domain and Full-length NES.

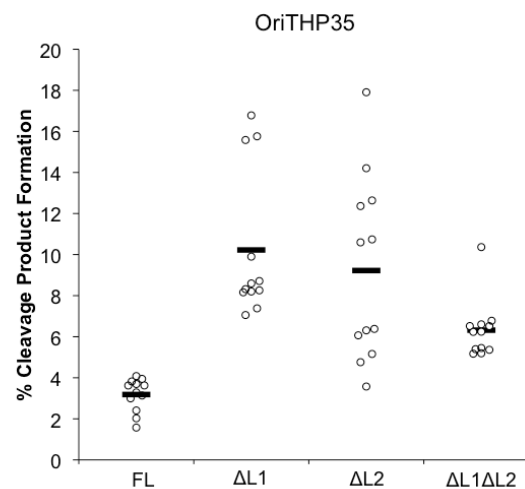
- A. Individual data points for the effect of oligonucleotide length on cleavage by NES 1-220 (relaxase domain) protein as shown in Figure S1.
- B. Individual data points for the effect of oligonucleotide length of cleavage by NES 1-665 (full-length) protein as shown in Figure S1. Data is the same as presented in Figure S3.
- C. Individual data points for the effect of oligonucleotide length on strand transfer by NES 1-220 (relaxase domain) protein as shown in Figure S1.
- D. Individual data points for the effect of oligonucleotide length on strand transfer by NES 1-665 (full-length) protein as shown in Figure S1. Data is the same as presented in Figure S4.

Figure S3

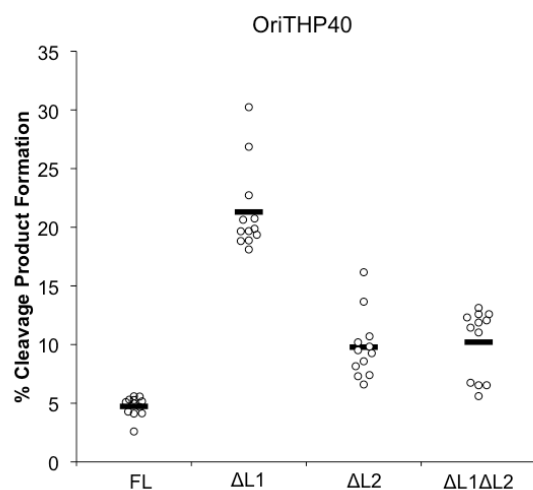
A.



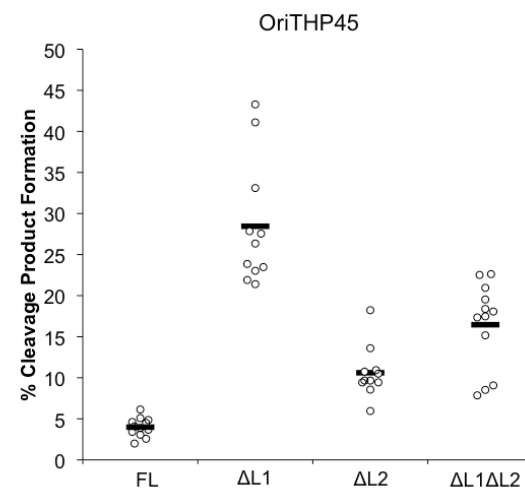
B.



C.



