

**Supplementary table 1.** Primers used to construct the TIGR4  $\Delta$  *pspC* deletion mutant.

Strain or primer	Relevant features or nucleotide sequence (5' to 3') <sup>a</sup>	Reference/ source or target gene <sup>b</sup>
PBCN0094	Serotype 4	(1)
PBCN0133	Serotype 6B	(1)
PBCN0031	Serotype 3	(1)
TIGR4	Serotype 4	(2)
R6	Unencapsulated	(3)
TIGR4 $\Delta$ cps	Unencapsulated TIGR4 with kanamycin	(4)
TIGR4 $\Delta$ pspC (spec)	Serotype 4	This study
TIGR4 $\Delta$ pspC (kan)	Serotype 4	This study
TIGR4 pspC2	Serotype 4	This study
TIGR4 pspC6	Serotype 4	This study
TIGR4 pspC9	Serotype 4	This study
TIGR4 pspC11	Serotype 4	This study
R6 pspC2	Unencapsulated	This study
R6 pspC6	Unencapsulated	This study
R6 pspC9	Unencapsulated	This study
R6 pspC11	Unencapsulated	This study
<b>Primers mutant construction</b>		
Spn2190_L1	TTG AGG CAA TGG TGC ACA AG	TIGR4 <i>pspC</i> ; left flank
EMspn2190_L2	<u>CCACTAGTCTAGAGCGGCTACACTAGCTACTCCAACAC</u>	TIGR4 <i>pspC</i> ; left flank; overlap Spec <sup>res</sup> cassette
EMspn2190_R1	CAAGATGAAGATCGCCTACG	TIGR4 <i>pspC</i> ; right flank
EMspn2190_R2a	<u>GCGTCAATTTCGAGGGGTATCGCTATGGAGTCAATGCCAAT</u>	TIGR4 <i>pspC</i> ; right flank; overlap Spec <sup>res</sup> cassette
EMspn2190_C	TCG TTC TCT GTC GCA TGA AC	TIGR4 <i>pspC</i> ; control
PBMrTn9	CAATGGTTCAGATACGACGAC	Spec <sup>res</sup> cassette; control
PBpR412_L	GCCGCTCTAGAACTAGTGG	Spec <sup>res</sup> cassette; pR412 plasmid
PBpR412_R	GATACCCCTCGAATTGACGC	Spec <sup>res</sup> cassette; pR412 plasmid
HBKanF4	GGAATTCGATATCAAGCTTA	Kan <sup>res</sup> cassette; pR410 plasmid
HBKanR	AGGTACTAAAACAATTCATCC	Kan <sup>res</sup> cassette; pR410 plasmid
HBKanR3	ATCCACATCGGCCAGATCGT	Kan <sup>res</sup> cassette; control
EMspn2190_pspC_kan_L2	<u>TAAGCTTGATATCGAATTCCTACACTAGCTACTCCAACAC</u>	TIGR4 <i>pspC</i> ; left flank; overlap Kan <sup>res</sup> cassette
EMspn2190_pspC_kan_R2	<u>GGATGAATIGTTTTAGTACCTGCTATGGAGTCAATGCCAAT</u>	TIGR4 <i>pspC</i> ; right flank; overlap Kan <sup>res</sup> cassette
EM_pspC_2_3_L2 new	<u>CTTTCGCTTTTIGATGCAAACAIGTTTTATTCTCTATATTT</u>	TIGR4; left flank; overlap <i>pspC</i> PBCN0094
EM_pspC_6_11_L2	<u>CTTTCGTTTTTIGATGCAAACAIGTTTTATTCTCTATATTT</u>	TIGR4; left flank; overlap <i>pspC</i> PBCN000133 & PBCN0031
EM_pspC_9_L2	<u>CGCTCATGATTTGATTTAAACAIGTTTTATTCTCTATATTT</u>	TIGR4; left flank; overlap <i>pspC</i> PBCN0133
EM_pspC_2_3_Fw	ATGTTTGCATCAAAAAGCGAAAG	PBCN0094 <i>pspC</i> ; forward
EM_pspC_6_11_Fw	ATGTTTGCATCAAAAACGAAAG	PBCN0133 & PBCN0031 <i>pspC</i> ; forward
EM_pspC_9_Fw	ATGTTTAAATCAAATCATGAGCG	PBCN0133 <i>pspC</i> ; forward
EM_pspC_2_3_6_R2_spec	<u>CAATGGTGAATGGGTAACATAAGCCGCTCTAGAACTAGTGG</u>	TIGR4; right flank; overlap PBCN0094 & PBCN0133
EM_pspC_9_11_R2_spec	<u>CTTGCTAAGAAAAGAATGAAATAGCCGCTCTAGAACTAGTGG</u>	TIGR4; right flank; overlap PBCN0133 & PBCN0031

EM_pspC_2_3_6_Rev	TTAGTTTACCCATTACCATTTG	PBCN0094 & PBCN0133 <i>pspC</i> ; reverse
EM_pspC_9_11_Rev	CTATTTTCATTCTTTTCTTAGCAAG	PBCN0133 & PBCN0031; <i>pspC</i> reverse
EM_pspC_2_Crev	GGTACTTGGGTAGCTCCCTC	<i>pspC2</i> ; control
EM_pspC_3_9_Crev	CTGCTTGGGTACTTCCCTC	<i>pspC6</i> ; control
EM_pspC_6_Crev	CGTGGAGTTATTCCCAATTCT	<i>pspC9</i> ; control
EM_pspC_11_Crev	GCTACTTGGGTAGTTACCTC	<i>pspC11</i> ; control
<b>Mutant validation by qPCR</b>		
Q- <i>pspC</i> -V2-F	CAGGCAGAACAAGGAGAACA	<i>pspC2</i> ; forward
Q- <i>pspC</i> -V2-R	GCATAGCTCTCACCCACTATT	<i>pspC2</i> ; reverse
Q- <i>pspC</i> -V6-F	ACCGTAACTACCAACCAATAC	<i>pspC6</i> ; forward
Q- <i>pspC</i> -V6-R	CGAGATTCTTAGCTTCCTCTT	<i>pspC6</i> ; reverse
Q- <i>pspC</i> -V9-F	TGGAAGTCAGGCAGAACAAC	<i>pspC9</i> ; forward
Q- <i>pspC</i> -V9-R	GCATAGCTCTCACCCACTATT	<i>pspC9</i> ; reverse
Q- <i>pspC</i> -V11-F	TGGTTCATGCGACAGAGAAG	<i>pspC11</i> ; forward
Q- <i>pspC</i> -V11-R	CATCGACTGTATTAGCAGCTTTC	<i>pspC11</i> ; reverse
Q-Sp- <i>gyrA</i> -F	AATGAACGGGAACCCTTGGT	<i>gyrA</i> ; forward
Q-Sp- <i>gyrA</i> -R	CCATCCCAACCGGATAC	<i>gyrA</i> ; reverse

## References

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