

**Table 1: Primers and PCR programs used in this study for deletion mutagenesis and cloning.**

Primer	Nucleotide sequence (5' - 3')	Target	PCR program
del- <i>nanS</i> -for1	CGCCCGATTATTACTATGTTCTTACCGTTGCTG GTCAGTCgcgattgtgtaggctggagc <sup>1</sup>	FRT-flanked kanamycin resistance cassette on pKD4 with homologies to <i>nanS</i> of <i>E.</i> <i>coli</i> C600	1 x 95 °C, 300 s  30 x (95 °C, 30 s ; 59 °C, 60 s; 72 °C, 90 s)
del- <i>nanS</i> -rev1	AGTGCTGAAATGACTGCTTCGCAGTGCCGTCG TCCAGTTCcatggtccatagaatcctcc <sup>1</sup>		
del- <i>nanS</i> -for2	TTACTATGTTCTTACTGTTGCTGGTCAGTCTAA TGCCATgtgtaggctggagctgctcg <sup>1</sup>	FRT-flanked kanamycin resistance cassette on pKD4 with homologies to <i>nanS</i> of <i>E.</i> <i>coli</i> EDL933	1 x 72 °C, 300 s <sup>4</sup>
del- <i>nanS</i> -rev2	AACTGCAAAATTGCTTCTACAAACCTGTCAGAA ATAATCCCatatgaatcctccttag <sup>1</sup>		

Del-Z1466-for	ATGGCATTAAACACTATGATGTTGTCAGGGCG	FRT-flanked kanamycin resistance cassette on pKD4 with homologies to <i>nanS</i> -p1 of <i>E. coli</i> EDL933
Del-Z1466-rev	GCGTCGCCGgcgattgttaggctggagc <sup>1</sup>  TGCCGCAGTGTCTGTGCTGTTCACTCCCACCG CAATGCTGTCcatggtccatatgaatatcctcc <sup>1</sup>	
Del-Z3342-for	ATGGCATTAAACACTACGATGTGGTCAGGGC	FRT-flanked kanamycin resistance cassette on pKD4 with homologies to <i>nanS</i> -p2 of <i>E. coli</i> EDL933
Del-Z3342-rev	GGCATCGCCGgcgattgttaggctggagc <sup>1</sup>  TGCCGCAGTGTCTGTGTTGTTCACTTCCACCG CAATGCTGTCcatggtccatatgaatatcctcc <sup>1</sup>	
Del-Z1466-for	See above	FRT-flanked kanamycin resistance cassette on pKD4 with homologies to <i>nanS</i> -p3 of <i>E. coli</i> EDL933
Del-Z1793-rev	TTATGATGATGCCGGTGCATTTATCTCCAGCAC CAGACTTTCcatggtccatatgaatatcctcc <sup>1</sup>	

Del-Z2377-for	CGCCGTCAGACCTTGCGGAAAAGCTGACACAC AAACTGAAAGgcgattgtgtaggctggagc <sup>1</sup>	FRT-flanked kanamycin resistance cassette on pKD4	
Del-Z2377-rev	GCATTTATCTCCAGCATCAGACTTTCTATCTCA ACGCCATACcatggtccatatgaatatcctcc <sup>1</sup>	with homologies to <i>nanS</i> -p4 and <i>nanSp</i> -7 of <i>E. coli</i> EDL933	
Del-Z3926/27-for	GTGTGTCATTCAGGCTTCCGGACTACAGCCCA CTCCATATgcgattgtgtaggctggagc <sup>1</sup>	FRT-flanked kanamycin resistance cassette on pKD4	
Del-Z2377-rev	See above	with homologies to <i>nanS</i> -p5 of <i>E. coli</i> EDL933	
Del-Z2377-for	See above	FRT-flanked kanamycin resistance cassette on pKD4	
Del-Z1793-rev	See above	with homologies to <i>nanS</i> -p6 of <i>E. coli</i> EDL933	

<p><i>nanS</i>-for</p> <p><i>nanS</i>-r</p>	<p>ATGAACGCAATAATATCGCC</p> <p>TCGCGCCAAAAGTGC AAAAT</p>	<p>Confirmation of <i>nanS</i> deletion</p>	<p>1 x 95 °C, 300 s</p> <p>30 x (95 °C, 30 s ; 49 °C, 30 s; 68 °C, 60 s)</p> <p>1 x 68 °C, 300 s<sup>6</sup></p>
<p>Stx2b-182-for</p> <p>Z1466-r</p>	<p>CTCAGTTGACAGGAATGAC</p> <p>TGCCGCAGTGTCTGTGCTG</p>	<p>Confirmation of <i>nanS</i>-p1a deletion</p>	<p>1 x 95 °C, 300 s</p> <p>30 x (95 °C, 30 s ; 57 °C, 30 s; 68 °C, 150 s)</p> <p>1 x 68 °C, 300 s<sup>6</sup></p>
<p>Stx1b-168-for</p> <p>Z3342-r</p>	<p>TCAGTCTCTTCTTCTCAGT</p> <p>TGCCGCAGTGTCTGTGTTG</p>	<p>Confirmation of <i>nanS</i>-p2 deletion</p>	<p>1 x 95 °C, 300 s</p> <p>30 x (95 °C, 30 s ; 49 °C, 30 s; 68 °C, 150 s)</p> <p>1 x 68 °C, 300 s<sup>6</sup></p>