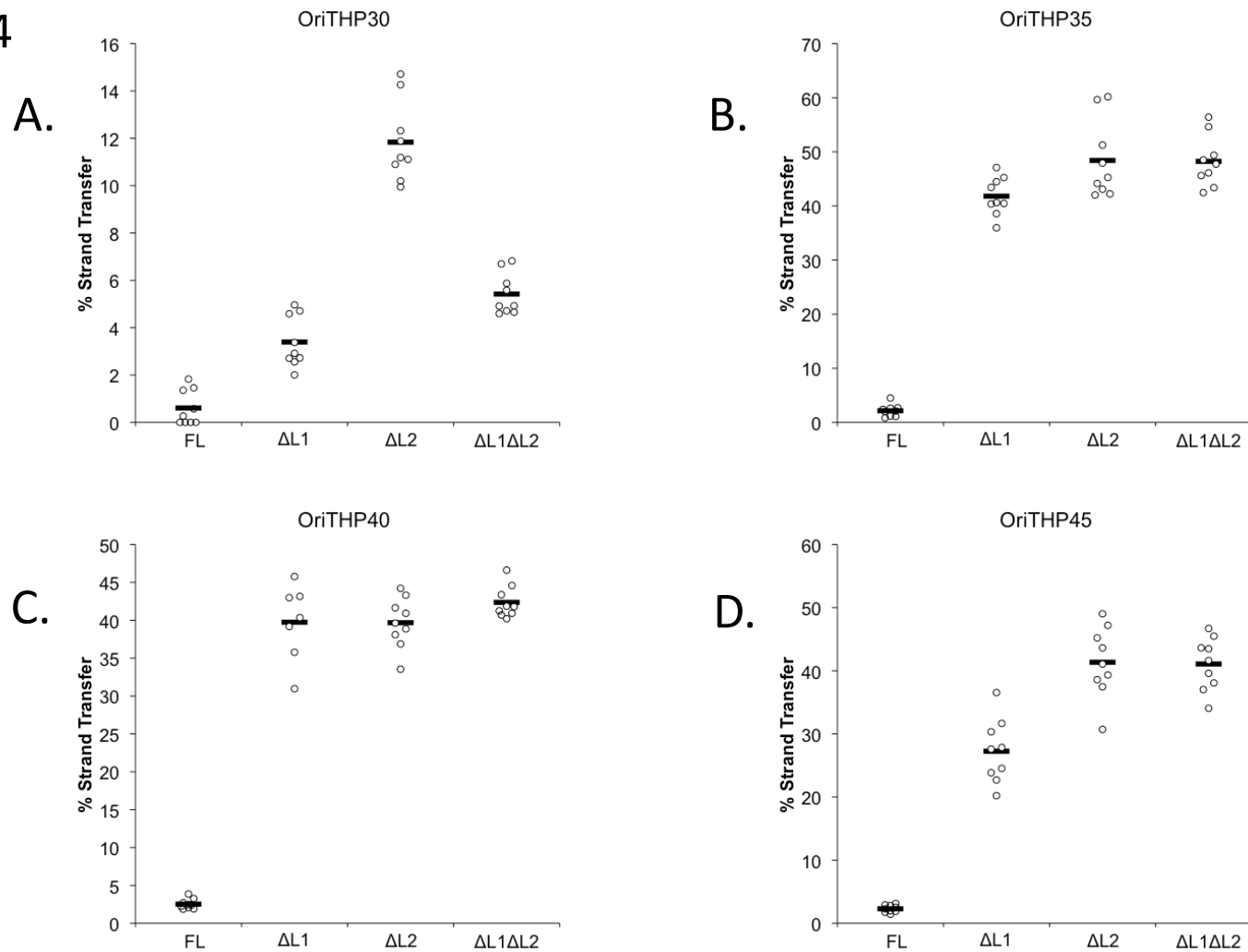
D.



Supplemental Figure S3. Cleavage by NES Loop Deletion Mutants.

- A. Cleavage activity of NES mutants on OriTHP30 as shown in Figure 2C.
- B. Cleavage activity of NES mutants on OriTHP35 as shown in Figure 2C.
- C. Cleavage activity of NES mutants on OriTHP40 as shown in Figure 2C.
- D. Cleavage activity of NES mutants on OriTHP45 as shown in Figure 2C.

