Table S2. Oligonucleotides used in this study

|  |  |
| --- | --- |
| Purpose | Oligonucleotide pair(s) |
| *LVS null mutants* |  |
| DotU Δ4-203 | DotU\_a: 5´-*CTC GAG* GAT ATT ATT GAT GTG TTG CAT ATT-3´ (*Xho*I) and DotU\_b:5´-*GGA TCC* TTA TTA AGC TGG TAA TAA GGG TA-3´ (*Bam*HI)  DotU\_c: 5´-*GGA TCC* GTC TTT CAT TTA TAA TAT CCT TTA T-3´ (*Bam*HI), DotU\_d: 5´-*GAG CTC* ATA GAA GAC AAG AGA ACA GGT A-3´ (*Sac*I) |
| VgrG Δ4-162 | Del\_pigB\_a: 5´-*CTC GAG* TAG TGT TTC TTC ATC AAA CAT TA-3´(*Xho*I) and Del\_pigB\_b: 5´-ATT CTT ATG TCA AAA GTT GGA TAA TAA TAT GAA TAA T-3´  Del\_pigB\_c:5´-TTT TGA CAT AAG AAT ATC CCC T-3´ and Del\_PigB\_d: 5´-*GAG CTC* ATC AAT TCC CTT TTA ATA AGG AT-3´(*Sac*I) |
| *Complementation in* cis |  |
| VgrG | Del\_pigB\_a (*Xho*I) and Del\_PigB\_d (*Sac*I) |
| *Complementation in* trans |  |
| DotU | PigF\_F: 5´-*ATT AAT* GAA AGA CTT TAA AGA GAT AGA-3´ (*Ase*I) and PigF\_R: 5´-*GAA TTC* TTA CCA GCT TAA TAA AAT TAG TAA-3´ (*Eco*RI) |
| DotU-GSK | PigF\_F (*Ase*I) and PigF\_GSKrev: 5´-*GGT ACC* CCA GCT TAA TAA AAT TAG TAA G-3´ (*Kpn*I) |
| DotU DE70-71AA-GSK | DotU\_NdeI\_F :5´-*CAT ATG* AAA GAC TTT AAA GAG ATA GAA ATT-3´ (*Nde*I) and DotU\_7071AA\_b:5´-**G**CA **G**CA ACA TAA GCA AGT AAC GGA AAA AC-3´  DotU\_7071AA\_c: 5´-TTG CTT ATG TTG **C**TG **C**AA AAT TGA TGC TAC TTA GGG AA-3´ and PigF\_GSKrev (*Kpn*I) |
| DotU DE70-71KK-GSK | DotU\_NdeI\_F (*Nde*I) and DotU\_7071KK\_b: 5´-**TC**T **T**AA CAT AAG CAA GTA ACG GAA AAA C-3´  DotU\_7071KK\_c: 5´-CTT GCT TAT GTT **A**A**G A**AA AAA TTG ATG CTA CTT AGG GA-3´ and PigF\_GSKrev (*Kpn*I) |
| DotU DE70-71SS-GSK | DotU\_NdeI\_F (*Nde*I) and DotU\_7071SS\_b: 5´-**GA**A **GA**A ACA TAA GCA AGT AAC GGA AAA AC-3´  DotU\_1771SS\_c: 5´-TTG CTT ATG TT**T C**T**T** **C**AA AAT TGA TGC TAC TTA GGG AA-3´ and PigF\_GSKrev (*Kpn*I) |
| DotU G134A-GSK | DotU\_NdeI\_F (*Nde*I) and DotU\_134A\_b: 5´-G**G**C ATA GAA GTC ATT GTG TAG GAT A-3´  DotU\_134A\_c: 5´- CAC AAT GAC TTC TAT G**C**C AAA TAC TAT GAC AAT ATA TAT AAC-3´ and PigF\_GSKrev (*Kpn*I) |
| DotU G134S-GSK | DotU\_NdeI\_F (*Nde*I) and DotU\_134S\_b: 5´-**T**AT AGA AGT CAT TGT GTA GGA TA-3´  DotU\_134S\_c: 5´-CAC AAT GAC TTC TAT **A**GC AAA TAC TAT GAC AAT ATA TAT AAC-3´ and PigF\_GSKrev (*Kpn*I) |
| DotU G134K-GSK | DotU\_NdeI\_F (*Nde*I) and DotU\_134K\_b: 5´-**TTT** ATA GAA GTC ATT GTG TAG GAT A-3´  DotU\_134K\_c: 5´-CAA TGA CTT CTA T**AA A**AA ATA CTA TGA CAA TAT ATA TAA C-3´ and PigF\_GSKrev (*Kpn*I) |
| DotU G134D-GSK | DotU\_NdeI\_F (*Nde*I) and DotU\_134D\_b: 5´- G**T**C ATA GAA GTC ATT GTG TAG GAT A-3´  DotU\_134D\_c: 5´-CAC AAT GAC TTC TAT G**A**C AAA TAC TAT GAC AAT ATA TAT AAC-3´ and PigF\_GSKrev (*Kpn*I) |
| VgrG | PigB\_F: 5´-*CAT ATG* TCA AAA GCA GAC CAT ATT T-3´ (*Nde*I) and PigB\_R: 5´-*GAA TTC* TTA TCC AAC CAT TGT TGC TGT AG-3´ (*Eco*RI) |
| VgrG-GSK | PigB\_F (*Nde*I) and PigB\_GSKrev: 5´-*GGT ACC* TCC AAC CAT TGT TGC TGT AG- 3´ (*Kpn*I) |
| IglA-GSK | IglA\_GSK\_F: 5´-*CAT ATG* GCA AAA AAT AAA ATC CCA AAT TCA AGG-3´ (*Nde*I) and IglA\_GSK\_R: 5´-TAT GAT TCA GCA AAT GAA GTA GTT CTT GGT CTA CCT GAC ATC TTA CCA TCT ACT TGT TGA TTA CTT AAG TC-3´ |