Z1793-for (2) Z1793-r	AAAAGTAAAAATGAAGGGC ATCTCCAGCACCAGACTTTC	Confirmation of <i>nanS</i> -p3 deletion	1 x 95 °C, 300 s 30 x (95 °C, 30 s ; 50 °C, 30 s; 68 °C, 180 s) 1 x 68 °C, 300 s <sup>6</sup>
Z6054-for Z6054-rev	TACGGAGTGGTCAGTGTG CCGACAGAAAATGAAAGTAA	Confirmation of <i>nanS</i> -p4 deletion	1 x 95 °C, 300 s 30 x (95 °C, 30 s ; 57 °C, 30 s; 68 °C, 210 s) 1 x 68 °C, 300 s <sup>6</sup>
Z3926/27-for Z3926/27-rev (2)	TGTTGGTACTGACTGATGGC AGCCACCAGTTAACTACTGATG	Confirmation of <i>nanS</i> -p5 deletion	1 x 95 °C, 300 s 30 x (95 °C, 30 s ; 55 °C, 30 s; 68 °C, 150 s) 1 x 68 °C, 300 s <sup>6</sup>

1985-for 1985-rev	GAGATTAGGCATATATCCAGGCTTC TGGCTCTGATAACTCATGCAGGCG	Confirmation of <i>nanS</i> -p6 deletion	1 x 95 °C, 300 s 30 x (95 °C, 30 s ; 59 °C, 30 s; 68 °C, 120 s) 1 x 68 °C, 300 s <sup>6</sup>
3022-for (2) 3022-rev (2)	GGTGGGGCAATCAGCAAGAAGGA CCAGTATGAACCGCCCCGG	Confirmation of <i>nanS</i> -p7 deletion	1 x 95 °C, 300 s 30 x (95 °C, 30 s ; 58 °C, 30 s; 68 °C, 270 s) 1 x 68 °C, 300 s <sup>6</sup>
z6054- <i>Nde</i> I-for z6054- <i>Xho</i> I-rev	<b>CCCCATATG</b> TCGATTAACATTATGATGTTG <sup>2</sup> AA <b>ACTCGAG</b> TGCTGCCGGTGCATTTATC <sup>2</sup>	<i>nanS</i> -p4	1 x 98 °C, 60 s 30 x (98 °C, 10 s; 66 °C, 30 s; 72 °C, 60 s) 1 x 72 °C, 600 s <sup>5</sup>

z3342-for z3342-rev	GCGAATAATATCCGACAGTAATCAC AAACCAGAGCACGAAAGTATCG	<i>nanS</i> -p2 with flanking region	1 x 98 °C, 60 s 28 x (98 °C, 10 s; 67 °C, 30 s; 72 °C, 60 s) 1 x 72 °C, 600 s <sup>5</sup>
z3342-18A/C-for z3342-13T/G-rev	CTTCCGCTGCCGGAGACCTATGACCGTCCG <sup>3</sup> CGGACGGTCATAGGTCTCCGGCAGCGGAAG <sup>3</sup>	<i>nanS</i> -p2 in pGEM <sup>®</sup> -T easy	1 x 98 °C, 30 s 12 x (98 °C, 30 s; 55 °C, 60 s; 72 °C, 320 s) 1 x 72 °C, 300 s <sup>5</sup>
z3342- <i>Nde</i> I-for z3342- <i>Xho</i> I-rev	CCCC <b>ATATGG</b> CATTTAAACACTACGATGTG <sup>2</sup> AA <b>ACTCGAGT</b> GCCGCAGTGTCTGTGTT <sup>2</sup>	<i>nanS</i> -p2	1 x 98 °C, 30 s 30 x (98 °C, 10 s; 66 °C, 60 s; 72 °C, 60 s) 1 x 72 °C, 600 s <sup>5</sup>

M13-for M13-rev	GTTTTCCCAGTCACGAC <sup>7</sup> AACAGCTATGACCATG	pGEM <sup>®</sup> -T easy next to insert region	1 x 95 °C, 300 s 30 x (95 °C, 30 s; 55 °C, 30 s; 68 °C, 150 s) 1 x 68 °C, 300 s <sup>6</sup>
nanShom1-for nanShom1-rev	CAGGATATGAGTACGCTGAATCATCCG GGAATATCCGGATCTTCTGCCGGCGC	<i>nanS</i> -p3-p7	1 x 95 °C, 300 s 30 x (95 °C, 30 s; 64 °C, 30 s; 68 °C, 45 s) 1 x 68 °C, 300 s <sup>6</sup>
nanShom3-for nanShom3-rev	AACCGTGGGGCAGGGGCTGCATAT TCCGGGTCTTCTTCCGGTTTGTTTCGT	<i>nanS</i> -p1a-p2	1 x 95 °C, 300 s 30 x (95 °C, 30 s; 65 °C, 30 s; 68 °C, 45 s) 1 x 68 °C, 300 s <sup>6</sup>

<sup>1</sup> Capital letters depict the complementary parts to the gene which should be deleted; small letters depict the complementary part to the FRT-flanked kanamycin resistance cassette on plasmid pKD4. <sup>2</sup> Bold letters indicate the restriction site of *Nde*I or



*Xho*I, respectively. <sup>3</sup> The bold letter indicates the base used for mutagenesis. <sup>4</sup> Taq DNA polymerase from Genaxxon. <sup>5</sup>

Phusion DNA polymerase from Thermo Scientific; <sup>6</sup> Taq DNA polymerase from New England Biolabs® GmbH ; <sup>7</sup>Promega

GmbH