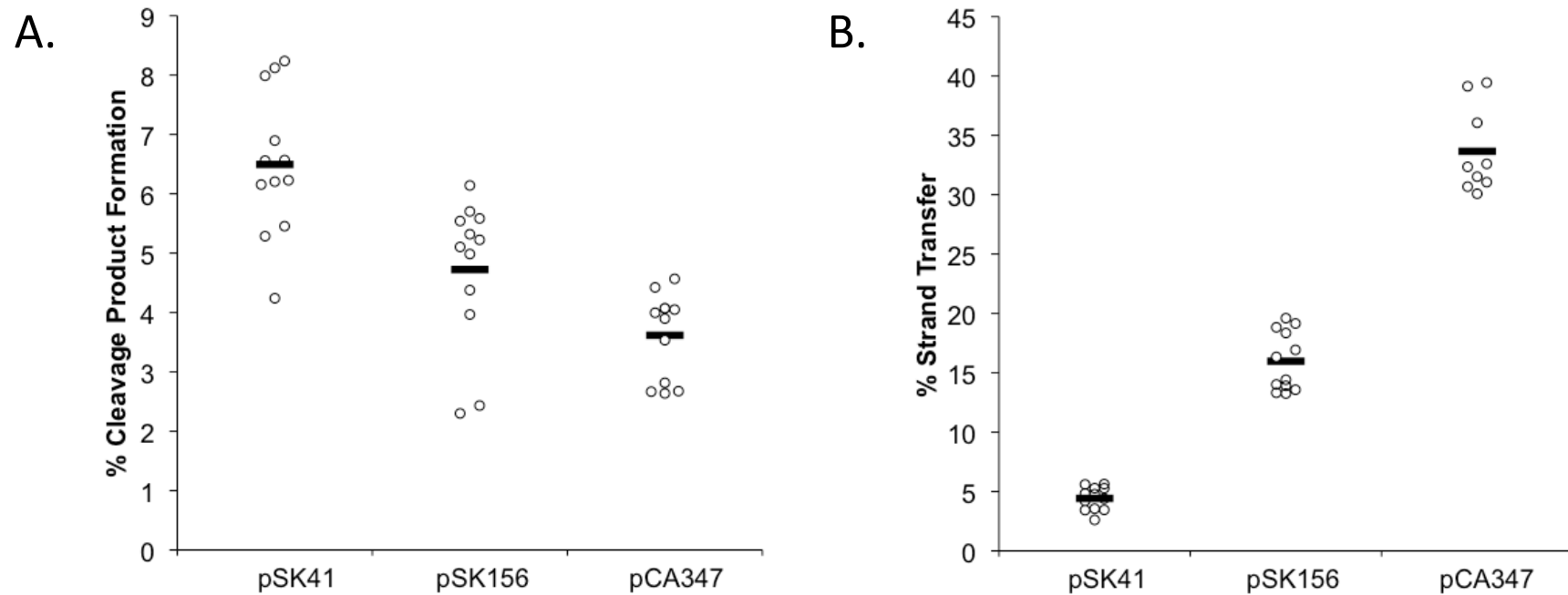
Figure S4



Supplemental Figure S4. Strand Transfer by NES Loop Deletion Mutants.

- A. Strand transfer activity of NES mutants on OriTHP30 as shown in Figure 2D.
- B. Strand transfer activity of NES mutants on OriTHP35 as shown in Figure 2D.
- C. Strand transfer activity of NES mutants on OriTHP40 as shown in Figure 2D.
- D. Strand transfer activity of NES mutants on OriTHP45 as shown in Figure 2D.

Figure S5



Supplemental Figure S5. Cleavage and Strand Transfer Processing of pSK41, pSK156, and pCA347 *oriT* Oligonucleotides.

- A. Cleavage activity of the pSK41-encoded NES protein on the pSK41, pSK156, and pCA347 *oriTs* as shown in Figure 4B.
- B. Strand transfer activity of the pSK41-encoded NES protein on the pSK41, pSK156, and pCA347 *oriTs* as shown in Figure 4C.

Figure S6

pSK41 *oriT*-string

cccaagcttAGC**ACGCGAACGGAACGTTTCGCATAAGTGCGCCCTTACGGGAT**TTAACTAGATTATAACGG
TAAACTTGAATCTGTCAAACGAAggatccgc

pSK156 *oriT*-string

gatcggatccTTTTTCTTTCGACGCCGTATCGTCGATGTAATTCAAAAAGTTATGGGCTATAAATCTAC
ATCACTTTTTCAAGAATGTAGTAGCAATATTC AACGCAAATTAATTTTTATAACTGCTCGGAATATCTCA
AGCCGTTTCTTTAATTTTGAAATAAAAAAATCGACGAAGGTCGATTACGTTTTTGTAC**ACGTCGATTTA**
TCCGACGTATAAGTGCGCCCTTACGGGATTTAACTAGATTATAACGACGAATTTTAGACCTGTAAAGCAA
Tggtaccaagcttggatccgatc

pCA347 *oriT*-string

gatcggatccTTTTTCTTTCGACGCCGTATCGTCGATGTAATTCAAAAAGTTATGGGCTATAAATCTAC
ATCACTTTTTCAAGAATGTAGTAGCAATATTC AACGCAAATTAATTTTTATAACTGCTCGGAATATCTCA
AGCCGTTTCTTTAATTTTGAAATAAAAAAATCGACGAAGGTCGATTACGTTTTTGCAC**ATGTCGATTTAT**
CCGACGTATAAGTGCGCCCTTACGGGATTTAACTAGATTATAACGACGAATTTTAGACCTGTAAAGCAAT
ggtaccaagcttggatccgatc