| IglB-GSK | IglB\_Y2H\_F: 5´-*CAT ATG* ACA ATA AAT AAA TTA AGT CTC ACT GAT G-3´ (*Nde*I) and IglB\_KpnI\_R: 5´-*GGT ACC* GTT ATT ATT TGT ACC GAA TAA TTC-3´ (*Kpn*I) |
| IglC-GSK | IglC\_GSK\_F: 5´-*CAT ATG* AGT GAG ATG ATA ACA AGA CAA CAG GTA-3´ (*Nde*I) and IglC\_GSK\_R: 5´-TAT GAT TCA GCA AAT GAA GTA GTT CTT GGT CTA CCT GAC ATT GCA GCT GCA ATA TAT CCT ATT TTA GCA-3´ |
| IglD-GSK | IglD\_Y2H\_F: 5´-*CAT ATG* TTT CTA GAA AGG ATT TAT TGG GAA GAT-3´(*Nde*I) and IglD\_KpnI\_R: 5´-*GGT ACC* AGA AAA GGC TAT AAA GAA ATC AA-3´ (*Kpn*I) |
| *Yeast two-hybrid interaction studies* |  |
| DotU | PigF\_F (*Nde*I) and PigF\_R (*Eco*RI) |
| VgrG | PigB\_F (*Nde*I) and PigB\_R (*Eco*RI) |
| IcmF | PdpB\_NdeI\_F: 5´-*CAT ATG* AAT TTT ATT AAA AAT CAT CAA ATA TT-3´ (*Nde*I) and PdpB\_XmaI\_R: 5´-*CCC GGG* TTA TTG TAC ATT GAC TTC TCC TTG T-3´ (*Xma*I) |
| *Bacterial two-hybrid interaction studies* |  |
| DotU | DotU\_NdeI\_F (NdeI) and DotU\_mut\_b: ´-**A**GC AAG TAA CGG AAA AAC TAT ATA TT-3´  DotU\_mut\_c: 5´-TTT CCG TTA CTT GC**T** TAT GTT GAT GAA AAA TTG ATG CTA-3´ and DotU\_NotI\_R: 5´-*GCG GCC GC* CCA GCT TAA TAA AAT TAG TAA GCT T-3´ (*Not*I) |
| VgrG | VgrG\_NdeI\_F: 5´-*CAT ATG* TCA AAA GCA GAC CAT ATT TTC AAC-3´ (*Nde*I) and VgrG\_NotI\_R: 5´-*GCG GCC GC* TCC AAC CAT TGT TGC TGT AGA AC-3´ (*Not*I) |
| IcmF | PdpB\_NdeI\_F: 5´-*CAT ATG* AAT TTT ATT AAA AAT CAT CAA ATA TT-3´ (*Nde*I) and PdpB\_mut\_b: 5´- **A**GT ATC ATT ATA TTT TGG TAA GAT TAC-3´  PdpB\_mut\_c: 5´-AAA ATA TAA TGA TAC **T**TA TGA CTT ATC AAT GAT TAC ATC A-3´ and PdpB\_NotI\_R: 5´-*GCG GCC GC* TTG TAC ATT GAC TTC TCC TTG T-3´ (*Not*I) |
| IglA | IglA\_GSK\_F (*Nde*I) and IglA\_NotI\_R: 5´-*GCG GCC GC* CTT ACC ATC TAC TTG TTG ATT ACT-3´ (*Not*I) |
| IglB | IglB\_Y2H\_F (*Nde*I) and IglB\_NotI\_R: 5´-*GCG GCC GC* GTT ATT ATT TGT ACC GAA TAA TTC TG-3´ (*Not*I) |
| IglC | IglC\_GSK\_F (*Nde*I) and IglC\_NotI\_rev: *GCG GCC GC* TGC AGC TGC AAT ATA TCC TAT-3´(*Not*I) |
| *qPCR* |  |
| *icmF* | FTT1345-F: 5´-TTG AGT CTA AGA GTT ATG CG ACT-3´ and FTT1345-R: 5´-TGT AGT GGT TCA TAT CCT TGT TTG-3´ |
| *dotU* | FTT1351-F: 5´-CTA CAC AAT GAC TTC TAT GGC AAA-3´ and FTT1351-R: 5´-ATT AAC CGA ATC AAT TGT CGA AT-3´ |
| *vgrG* | FTT1347-F: 5´-TTG TTA GCT ACT AAG AAA TCA AGC ATT and FTT1347-R:5´-TTA ATA GTT GCA GAC TCT AGC GAA A-3´ |
| *tul4* | 17kD-F: 5´-GTG CCA TGA TAC AAG CTT CC-3´ and 17kD-R: 5´-GCT GTC CAC TTA CCG CTT CA-3´ |

The nucleotide sequences in italics represent the incorporated *Nde*I, *Eco*RI, *Bam*HI, *Sac*I, *Xma*I, *Xho*I, *Not*I, *Ase*I and *Kpn*Irestriction sites used for cloning of the PCR amplified DNA fragments. The underlined sequence indicates the complementary overlap between respective primers in the overlap PCR reactions. In primers used to generate amino acid substitutions, the nucleotides substituted are indicated in boldface. To optimise expression, all substitutions were adapted according to the codon usage preferences of *F*. *tularensis* (http://www.kazusa.or.jp/codon).