Supplemental Figure S6. Nucleotide Sequences for *in vivo* Transfer Assays. Nucleotide sequences of the GeneArt Strings (Invitrogen) encompassing the *oriT* regions of plasmids pSK41 (GenBank Acc. AF051917, nt 10189-10273), pSK156 (GenBank Acc GQ900448, nt 13396-13126) and pCA347 (GenBank Acc. CP006045, nt 3825-4094). Sequences in lower case were added to the DNA fragments to facilitate cloning and the *Bam*HI and *Hind*III restriction sites used are underlined. Sequence in bold correlates to the oligonucleotide *oriTs* shown in Fig. 3A.

Supplemental Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description ^a	Reference or source
<i>Escherichia coli</i>		
DH5 α	F ⁻ <i>endA hsdR17 supE44 thi-1 λ recA1 gyrA96 relA1 ϕ80 dlacZAM15</i>	Bethesda Research Laboratories
BL21(DE3)	F ⁻ <i>ompT hsdSB (r_B⁻ m_B⁻) dcm gal λ(DE3)</i>	Novagen
<i>Staphylococcus aureus</i>		
RN4220	Restriction-deficient derivative of NCTC8325-4	Kreiswirth <i>et al.</i> , 1983
SK5428	SK982 harboring pSK41	Lyon <i>et al.</i> , 1984
WBG541	Sm ^R /Nb ^R derivative of NCTC8325-4	Townsend <i>et al.</i> , 1983
WBG4515	Fs ^R /Rf ^R derivative of NCTC8325-4	Townsend <i>et al.</i> , 1983
Plasmids		
pSK41	Gm ^R , Tb ^R , Km ^R , Nm ^R , <i>tra</i> ⁺ , conjugative multiresistance plasmid	Berg <i>et al.</i> , 1998
pSK5632	pSK1-based <i>S. aureus</i> (Cm ^R)/ <i>E. coli</i> (Ap ^R) shuttle vector	Grkovic <i>et al.</i> , 2003
pSK6877	pSK41 <i>oriT</i> -string cloned into <i>Hind</i> III and <i>Bam</i> HI of pSK5632	This study
pSK6879	pCA347 <i>oriT</i> -string cloned into <i>Bam</i> HI of pSK5632	This study
pSK6881	pSK156 <i>oriT</i> -string cloned into <i>Bam</i> HI of pSK5632	This study

^a Sm^R, streptomycin resistance; Nb^R, novobiocin resistance; Fs^R, fusidic acid resistance; Rf^R, rifampin resistance; Gm^R, gentamycin resistance; Tb^R, tobramycin resistance; Km^R, kanamycin resistance; Nm^R, neomycin resistance; Cm^R, chloramphenicol resistance; Ap^R, ampicillin resistance; *tra*⁺ conjugative transfer genes.

References:

Kreiswirth, B.N., Lofdahl, S., Betley, M.J., O'Reilly, M., Schlievert, P.M., Bergdoll, M.S., and Novick, R.P. (1983) The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* 305:709-712.

Lyon, B.R., May, J.W., and Skurray, R.A. (1984) Tn4001: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. *Mol. Gen. Genet.* 193:554-556.

Townsend, D.E., Grubb, W.B. and Ashdown, N. (1983) Gentamicin resistance in methicillin-resistant *Staphylococcus aureus*. *Pathology*, **15**, 169-174.

Berg, T., Firth, N., Apisiridej, S., Hettiaratchi, A., Leelaporn, A., and Skurray, R.A. (1998) Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multiresistance plasmids. *J. Bacteriol.* 180:4350-4359.

Grkovic, S., Brown, M.H., Hardie, K.M., Firth, N., and Skurray, R.A. (2003) Stable low-copy-number *Staphylococcus aureus* shuttle vectors. *Microbiol.* 149:785-794.